HORMONES, BRAIN AND STRESS

E. RONALD DE KLOET

Division of Medical Pharmacology, LACDR/LUMC, Leiden University, 2300 RA Leiden, The Netherlands
E-mail: e.kloet@lacdr.leidenuniv.nl

Abstract. The stress system orchestrates body and brain responses to the environment. This action exerted by the mediators of the stress system has two modes of operation. The immediate response mode driven by corticotropin-releasing hormone (CRH) organises via CRH-1 receptors the behavioural, sympathetic and hypothalamic-pituitary-adrenal (HPA) responses to a stressor. In the other - slower - mode, which facilitates behavioural adaptation, the urocortins acting through CRH-2 receptors seem prominent. Corticosteroid hormones secreted by the adrenal cortex are implicated in both modes through their high affinity type 1 (mineralocorticoid receptors - MR) and lower affinity type 2 (glucocorticoid receptors - GR) receptors that are co-localised in limbic neural circuitry. Current data suggest that MR controls in specific afferents the threshold or sensitivity of the fast CRH-1 driven stress system mode and thus prevents disturbance of homeostasis, while GR facilitates its recovery by restraining in these very same circuits stress responses and by mobilising energy resources. In preparation for future events GR facilitates behavioural adaptation and promotes storage of energy. The balance in the two stress system modes is thought to be essential for cell homeostasis, mental performance and health. Imbalance induced by genetic modification or chronic stressors changes specific neural signalling pathways underlying psychic domains of cognition and emotion, anxiety and aggression. This Yin-Yang stress concept is fundamental for genomic strategies to understand the mechanistic underpinning of cortisol-induced stress-related disorders such as i.e. severe forms of depression and co-morbid diseases.

Keywords: Stress – Behaviour – Corticosteroids – Brain - Genes

Introduction

It is a great honour to be invited as introductory lecturer for the triennial conference on catecholamines and other transmitters in stress. While preparing for this 8th edition of the Smolenice Castle Conference I sat back for a moment reflecting on the very first Conference more than twenty years ago. I see the castle's facilities, the atmosphere and the hospitality, images that stand out so characteristic in the Slovakian hills. Images that trigger the memory of research life at that time. No laptop, fax and e-mail. Corrections and literature references were endless, tedious jobs. Ima-ges that also give me a chance to start from a historical perspective, to phrase fundamental questions and to sketch some future directions, at least towards June 2006. Images reminding me that at each Conference the most imminent question asked by the participants is: when will the next meeting be Richard?

A fundamental question for the castle meeting is how the stress system can change its function from protection to damage and disease. It is common knowledge that the duration and nature of the stressor are important determinants, but then the question remains why one individual copes

and remains in excellent health, while the other suffers and breaks down under similar adverse conditions. Current wisdom predicts an effective stress reaction as a healthy condition. "Effective" meaning a highly reactive system that readily turns on and off its behavioural, autonomous and endocrine responses to stressors. If the system responds sluggish, or when stress reactions are slowly terminated and prolonged, its mediators enhance vulnerability to damage and disease, for which the individual is predisposed. On the genomic level this implies that it is essential to understand how transient changes in expression of stress responsive genes are converted to prolonged maladaptive genomic changes (SABBAN and KVETNANSKY 2001).

In the control of stress reactions the corticosteroid hormones operating in concert with catecholamines and other transmitter are very potent players. If corticosteroid control is insufficient stress reactions are much too strong (HEIM et al. 2000). Alternatively, if adaptation to stress fails circulating corticosteroid levels remain elevated for a prolonged period of time (SELYE 1952). This excess corticosteroid has catabolic consequences and leads to breakdown of vital functions (SAPOLSKY et al. 2000). At least 50% of the depressed patients have elevated cortisol due to feedback resistance and a flattened circadian rhythm in the face of sympathetic hyperactivity (BELANOFF et al. 2001; HOLSBOER 2000; 2001; CHROUSOS and GOLD 1992; GOLD and CHROUSOS 1999). These include the patients suffering from melancholic depression as opposed to the pathophysiological mirror image, the atypical depressed patient with the signs and symptoms of hypocortisolemia (GOLD and CHROUSOS 1999). Hypercorticism is in particular often a hallmark of severe depression with psychotic features (SCHATZBERG et al. 1985). The psychotically depressed patient appears to respond favourably to anti-glucocorticoid therapy (BELANOFF et al. 2002) as has been previously reported for Cushing patients (VAN DER LELY et al. 1991). If further validated (GOLD et al. 2002), the finding actually represents the first pathophysiological substrate for a psychiatric disorder caused by excess cortisol that can be rescued by anti-glucocorticoids.

Thus, the initial question on the role of the stress system can be rephrased to cortisol: how does its action change from protective into harmful. What is the cause and what are the consequences? To address these questions, I first discuss the action mechanism of the corticosteroids. These hormones act conditional, and accordingly the context they operate is extremely important. Second, some animal models for depression are discussed in which the stress system is dysregulated and I focus briefly on two representative models: mice genetically selected for extreme differences in coping style and mice subjected to early trauma. Third, new strategy's to identify novel molecular targets in stress circuitry for treatment of stress-related disorders are embedded in the section future directions. Most studies are in the mouse and rat that have corticosterone as the principal naturally occurring glucocorticoid, while man has cortisol.

Brain corticosteroid receptors as stress system nodes that integrate information in binary fashion

Steroid molecules can affect cell function through direct interactions with transmitter receptors, as is the case for instance with neurosteroids derived from progesterone and deoxycorticosterone that interact with the GABA receptor. Here we focus on the intracellular receptors that mediate a variety of signalisations. Activation can have immediate responses through re-aggregation and molecular remodelling of the receptor molecule in the cytoplasmic compartment or through direct interaction with the transcription machinery (BAULIEU 2000; AUPHAN et al. 1995).

Discovery of brain corticosteroid receptors. I started my studies in Holland when Bruce McEwen (MCEWEN et al. 1968) discovered corticosteroid receptors in the limbic brain. Much to the surprise of the established stress community these nuclear receptors were expressed in abundance in the hippocampus beyond the core of the hypothalamic-pituitary-adrenal (HPA) axis in the hypothalamic paraventricular nucleus (PVN) and the pituitary corticotrophs in which these receptors were expected. As a student my very first ex-

periment was with dexamethasone because in my opinion the much higher potency of the synthetic glucocorticoid would label the hippocampus even better than corticosterone. It did not, but rather labelled the pituitary corticotrophs and to some extent the CRH neurons in the PVN (DE KLOET et al.1975). Only years later we found out why. I give three intriguing twists in the story.

Multidrug resistance P-glycoprotein (mdr Pgp) is a gatekeeper for brain penetration. Dexamethasone in tracer amounts poorly labelled the brain (MEIJER et al. 1998). This is because mdr Pgp and related proteins in the blood-brain-barrier export circulating synthetic steroid after penetration in the endothelial cells out of the brain as it does with many exogenous compounds. While this was suspected many years ago the proof came from experiments with Pgp knockout mice. ^{3}H-Dexamethasone administered to mdr Pgp mutants accumulates in brain in a tenfold larger amount than in the wild types, and neurons of the hippocampus now weakly retain this steroid. Also ^{3}H-cortisol, not naturally occurring in rat and mouse, is poorly retained in wild type brains and appears a Pgp substrate. In the mutants profound labelling of hippocampal neurons occurs with cortisol, as is the case with corticosterone. To our surprise human MDR also recognises cortisol rather than corticosterone as substrate, and liquid chromatograph — mass spectrometry analysis of post-mortem human brain samples revealed that in fact corticosterone is the preferred brain corticosteroid (KARSSEN et al. 2001). One of the implications of the steroid gatekeeper is that moderate amounts of dexamethasone suppress stress-induced HPA activity at a pituitary rather than a brain site. Because of this pituitary HPA blockade body and brain are depleted of endogenous corticosterone. In the periphery glucocorticoid actions of corticosterone are substituted by dexamethasone, but the corticosterone-depleted brain is not. Hence, moderate amounts of dexamethasone produce an adrenalectomy-like state in the brain.

Mineralocorticoid and glucocorticoid receptors: co-localised binary system. Even in the mdr mutants dexamethasone does not label the limbic neurons as well as corticosterone does. We discovered that while both steroids are glucocorticoids, dexamethasone binds as expected with high affinity to the glucocorticoid receptor (GR); corticosterone prefers with highest affinity the mineralocorticoid receptors (MR) and has a ten fold lower affinity to GR (REUL and DE KLOET 1985). Hence in our original experiments corticosterone labelled the MR, but the tracer was too low in quantity to see the classical dexamethasone labelled GR. This discovery was possible because of the synthesis of "pure" glucocorticoids and the cloning of GR and MR (EVANS and ARRIZA 1989). The precise topography was revealed in brain with immunocytochemistry and in situ hybridisation (FUXE et al. 1985; VAN EEKELEN et al. 1988, ITO et al. 2000). With confocal microscopy MR and GR appeared to be co-localised in abundance in limbic neurons including hippocampal CA1 and dentate gyrus (VAN STEENSEL et al. 1995). Co-localisation occurred also in nuclei of the amygdala and medial prefrontal cortex (HELM et al. 2002), areas that have an important function in emotion and cognition.

MR binds aldosterone and corticosterone with similar affinity, yet in epithelial cells MR is aldosterone selective. In epithelial cells such as in kidney and the periventricular brain areas the enzyme 11-βHydroxysteroid dehydrogenase type 2 (HSD-2) oxidises corticosterone to the inactive 11-dehydro metabolite leaving these MR available only for aldosterone (EDWARDS et al. 1988). Hence these sites are aldosterone-selective in control of electrolyte homeostasis. Limbic brain regions do not express HSD-2 and therefore MR *sees* predominantly corticosterone that circulates in a 100 - 1000 fold excess. Rather a brain structure as the hippocampus contains HSD-1 which re-activates bioinactive corticosterone. Potentially this regeneration of corticosterone could lead under some circumstances to excess corticosteroid exposure and disease (SECKL 1997). This has been demonstrated with a mouse mutant with over-expressed HSD-1 in adipose tissue. The mutants developed visceral obesity with a high fat diet as well as insulin resistant diabetes, hyperlipedemia and hyperphagia. Thus 11-HSD may be common modulator of obesity and metabolic syndrome (MASUZAKI et al. 2001).

Receptors, transcription factors and co-regulators: the integrating pathway node. Some 8 years ago it was demonstrated that corticosteroid receptors could affect signalling pathways beyond activation of glucocorticoid response elements (GREs) by interaction with other transcription factors (AUPHAN et al. 1995). MR and GR both bind to these GREs, but only GR is capable to interact with transcription factors such as activating protein (AP-1) and nuclear factor κB (NFκB) to attenuate stress-induced signalisations through the membrane. This finding provided a firm mechanistic underpinning to the concept advanced by TAUSK (1951) and MUNCK et al. (1984) that glucocorticoids actually restrain primary stress reactions. It is now known that they achieve this restraint through interaction of the GR monomers with transcription factors driven by catecholamines and other transmitters.

Recently, co-activator and co-repressor molecules were identified that appeared powerful modulators of nuclear receptor function (MEIJER, 2002). Members of the steroid co-activator receptor (SRC) family of proteins promote agonist-induced receptor activation by permitting recruitment of e.g. CBP/p300 transcription activators and chromatin remodelling. The NCOR and SMRT co-repressor molecules do the opposite and promote repression of gene transcription. Current studies have shown uneven co-localisation of various SRC subtypes with MR and GR in brain suggesting some degree of cell specificity. *In vitro* transfection experiments suggest that variable stoechiometry of co-repressors and co-activators, either induced or congenital, underlies differential MR/GR functioning. The GR antagonist mifepristone (RU 486) with the receptors provides an interesting example of the possible modes of interaction with steroid receptor signalling. The antagonist may even act as an agonist bound to GR monomers in interaction with NFκB. It only acts as antagonist at GREs if sufficient co-repressor is available (figure 1).

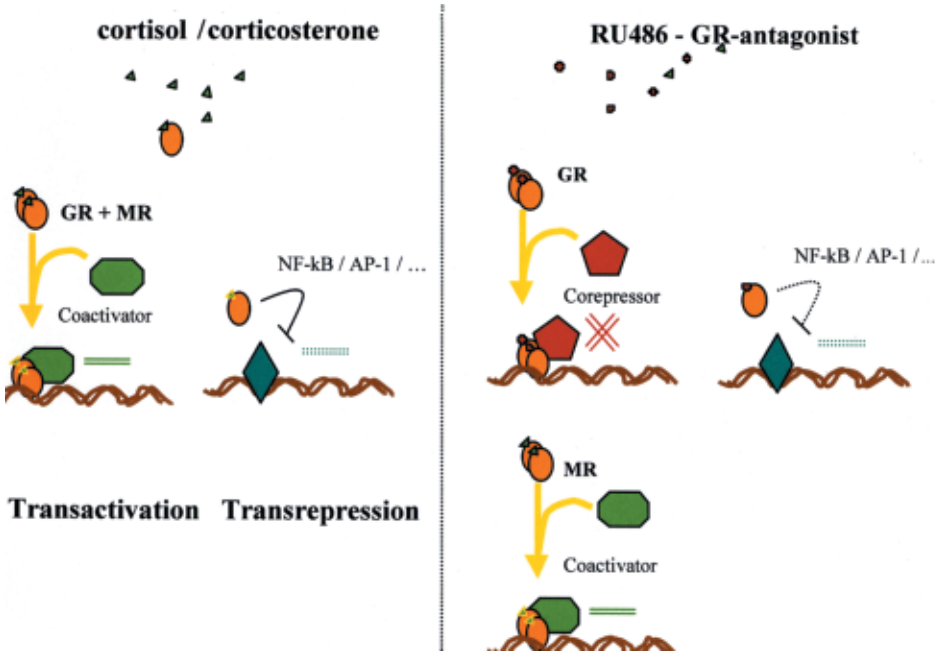

Fig 1 Action mechanism of corticosteroid hormones.
LEFT: **The naturally occurring glucocorticoid agonists cortisol and corticosterone interact with mineralocorticoid and glucocorticoid receptors (MR and GR), which upon dimerisation recruits co-activators to stimulate gene transcription (transactivation). As monomers GR, but not MR, can interfere with activation of gene transcription by transcription factors (NFκB / AP-1) triggered by membrane signalisations (transrepression).**
RIGHT: **GR dimerises upon glucocorticoid antagonist RU 486 and recruits co-repressors for blockade of agonist stimulated gene transcription. MR is not affected and escapes blockade, as does transrepression involving GR monomers and transcription factors. Courtesy of dr Onno Meijer.**

MR and GR operate in co-ordinate bi-directional fashion at the on- and offset of the stress response

Once properties, localisation and regulation were known we began detailed studies to explore the function on various levels of complexity. The approaches ranged from molecular changes to cellular homeostasis and adaptation to stress with the perspective to learn more about their implication on health and disease. We identified that one hormone (corticosterone) used two signalling systems (MR and GR). This work was inspired by Hans Selye's thesis on pro- and anti-inflammatory activity of mineralocorticoid and glucocorticoid hormones that were considered the exponents of opposing hormone actions crucial for maintenance of homeostasis. The work and viewpoints of TAUSK (1951), INGLE (1954), MUNCK et al. (1984) further highlighted the role of glucocorticoids in stress. It was common knowledge that any condition that (threatens) to disturb homeostasis triggered primary stress reactions that were prevented from overshooting by GR-mediated effects requiring stress levels of corticosterone. Thus inflammation, infection, energy need and psychological challenges all trigger via stressor-specific pathways the "non-specific" HPA response, and glucocorticoids feedback to facilitate elimination of the primary stress reaction. If the inflammation subsides the source of the stressor is eliminated.

An elegant demonstration of this principle was provided by Dallman and colleagues (LAUGERO et al. 2001; 2002). Creating energy demand by adrenalectomy imposes a new steady state condition characterised by profound activation of CRH and ACTH as if demanding the need for glucocorticoids that are not there. Surprisingly, the neuroendocrine activation is eliminated by providing the energy substrate sucrose, which the animals select to consume when offered together with saline. The brains of the sucrose-fed adrenalectomized rats adapt in behaviour (feeding, sucrose preference) and in HPA regulation. On the basis of this finding LAUGERO et al. (2001) propose that many of the corticosterone actions actually may be indirect and result from its metabolic action. Because the hormone restores energy metabolism the adrenalectomy-induced ACTH/CRH hyperactivity.is also reinstated in a permissive manner. In contrast, corticosterone chronically infused intracerebroventricularly (icv) in the brains of adrenalectomized animals mimics the effect of stress on the brain. Corticosterone icv enhances basal and stress-induced ACTH release and blocks the effect of sucrose infusion (LAUGERO et al. 2002) suggesting that the hormone has activated a feed-forward loop in the brain. These findings have triggered an intriguing twist in the debate on the good and bad effects of corticosterone in brain.

Cells. On the *cellular* level using the hippocampal slice two general principles were revealed (JOËLS and DE KLOET 1989; 1992; 1994; JOËLS 1994; 2001). First, the control exerted by MR and/or GR appeared to proceed in a U- shaped manner. Ion conductance and transmitter responses were maximal in the absence of corticosterone when no receptor is active and in the presence of very high supraphysiological concentrations of the steroid when both receptors are active. Intermediate corticosterone concentrations occupying predominantly MR and little GR that reflect the average steroid concentration during the day, do minimise the cell responses. For instance the 5HT1A-induced hyperpolarisations of CA1 and dentate gyrus neurons were minimal with MR stimulation, while they were maximal in tissue from ADX animals or from acute stimulation by high corticosterone concentrations. The magnitude of the latter 5HT1A receptor mediated response diminished if tissue was obtained from animals that had experienced prior to the experiment a prolonged period of chronic stress or excess corticosterone.

Second, these responses form the mechanistic basis for phenomena on the network level such as LTP, that also have been demonstrated to show a U-shaped dose responsiveness to corticosterone (DIAMOND et al. 1990). On a more generalised note the cellular work demonstrates that MR stabilises excitability on the cell and circuit level in hippocampus, while GR suppresses excitability transiently raised by excitatory stimuli. The design of the experiments also revealed the conditional nature of the effects. The resting membrane potential was not changed by corticosterone exposure and the steroid effects only became detectable after stim-

ulation by membrane signalisations. Thus the nature and context of these signalisations suggest an enormous diversity of corticosterone action in the control of cell homeostasis.

Neuroendocrine regulation. In neuroendocrine regulation the intracerebral MR and GR blockade using rather selective antagonists exerts a profound and differential effect on measures of HPA activity. In all experiments adrenally intact animals were used. The basis of our experiments was that we distinguished the blockade of GR in the HPA core (i.e. pituitary corticotrophs and PVN micro-environment) from blockade of MR/GR in stressor-specific afferents from brain stem, amygdala-locus coeruleus, prefrontal cortex and hippocampus. The latter blockade interferes with processing of information and behavioural responses with consequent changes in HPA regulation. Thus, exposure to a novel environment was used as stressor, since the limbic-cortical brain circuits involved in e.g. attention, appraisal, fear, and reward abundantly express MR and GR. The studies showed that the MR antagonist RU 28318 causes a rise in circadian basal trough and peak levels of HPA activity as well as an enhanced response to a novelty stressor (RATKA et al. 1989). This effect after central MR blockade was maintained after intrahippocampal administration (VAN HAARST et al. 1997). The GR antagonist mifepristone had no effect on basal trough activity as expected because no GR is occupied under these conditions. Rather GR blockade attenuated and prolonged the response to the novelty stressor (RATKA et al. 1989). The attenuation of the novelty-induced response was mimicked with antagonist application in the dorsal hippocampus, while the prolonged response required GR blockade in the PVN (DE KLOET et al. 1988; VAN HAARST et al. 1997, OITZL et al. 1995). Upon continuous infusion of a few ng icv of mifepristone the amplitude of the circadian rhythm became after four up to 14 days much more enhanced because the peak rather than trough levels in HPA activity did rise (VAN HAARST et al. 1996). This phenomenon very well could be an aspect of the beneficial therapeutic effect of mifepristone in psychotic depression. As is the case in the rat, chronic mifepristone enhanced reactivity of the flattened circadian rhythm in cortisol characteristic for the disease (BELANOFF, unpublished).

These experiments provide only a glimpse of what MR and GR activation in brain potentially can do to basal and stress-induced HPA activity. A number of points can be made in this respect.

First, there is a rich diversity in stressor-specific pathways activating the neurosecretory parvocellular CRH neurons of the PVN through predominant aminergic and GABA-ergic innervations (PALKOVITS 1999; COLE and SAWCHENKO 2002). These pathways include (I) the ascending brain stem aminergic inputs thought to mediate 'systemic' stressors as opposed to the 'processive' or 'psychological' stressors requiring processing of information in higher brain structures (HERMAN and CULLINAN 1997). (II) The input from the locus coeruleus-amygdala circuit that is important for the sympathetic tone. (III) The excitatory input of the left and right medial frontal cortex important for shifts of internal to external cues. (IV) The excitatory input from the hippocampus that depends on context. The aminergic pathways directly project to the CRH neurons, but many of the networks affect transsynaptically via an inhibitory GABA-ergic network the PVN. The excitatory inputs from limbic-cortical regions modulate the GABA-ergic interneuronal network in the PVN micro-environment (COLE and SAWCHENKO 2002; HERMAN et al. 2002) providing a stressor specific neurochemical signature to the neurosecretory neurons (PACAK and PALKOVITS 2001).

Second, tonic influences and feedback actions (DE KLOET and REUL 1987) exerted by corticosterone on the brain have an enormous diversity, since their rising levels feed back on the very same circuits that have initially led to their secretion. Thus corticosterone control depends on the phase of the CRH pulse generator (WINDLE et al. 1998), the nature and duration of the stimulus (LAUGERO et al. 2001) and mechanism involved in processing stressful information (KOVÁCS et al. 2000). AKANA et al. (2001) differentiated between frontal cortex and amygdala on the basis of metabolic and autonomic parameters from the perspective of stress-induced state dependent pathways. Our studies have addressed the hippocampus, because this structure plays an important

role in response to novelty and adaptation to stress. The inhibitory GABA-ergic network in the PVN micro-environment is a corticosterone target in its own right.

Third, the PVN is considered a dynamic entity integrating a diversity of signals that provide a neuroendocrine signature (ROMERO et al. 1996) to exert control over adrenocortical activation by neuroendocrine and autonomic pathways (BUIJS et al. 1999, HERMAN et al. 2002). In particular the stress-induced AP-1 pathway is blocked by corticosterone (KOVÁCS et al. 2000) suggesting a powerful vasopressin link in feedback regulations on the HPA core. At the same time by acting on the PVN corticosterone is capable to control the integrative function of the PVN both with respect to its afferent inputs as its central efferents controling neuroendocrine, autonomic and behavioural functions (DUAN et al. 1999). Corticosterone feedback is context-dependent and has an enormous diversity due to the great number of afferents stemming from peripheral (immune, metabolic, and inflammatory) and central sources. Given all these mutually interactive networks it will be a tall order to sort out the contribution of corticosterone to feedback in the HPA core *versus* each individual pathway under the great variety of stressor specific conditions.

Behaviour. Central MR activation stimulates autonomic outflow (VAN DEN BERG et al.1994; VAN DEN BUUSE et al. 2002), facilitates the conservation/withdrawal response if animals are exposed to a severe stressor (KORTE 2001) and enhances aggressive behaviour of a resident mouse to an intruder (HALLER et al. 2000). In the spatial learning tests MR affects interpretation of environmental information and selection of the appropriate behavioural response to deal with the stressor. Experimental evidence for this comes from the administration of a few ng mineralocorticoid antagonist icv immediately before testing, which altered the behavioural pattern in a maze in search for a route to escape or to find food that the animal had learned the previous day (OITZL et al. 1994; see figure 2). The neural mechanism underlying the latter MR-mediated action is not known and neither is known how the autonomous, neuroendocrine and behavioural consequences of central MR blockade mutually affect each other.

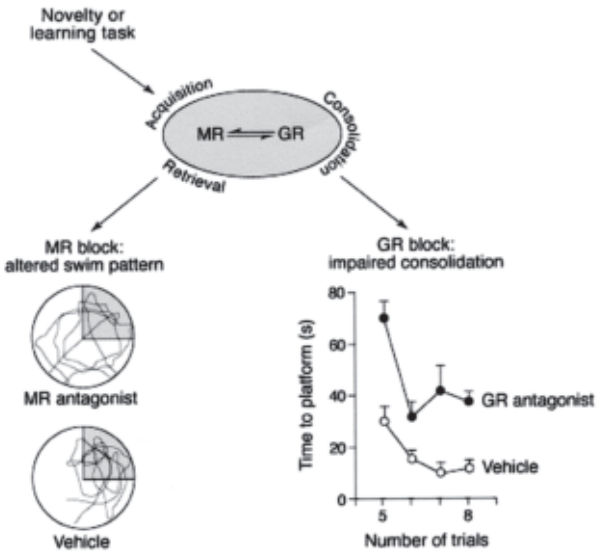

Fig 2 MR and GR affect different aspects of information processing. Following exposure to a learning task MR blockade, 30 to 45 minutes before retrieval on day 2, changed the swim pattern. Inhibition of GR immediately after acquisition on day 1 results in impaired performance 24 h later. These MR- and GR-mediated effects on information processing facilitate behavioural adaptation (from DE KLOET et al. 1999).

Blockade of brain GR impairs the storage of new information (Oitzl and de Kloet 1992; Sandi et al. 1997). A glucocorticoid antagonist administered around the time of learning in the hippocampus impaired the consolidation of newly acquired information As a consequence 24 hours later, the rat is unable to retrieve the information that was learned the previous day and has to learn the maze problem all over again. Likewise, mutant mice with a point mutation in GR, which obliterates binding to DNA, are unable to store learned information (Oitzl et al. 2001). This suggests that corticosteroid-induced cognitive performance requires transactivation as was previously found in the cellular responses to corticosterone in hippocampus (Karst et al. 2000) because such mutants lack the direct activation of GREs, but still have a GR that can interact with other transcription factors (Reichardt et al. 2000). Transgenic mice with downregulated GRs (knockdown) show also cognitive defects and elevated plasma ACTH and corticosterone concentrations in response to stress. After treatment with an antidepressant GRs are increased in concentration and simultaneous behavioural and hormonal corrections (Montkowski et al.1995). Mice exposed to chronic stress and high corticosterone deteriorate in spatial learning, while the reverse occurs after chronic treatment with GR antagonists which appears to result in enhancement of cognitive performance (Oitzl et al. 1998).

How do these MR- and GR-mediated effects on cognition relate to emotional behaviour? This can be illustrated in the following experiment measuring an anxiety paradigm. After exposure to a stressor activation of GR-dependent mechanisms promotes storage of newly acquired information (Korte 2001). This memory is helpful to predict the nature of upcoming events if the animal is exposed to the same place and context. During subsequent visits to the same "stressful" situation the individual's initial response is triggered by the previous experience. It depends on MR, because this behavioural repertoire is blocked by an MR-antagonist given just before behavioural testing suggesting anxiolytic activity of such antagonists. It is also blocked by exogenous GR antagonists prior to the initial stressful event the day before. One day later memory to the previous stressful experience is extinguished. Accordingly, blockade of brain GR interferes with cognitive aspects of fear and anxiety. This action likely takes place in the amygdala (Roosendaal 1999; McGaugh 2002), while the examples from spatial learning relate to the hippocampus (de Kloet et al. 1999).

MR and GR operate in two stress system modes

The key CNS systems generating the stress response have two modes of operation that involve two families of CRH-related peptides (Holsboer 2003; Hauger et al. 2003) (table 1). One mode in-

Table 1

STRESS	ADAPTATION
CRH	Stresscopin
CRH-1 receptor	CRH-2 receptor
sympathetic	para-sympathetic
immediate	late sustained
fight/flight	coping
MR	GR

CRH-1 and CRH-2 receptor systems drive the immediate response mode and the late adaptive mode of the stress system (see also Hsu and Hsueh 2001). MR determines the threshold or sensitivity of the fast response; GR represents the slow adaptive mode that terminates the fast response and that prepares for the future through storage of energy and information.

volves the fast, CRH-driven, neuroendocrine/sympathetic 'fight-flight' response mediated by CRH-1 receptors The fast responding system includes CRH-producing neurons located in the PVN, the amygdala, the noradrenergic neurons located in the locus coeruleus and other aminergic cells in the brain stem. In the periphery the adrenal cortex producing, amongst others, cortisol and the adrenal medulla secreting catecholamines, particularly adrenalin are the principal pacemakers.

The other slower system promotes recovery and adaptation and seems activated by the recently discovered urocortins acting via CRH-2 receptors (Hsu and Hsueh 2001; Reul and Holsboer, 2002). The urocortin II (stresscopin-related) and urocortin III (stresscopin) peptides have a distinctly different localisation from CRH and were identified as selective ligands for the CRH-2 receptor system (Hsu and Hsueh 2001; Lewis et al. 2001). Urocortin I is synthesised in a discrete region in the midbrain, the Eddinger Westphal nucleus, and binds to both CRH receptor sites. Urocortin II is expressed in PVN and locus coeruleus and urocortin III in the hypothalamic area rostral of the PVN, the preoptic nucleus and medial amygdala, but not in cerebellum, cerebral cortex or pituitary. Their terminal fields innervate hypothalamic and brain stem areas matching CRH-2 receptor distribution (Li et al. 2002).

Administration of the urocortins II and III evokes anxiolytic responses as opposed to the anxiogenic depression-like behaviour and hypersensitivity evoked by CRH (Hsu and Hsueh 2001). Some phenomena after CRH are also observed in animal models of depression (e.g. decreased food intake, inhibition of sexual behaviour, sleep disturbances and psychomotor activation). Opposing actions are being recorded for the urocortins II and III and that has led some (Hsu and Hsueh 2001) to suggest that CRH and urocortin are two anti-parallel stress systems that function as organisers of the sympathetic and parasympathetic response, respectively. These data strongly suggest a role for balanced CRH/urocortin family of peptides in the pathophysiology of states of anxiety and depression.

Synthesis. How are the corticosteroids implicated? The cellular data in various limbic regions suggest globally that MR prevents disturbance of homeostasis, while GR promotes its recovery. On the systems physiology and behavioural level this would imply that MR is implicated in a mechanism determining the threshold or sensitivity of the CRH - CRH-1 receptor-driven stress system response. Through GR the stress-induced activation in the various modalities of stress system afferents and in the hypothalamic-pituitary CRH/POMC core of the system are facilitated in termination. In this way GR is assumed to act synergistically to the late responding urocortin II/III - CRH-2 system promoting recovery and adaptation (Table 2).

Table 2

- MINERALOCORTICOID RECEPTORS (type 1)
  - prevent disturbance of cellular homeostasis
  - control sensitivity stress response systém
  - help to select behavioural response
- GLUCOCORTICOID RECEPTORS (type 2)
  - control energy metabolism
  - facilitate recovery of cellular homeostasis
  - restrain stress-induced responses
  - promote information storage
  - promote behavioural adaptation

Function of MR and GR on the cellular, neuroendocrine and behavioural level (see also de Kloet et al. 1998; 1999).

We postulate that the balance in these two stress systems is important for maintenance of health and homeostasis (DE KLOET et al. 1998). Figure 3 depicts this stress system balance idea in its stable (homeostasis) and labile (allostasis?) (MCEWEN and WINGFIELD 2003) version. It implies that in case of imbalance that the individual loses the ability to maintain homeostasis, if challenged by an adverse event. This may lead to a condition of neuroendocrine dysregulation and impaired behavioural adaptation as risk factor for the precipitation of depression (HOLSBOER 2001; 2001; 2003). It is in

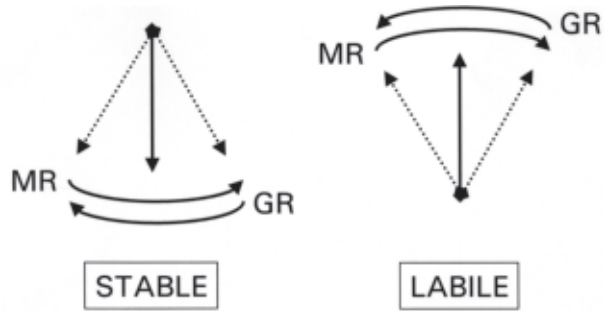

Fig 3 MR- and GR-mediated effects as indices for stress system activity. Figure depicts stable (homeostasis) and labile (allostasis?) representations.

this arena that the conversion of good vs. bad corticosteroid effect occurs. If coping with stress fails, corticosteroids fail to terminate the stress reactions and targets are exposed to elevated corticosteroid concentrations for a prolonged period of time. It is thought to sustain positive reverberating feedback loops (GOLD et al. 2002) that further aggravate the condition of imbalance. In the next sections I describe two animal models generated by 'nature-nurture' inputs that may be instrumental to dissect further the signalling pathways involved in stress system imbalance.

Animal models

Genetically selected mouse lines. Stress system responses display a large inter-individual variation in a normal population. In males the extremes display either an active fight/flight or a passive/conservation withdrawal response to a psychosocial challenge. Active animals rely on stable living conditions, show impaired adaptation to changing environment, display territorial aggression and flee after defeat. Their sympathetic response pattern dominates. Passive animals thrive better on changing conditions and they seem to be more dominated by parasympathetic activity and have high circulating cortisol levels after stress. Mouse and ratlines have been selected that represent these extremes in stress system activity (LANDGRAF et al. 2002; BOHUS et al. 1987).

In the research of the late professor Bohus the selection of male wild house mice for long and short latency before attack of the intruder in the home territory also did accumulate many of the traits characteristic for active and passive coping styles. The Short Attack Latency (SAL) mice display an innate active coping style towards environmental challenges with high stress-induced sympathetic and low adrenocortical activity. The Long Attack Latency (LAL) mice show a passive coping style and higher stress-induced corticosterone level. The SAL and LAL mice differ in many other parameters. For instance, the 5HT1A receptor expression and responsiveness in hippocampal CA1 neurons is more than 30% lower in LAL than in SAL (Veenema). Interestingly LANDGRAF et al. (2002) have selected rat lines based on emotional responses and these line differences could be eliminated with a V1A antagonist. This finding supports the evidence that in males vasopressin is also involved in genetic differences in anxiety and aggression e.g. fight or flight responses. In females oxytocin is more prominent in coping with a psychological stressor. Oxytocin promotes aspects of social behaviour, leading some to formulation of the tend-to-be-friend concept (CARTER 1998).

These extremes selected for aggressive and emotional behavioural traits may actually represent individuals in which either the CRH-1 or CRH-2 stress system modes dominates. In Veenema's studies basal hippocampal MR and GR, and hypothalamic CRH expression was not different. In subsequent experiments the LAL mice were repeatedly exposed to defeat or to the threat of defeat for 25 days. It appeared that the threat of defeat that is LAL's living next-door to the SAL only in sensory, but not physical, contact generated some of the features demonstrated in patients

suffering from depression notably an enhanced adrenocortical output and a lower MR/GR ratio (VEENEMA et al. 2003; VEENEMA, this conference). Hence, the passive behavioural coping style combined with low hippocampal 5HT1A receptor function and elevated circulating corticosterone levels predicts to some extent enhanced stressor susceptibility. These measures if further validated may match in males the criteria for an animal model for depression. Whether the same reasoning holds for female mice remains to be seen, because of their entirely different coping style, i.e. the formation of female-female social bonds involving estrogens and oxytocin rather than the sympathetic fight-flight response of males that was the basis for the SAL vs LAL selection.

Early life trauma. Individual differences in stress system activity are not only determined by genotype, but also by cognitive and non-cognitive (e.g. metabolic, immune) inputs, and experience related factors (LEVINE et al. 2000) Rearing experiences are in animals particularly potent and may permanently alter behavioural and endocrine stress responses. For instance, handling of rats (i.e. daily separation for 15 min from the mother) provides a brief intermezzo in mother-pup interaction, which subsequently results in increased sensory stimulation by intensified maternal care. As adults, the neonatally handled animals exhibit reduced fearfulness more rapid activation and termination of the adrenocortical responses than their non-handled littermates, while spatial learning is improved. These permanent effects on the stress system and cognitive performance seem to be associated with increased GR number, increased synaptogenesis and increased expression of BDNF mRNA in hippocampus (LIU et al. 2000). These findings have led to the concept that variations in maternal care form the basis for stable inter-individual differences in later stress reactivity and cognition through changes in gene expression patterns in the stress system (LEVINE et al. 2000, MEANEY 2001). Maternal care also affects the maternal behaviour of female offspring. Maternal behaviour is dependent on estrogen-oxytocin interactions, which form the basis of intergenerational transmission of individual differences in stress reactivity (MEANEY 2001).

In contrast, early adversity can lead to an anxious animal, more prone to develop depression-like behaviour if stressed, particularly so if the animal is genetically 'primed'. Adult rats that had been subjected to daily 6-hour separation from their mother during postnatal days 2-20 showed greater stress-induced increase of plasma ACTH and corticosterone levels, than rats raised under normal conditions. Moreover, CRH concentrations in CSF and in the median eminence were elevated, expression of CRH mRNA in the PVN was increased, CRH binding in the pituitary decreased and that in the extrahypothalamic CRH system increased (ARBORELIUS et al. 1999). CRH and noradrenergic drive are obviously increased, and the GABA / benzodiazepine "tone" decreased. Behaviourally these animals are anxious, show decreased social interaction and impaired cognitive functioning (KAUFMAN et al. 2000).

We have examined the life long effect of a single maternal deprivation. Infant rats of the Brown Norway strain were maternally deprived for 24 hours at postnatal day 3. Spatial learning ability in the Morris water maze and circulating corticosterone was measured at 3, 12, 24 and 32 months of age. The results show that until midlife the cognitive performance of rats deprived as pups declined faster in the face of a dramatically enhanced HPA responsiveness to stress than in the mother reared control animals. During aging this difference in cognition and HPA responsiveness between deprived and control animals vanished, but instead the deprived animals showed a strongly enhanced individual variation in performance (figure 4). Thus, the majority of the mother-reared senescent animals were partially impaired with few animals either unimpaired or fully impaired. In contrast, maternal deprivation drives spatial learning ability to the extremes, at the expense of the average. This implies that in the deprived senescent group there are almost no partially impaired animals. About 50% is impaired and 40% shows excellent performance (OITZL et al. 2000). The latter "successful agers" have the highest expression of brain-derived neurotrophic factor (BDNF) in the hippocampus (SCHAAF et al. 2001) The high expression of BDNF in the cognitively unimpaired senescent animals supports the con-

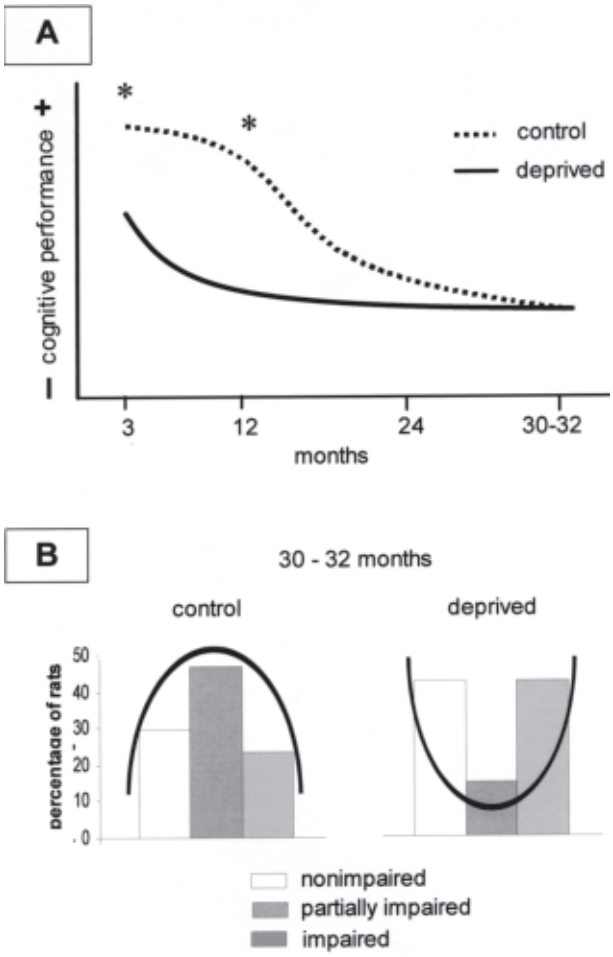

Fig 4 Cognitive performance of male Brown Norway rats deprived as pups from maternal care.
A**: Rats were maternally deprived at post natal day 3 for 24 hours (deprived) or were maternally reared (controls). Note the impairment of performance in the spatial learning test at 3 and 24 months of age. At senescence control and deprived rats are partially impaired and in average not different.**
B**: Maternally deprived rats display during aging enhanced individual variation in performance. The majority of the controls is partially impaired. In the deprived animals aging drives cognitive performance to the extreme; the senescent rats are either impaired or not impaired, at the expense of the average partially impaired performance (from** OITZL **et al. 2001).**

cept that this growth factor is implicated in the regulation of synaptic plasticity underlying memory performance.

The results show that healthy senescent Brown Norway rats do not have elevated corticosterone levels, but rather that corticosterone levels are lower than in the controls (WORKEL et al. 2001; VAN EEKELEN et al. 1995). These low levels at senescence were attained after that the maternally deprived rats had at mid-age (12 months) a period of strongly enhanced adrenocortical activation in response to a novelty stressor. In other rat strains some aspects of cognitive decline are linked to elevated corticosterone levels in a subgroup of rodents. Long-term amitryptiline treatment from midlife onwards improves cognition, decreases indices for anxiety and restores HPA activity in this subgroup (YAU et al. 2002). Collectively the data suggest that adrenocortical activity at midlife determine a trajectory of aging which can be influenced by pharmacological intervention. This line of reasoning finds support from a recent study of LUPIEN et al. (1999) showing that memory function of the elderly can be intensely modified by pharmacological treatment with glucocorticoids, although the direction of the effects depends on the cortisol history of each individual. When aged individuals are treated with metyrapone their resulting memory deficits were corrected with cortisol if they had a 5-year history of moderate cortisol levels. If cortisol levels had been high over that same period cortisol administered to the metyrapone-treated elderly further deteriorated cognitive function.

In conclusion, in rodent pups maternal care programs for life inter-individual differences in adrenocortical and emotional reactivity, and in cognitive performance. These individual differences are amplified at senescence in case of deprivation of maternal care. In this trajectory of aging that leads to a more clear-cut dissociation between good and bad performers, the history of cortisol exposure seems to be an important determinant. Deprivation from maternal care is a laboratory model of neglect, which can be taken as model for abuse. The outcome of this type of early trauma depends on the gender and strain (genetic background) of the pups as well as the time point and the duration the pup is deprived from maternal care.

Future directions

This essay is based on the thesis that the stress system operates in an immediate fast responding and a slower adaptive mode in which the balance

in brain corticosteroid receptor-mediated actions is one of the control nodes. This balance could be viewed as the set-point of the stress system in maintaining stabile or labile equilibrium in life processes (figure 5), a discussion which is presently gaining momentum (McEwen and Wingfield 2003). Selye advocated the opposing actions of mineralocorticoid and glucocorticoid hormones and took as criterion their pro- and anti-inflammatory effects respectively. Our work has given a central position to MR- and GR-mediating the action of one single hormone: corticosterone. Collectively, the data suggest that MR-mediated actions are directed to maintain equilibrium and health, while GR promotes their recovery.

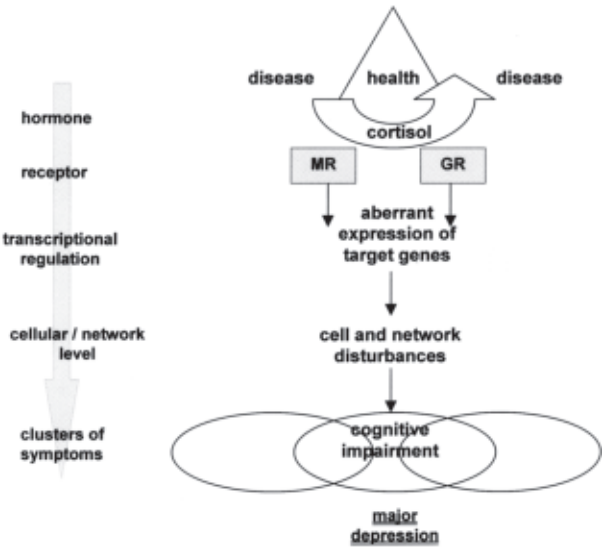

Fig 5 The MR – GR balance hypothesis.
The balance in MR – GR-mediated effects is thought crucial for homeostasis and health. Imbalance either by excess MR or excess GR stimulation is proposed to enhance vulnerability to disease the individual is predisposed. The scheme depicts the various levels of analysis.

Evidence was shown that on different levels of biological complexity the properties and localisation of MR and GR direct the type of molecular changes and cellular/network properties that underlie behavioural adaptation. Stress system imbalance induced by chronic stressors compromises these processes from gene to behaviour and increases disease vulnerability for which the individual is predisposed. Thus aberrant corticosteroid concentrations cause altered function and structure of discrete brain areas affecting such fundamental processes as apoptosis, neurogenesis and synaptic plasticity (McEwen 1999; 2002; Sapolsky 2000; Czeh et al 2001; de Kloet et al. 1999).

The challenge today is to combine knowledge on the function of individual gene products identified by genomic screening with holistic approaches in the analysis of higher brain functions i.e. emotions and cognitive processes. Technical innovations, such as the application of *in vivo* si-RNA technology and candidate gene single nucleotide polymorphism (SNP) analysis will greatly facilitate progress in the molecular arena. On the other hand neuro-imaging technology (Drevets et al. 1997) will help to pinpoint responsive brain circuitry in patients that have been diagnosed with greater precision using neuropsychological and systems physiology approaches. I see new developments in many areas, but would like to mention three aspects with particular bearing for the field of stress hormones.

Genetics of the corticosteroid receptor system

I expect that the coming years SNP's will be identified in the corticosteroid - MR/GR transcription machinery that bias corticosteroid control of the stress response. The 11-HSD defect providing local excess of cortisol resulting in metabole syndrome is an excellent example (Masuzaki et al. 2001). Likewise one could predict that certain SNP's may bias aspects of receptor signalling which may cause local imbalances in homeostatic control (de Rijk et al. 2001; van Rossum et al. 2002).

Genomic screening

The initial observations have opened up a bewildering array of stress-responsive genes (Datson et al. 2001; Feldker et al. 2003) that need to be analysed to answer questions such as how, where and when these genes become active in stress-induced signalling pathways, and foremost what their precise function is (Sabban and Kvetňanský 2001). Recently, in a hippocampal transcriptome both SAGE and GeneChip analysis showed under

basal conditions a higher expression of several cytoskeleton genes in LAL (the passive copers) hippocampi than in SAL (active copers), as well as higher expression of a number of calmodulin-related genes and genes encoding components of a MAPK-cascade. Accordingly, this differential regulation of a raf/ERK pathway may be related to structural differences in hippocampus of LAL and SAL mice that can be taken marker for *predisposition*. A chronic psychosocial stressor produced a phenotype characterised by a dysregulated stress system. In the hippocampus down regulation of CHUK a kinase involved in the regulation of NFkB was observed, as well as down regulation of several Ras oncogene family members. These genes may be considered a marker for stress-induced change or a molecular marker for e.g. *pathogenesis* (FELDKER et al., in press).

Animal models

Behavioural tasks in which the analysis of simultaneous emotional and cognitive processes is combined with neurophysiological network analysis may lead to better animal models. This may open up questions on the mode of action of stress hormones in control of cognitive processes leading to the precipitation of emotional disturbances characteristic of for example depression. A distinction should be made in this respect between (i) the core of the HPA axis with emphasis on dysregulations in the PVN microenvironment in its organization of the stress response and (ii) dysregulations in specific afferent stress circuits to the PVN e.g. medial prefrontal cortex, hippocampus, amygdala and brain stem, that are targets for the stress hormones. This is important because it becomes increasingly clear that a novel generation of drugs may arise from targeting pathway nodes in stress regulation centers in the brain to treat stress-related brain disorders and co-morbid medical consequences such as metabolic, cardiovascular and neurodegenerative diseases. The potential success of the GR antagonists in the treatment of severe depression (BELANOFF et al. 2002) hints that the therapeutic focus on stress circuitry may be a rewarding approach.

Acknowledgements

The financial support of the Netherlands Organization for Scientific Research (NWO) and the EU is gratefully acknowledged. Over the years the enthusiasm of my collaborators and colleagues has been so important for the joy of research. I thank Dr Onno C. Meijer for critically reading this manuscript, and dedicate this article to my long-time friend the late professor Béla Bohus, who has always been a big supporter of the Smolenice Castle Conference. The editorial assistance of Ellen M. Heidema is gratefully acknowledged.

References

AKANA SF, CHU A, SORIANA L, DALLMAN: Corticosterone exerts site specific and state dependent effects in prefrontal cortex and amygdala on regulation of adrenocorticotropic hormone, Insulin and fat depots. J Neuroendocr **13**, 625-637, 2001

ARBORELIUS L, OWEN MJ, PLOTSKYPM, ET AL: The role of corticotropin-releasing factor in depression and anxiety disorders. J Endocrinology **160**, 1-12, 1999

AUPHAN N, DIDONATO JA, ROSETTE C, HELMBERG A, KARIN M: Immunosuppression by glucocorticoids: inhibition of NF-kappaB activity through induction of I kappa B synthesis. Science **270**, 286-290, 1995

BAULIEU EE: 'New' active steroids and an unforeseen mechanism of action. C R Acad Sci III **323**, 513-518, 2000

BELANOFF JK, KALEHZAN M, SUND B, FLEMING FICEK SK, SCHATZBERG AF: Cortisol activity and cognitive changes in psychotic major depression. Am J Psychiatry **158**, 1612-1616, 2001

BELANOFF JK, ROTHSCHILD AJ, CASSIDY F, DEBATTISTA C, BAULIEU EE, SCHOLD C, SCHATZBERG AF: An open label trial of C-1073 (mifepristone) for psychotic major depression. Biol Psychiatry **52**, 386-392, 2002

Bohus B, Benus RF, Fokkema DS, Koolhaas JM, Nyakas C, van Oortmerssen GA, Prins AJ, de Ruiter AJ, SCHEURINK AJ, STEFFENS AB. ET AL.: Neuroendocrine states and behavioural and physiological stress responses. Progr Brain Res **72**, 57-70, 1987

BUIJS RM, WORTEL J, VAN HEERIKHUIZE JJ, FEENSTRA MGP, TER HORST GJ, ROMIJN HJ, KALSBEEK A: Anatomical and functional demonstration of a multisynaptic suprachiasmatic nucleus - adrenal (cortex) pathway. Eur J Neurosci **11**, 1535-1554, 1999

CARTER CS: Neuroendocrine perspectives on social attachment and love. Psychoneuroendocrinol **23**,779-818, 1998

CHROUSOS G, GOLD PW: The concepts of stress and stress system disorder. JAMA **267**,1244-1252, 1992

COLE RL, SAWCHENKO PE: Neurotransmitter regulation of cellular activation and neuropeptide gene expression in the paraventricular nucleus of the hypothalamus. J Neurosci **22**, 959-969, 2002

CZEH B, MICHAELIS T, WATANABE T, FRAHM J, DE BIURRUN G, VAN KAMPEN M, BARTOLOMUCCI A, FUCHS E: Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proc Natl Acad Sci USA **98**, 12796-12801, 2001

DATSON NA, VAN DER PERK J, DE KLOET ER, VREUGDENHIL E: Identification of corticosteroid-responsive genes in rat hippocampus using serial analysis of gene expression. Eur J Neurosci **14**, 675-689, 2001

DE KLOET ER, DE KOCK S, SCHILD V, VELDHUIS HD: Antiglucocorticoid RU 38486 attenuates retention of a behaviour and disinhibits the hypothalamo-pituitary-adrenal axis at different brain sites. Neuroendocrinol **47**, 109-115, 1988

DE KLOET ER, REUL JM: Feedback action and tonic influence of corticosteroids on brain function: a concept arising from the heterogeneity of brain receptor system. Psychoneuroendocrinol **12**, 83-105, 1987

DE KLOET ER, OITZL MS, JOËLS M: Stress and cognition: are corticosteroids good or bad guys? Trends Neurosci **22**, 422-426, 1999

DE KLOET ER, VREUGDENHIL E, OITZL MS, JOËLS M: Brain corticosteroid receptor balance in health and disease Endocr Rev **19**, 269-301, 1998

DE KLOET ER, WALLACH G, MCEWEN BS: Difference in binding of corticosterone and dexamethasone to rat brain and pituitary. Endocrinology **96**, 598-596, 1975

DeRijk RH, Schaaf MJ, Turner G, Datson NA, Vreugdenhil E, Cidlowski J, de Kloet ER, Emery P, Sternberg EM, DETERA-WADLEIGH SD: A human glucocorticoid receptor gene variant that increases the stability of the glucocorticoid receptor beta-isoform mRNA is associated with rheumatoid arthritis. J Rheumatol **28**, 2383-2388, 2001

DIAMOND DM, BENNETT MC, STEVENS KE, WILSON RL, ROSE GM: Exposure to a novel environment interferes with the induction of hippocampal primed burst potentiation in the behaving rat. Psychobiol 18:273 286, 1990

DUAN YF, KOPIN IJ, GOLDSTEIN DS: Stimulation of the paraventricular nucleus modulates firing of neurons in the nucleus of the solitary tract. Am J Physiol. **277**, R403-R411,1999

DREVETS WC, PRICE JL, SIMPSON JR JR, TODD RD, REICH T, VANNIER M, RAICHLE ME: Subgenual prefrontal cortex abnormalities in mood disorders. Nature 386, 824-827, 1997

EDWARDS CRW, STEWART PM, BURT D, BRETT L, MCINTYRE MA, SUTANTO W, DE KLOET ER, MONDER C: Localisation of 11ß-hydroxysteroid dehydrogenase-tissue specific protector of the mineralocorticoid receptor. Lancet **2(8618)**, 986-989, 1988

EVANS RM, ARRIZA JL: A molecular framework for the actions of glucocorticoid hormones in the nervous system. Neuron **2**,1105-1112, 1989

FELDKER DE, DATSON NA, VEENEMA AH, MEULMEESTER E, DE KLOET ER, VREUGDENHIL E: Serial analysis of gene expression predicts structural differences in hippocampus of long attack latency and short attack latency mice. Eur J Neurosci **17**, 379-387, 2003

Fuxe K, Wikström AC, Ökret S, Agnati LF, Härfstrand A, Yu ZY, Granholm L, Zoli M, Vale W, Gustafsson JĹ: Mapping of glucocorticoid receptor immunoreactive neurons in the rat tel- and diencephalon using a monoclonal antibody against rat liver glucocorticoid receptor. Endocrinology **117**,1803-1812, 1985

GOLD PW, DREVETS WC, CHARNEY DS: New insights into the role of cortisol and the glucocorticoid receptor in severe depression. Biol Psychiatry **52**, 381-385, 2002

GOLD PW, CHROUSOS GP: The endocrinology of melancholic and atypical depression: relation to neurocircuitry and somatic consequences. Proc Assoc Am Physicians **111**, 22-34, 1999

HALLER J, MILLAR S, VAN DE SCHRAAF J, DE KLOET ER, KRUK MR: The active phase-related increase in corticosterone and aggression are linked. J Neuroendocrinol **12**, 431-436, 2000

HAUGER RL, GRIGORIADIS DE, DALLMAN MF, PLOTSKY PM, VALE WW, DAUTZENBERG FM: International Union of Pharmacology. XXXVI. Current Status of the Nomenclature for Receptors for Corticotropin Releasing Factor and Their Ligands. Pharmacol Rev **55**, 21-26, 2003

HEIM C, EHLERT U, HELLHAMMER DH: The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. Psychoneuroendocrinol **25**, 1-35, 2000

HELM KA, HAN JS, GALLAGHER M: Effects of cholinergic lesions produced by infusions of 192 IgG-saporin on glucocorticoid receptor mRNA expression in hippocampus and medial prefrontal cortex of the rat. Neurosci **115**, 765-774, 2002

HERMAN JP, CULLINAN WE: Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci **20**, 78-84, 1997

HERMAN JP, CULLINAN WE, ZIEGLER DR, TASKER JG: Role of paraventricular microenvironment in stress integration. Eur J Neurosci **16**, 381-385, 2002

HOLSBOER F: The corticosteroid receptor hypothesis of depression. Neuropsychopharmacol **23**, 477-501, 2000

HOLSBOER F: Stress, hypercortisolism and corticosteroid receptors in depression: implications for therapy. J Affect Disorders **62**, 77-91, 2001

HOLSBOER F: Corticotropin-releasing hormone modulators and depression. Curr Opin Invest Drugs **4**, 46-50, 2003

HSU SY, HSUEH AJ: Human stresscopin and stresscopin-related peptide are selective ligands for the type 2 corticotropin-releasing hormone receptor. Nat Med **7**,605-611, 2001

INGLE DJ: Permissibility of hormone action: a review. Acta Endocrinol **17**,172-194, 1954

ITO T, MORITA N, NISHI M, KAWATA M: In vitro and in vivo immunocytochemistry for the distribution of mineralocorticoid receptor with the use of specific antibody. Neurosci Res **37**,173-182, 2000

JOËLS M: Steroid hormones and excitability in the mammalian brain. Front Neuroendocrinol **18**, 2-48, 1997

JOËLS M: Corticosteroid actions in the hippocampus. J Neuroendocrinol **13**, 657-669, 2001

JOËLS M, DE KLOET ER: Effects of glucocorticoids and norepinephrine on the excitability in the hippocampus. Science. 1989 Sep 29;245(4925):1502-1505

JOËLS M, DE KLOET ER: Control of neuronal excitability by corticosteroid hormones. Trends Neurosci **15**, 25-30, 1992

JOËLS M, DE KLOET ER: Mineralocorticoid and glucocorticoid receptors in the brain. Implications for ion permeability and transmitter systems. Progr Neurobiol **43**, 1-36, 1994

KARSSEN AM, MEIJER OC, VAN DER SANDT IC, LUCASSEN PJ, DE LANGE EC, DE BOER AG, DE KLOET ER: Multidrug resistance P-glycoprotein hampers the access of cortisol but not of corticosterone to mouse and human brain. Endocrinology **142**, 2686-2694, 2001

KARST H, KARTEN YJ, REICHARDT HM, DE KLOET ER, SCHÜTZ G, JOËLS M: Corticosteroid actions in hippocampus require DNA binding of glucocorticoid receptor homodimers. Nat Neurosci **3**, 977-978, 2000

KAUFMAN J, PLOTSKY P, NEMEROFF CB, CHARNEY DS: Effects of early adverse experiences on brain structure and function: clinical implications. Biol Psych **15**, 778-790, 2000.

KORTE SM: Corticosteroids in relation to fear, anxiety and psychopathology. Neurosci Biobehav Rev **25**,117-142, 2001

KOVÁCS KJ, FOLDES A, SAWCHENKO PE: Glucocorticoid negative feedback selectively targets vasopressin transcription in parvocellular neurons. J Neurosci 15, 3843-3852, 2000.

LANDGRAF R, WIGGER A: High vs low anxiety-related behaviour in rats: an animal model of extremes in trait anxiety. Behav Genet **32**, 301-314, 2002.

LAUGERO KD, BELL ME, BHATNAGAR S, SORIANO L, DALLMAN MF: Sucrose ingestion normalizes central expression of corticotrophin releasing factor messenger ribonucleic acid and energy balance in adrenalectomized rats: a glucocorticoid-metabolic-brain axis? Endocrinology **142**, 2796-2804, 2001

LAUGERO KD, GOMEZ F, MANALO S, DALLMAN MF: Corticosterone infused intracerebroventricularly inhibits energy storage and stimulates the hypothalamo-pituitary axis in adrenalectomized rats drinking sucrose. Endocrinology **143**, 4552-4562, 2002

LEVINE S, DENT G, DE KLOET ER: Stress-hyporesponsive period. In: Encyclopedia of Stress (Ed. G. Fink), Vol. 3, pp. 518-526, Academic Press, New York 2000

Lewis K, Li C, Perrin MH, Blount A, Kunitake K, Donaldson C, Vaughan J, Reyes TM, Julyas J, Fischer W, BILEZKJIAN L, RIVIER J, SAWCHENCO PE, VALE WW: Identification of urocortin III, an additional member of the corticotropin releasing factor (CRF) family with high affinity for the CRH 2 receptor. Proc Natl Acad Sci **8**, 7570-7575,2001

LI C, VAUGHAN J, SAWCHECKO PE, VALE WW: Urocortin III- immunoreactive projections in rat brain: partial overlap with sites of type 2 corticotropin-eleasing factor receptor expression. J Neurosci **22**, 991-1001, 2002

LIU D, DIORIO J, DAY JC, FRANCIS DD, MEANEY MJ: Maternal care, hippocampal synaptogenesis and cognitive development in rats. Nature Neurosci **3**,799-806, 2000

LUPIEN SJ, NAIR NP, BRIERE S, MAHEU F, TU MT, LEMAY M, MCEWEN BS, MEANEY MJ: Increased cortisol levels and impaired cognition in human aging: implication for depression and dementia in later life. Rev Neurosci **10**,117-139, 1999

MASUZAKI H, PATERSON J, SHINYAMA H, MORTON NM, MULLINS JJ, SECKL JR, FLIER JS: A transgenic model of visceral obesity and the metabolic syndrome. Science **294**, 2166-2170, 2001

McEwen BS: Stress and hippocampal plasticity. Ann Rev Neurosci **22**,105-122, 1999

McEwen BS: Protective and damaging effects of stress mediators: the good and bad sides of the response to stress. J Clin Endocr Metabolism **51**, 2-4, 2002

McEwen BS, Weiss JM, Schwartz LS: Selective retention of corticosterone by limbic structures in rat brain. Nature **220**, 911-912, 1968

McEwen BS, Wingfield JC: The concept of allostasis in biology and biomedicine. Horm Behav 43, 2-15, 2003

Meaney MJ: Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Ann Rev Neurosci **24**, 1161-1192, 2001

McGaugh JL, Roozendaal B: Role of adrenal stress hormones in forming lasting memories in the brain. Curr Opin Neurobiol **12**, 205-210, 2002

Meijer OC, De Lange ECM, Breimer DD, De Boer AG, Workel JO, De Kloet ER: Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr1A P-glycoprotein knockout mice. Endocrinology **139**, 1789-1793, 1998

Meijer OC: Coregulator proteins and corticosteroid action in the brain. J Neuroendocrinol **14**, 499-505, 2002

Montkowski A, Barden N, Wotjak C, Stec I, Ganster J, Meaney M, Engelmann M, Reul JM, Landgraf R, Holsboer F: Long-term antidepressant treatment reduces behavioural deficits in transgenic mice with impaired glucocorticoid receptor function. J Neuroendocrinol **7**, 841- 848,1995

Munck A, Guyre PM, Holbrook NJ: Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr Rev **5**, 25-44, 1984

Oitzl MS, de Kloet ER: Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning. Behav Neurosci **106**, 62-71, 1992

Oitzl MS, Fluttert M, de Kloet ER: The effect of corticosterone on reactivity to spatial novelty is mediated by central mineralocorticosteroid receptors. Eur J Neurosci **6**,1072-1079, 1994

Oitzl MS, Fluttert M, Sutanto W, de Kloet ER: Continuous blockade of brain glucocorticoid receptors facilitates spatial learning and memory in rats. Eur J Neurosci **10**, 3759-3766, 1998

Oitzl MS, Reichardt HM, Joëls M, de Kloet ER: Point mutation in the mouse glucocorticoid receptor preventing DNA binding impairs spatial memory. Proc Natl Acad Sci USA **98**,12790-12795, 2001

Oitzl MS, van Haarst AD, Sutanto W, de Kloet ER: Corticosterone, brain mineralocorticoid receptors (MRs) and the activity of the hypothalamic-pituitary-adrenal (HPA) axis: the Lewis rat as an example of increased central MR capacity and a hyporesponsive HPA axis. Psychoneuroendocrinol **20**, 655-675,1995

Oitzl MS, Workel JO, Fluttert M, Frosch F, De Kloet ER: Maternal deprivation affects behaviour from youth to senescence: amplification of individual differences in spatial learning and memory in senescent Brown Norway rats. Eur J Neurosci **12,** 3771-3780, 2000

Pacak K, Palkovits M: Stressor specificity of central neuroenodcrine responses: implications for stress-related disorders. Endocr Rev 22, 502-548, 2001

Palkovits M: Interconnections between the neuroendocrine hypothalamus and the central autonomic system Front Neuroendocrinol **20**, 270-295, 1999

Ratka A, Sutanto W, Bloemers M, de Kloet ER: On the role of brain mineralocorticoid (type I) and glucocorticoid (type II) receptors in neuroendocrine regulation. Neuroendocrinol **50**,117-123, 1989

Reichardt HM, Tronche F, Berger S, Kellendonk C, Schütz G: New insights into glucocorticoid and mineralocorticoid signaling: lessons from gene targeting. Adv Pharmacol **47**,1-21, 2000

Reul JM, de Kloet ER: Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology **117**, 2505-2511, 1985

Reul JM, Holsboer F: Corticotropin-releasing factor receptors 1 and 2 in anxiety and depression. Curr Opin Pharmacol **2**, 23-33, 2002

Romero LM, Sapolsky RM: Patterns of ACTH secretagog secretion in response to psychological stimuli. J Neuroendocrinol **8**, 243-258, 1996

Roozendaal B: 1999 Curt P. Richter Award. Glucocorticoids and the regulation of memory consolidation. Psychoneuroendocrinol **25**, 213-238, 2000

Sabban EL, Kvetňanský R: Stress-triggered activation of gene expression in catecholaminergic systems: dynamics of transcriptional events. Trends Neurosci **24**, 91-98, 2001

Sandi C, Loscertales M, Guaza C: Experience-dependent facilitating effect of corticosterone on spatial memory formation in the water maze. Eur J Neurosci **9**, 637-642, 1997

Sapolsky RM: Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry **57**, 925-935, 2000

Sapolsky RM, Romero LM, Munck AU: How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev **21**, 55-89, 2000

Schaaf MJ, Cidlowski JA: Molecular mechanisms of glucocorticoid action and resistance. J Steroid Biochem Mol Biol **83**, 37-48, 2002

Schaaf MJ, Workel JO, Lesscher HM, Vreugdenhil E, Oitzl MS, de Kloet ER: Correlation between hippocampal BDNF mRNA expression and memory performance in senescent rats. Brain Res **915**, 227-233, 2001

Schatzberg AF, Rothschild AJ, Langlais PJ, Bird ED, Cole JO: A corticosteroid/dopamine hypothesis for psychotic depression and related states. J Psychiatr Res **19**, 57-64, 1985

Seckl JR, Yau J, Holmes M: 11Beta-hydroxysteroid dehydrogenases: a novel control of glucocorticoid action in the brain. Endocr Res **28**, 701-707, 2002

Selye H: The Story of the Adaptation Syndrome. Acta, Montreal, 1952

Tausk M: Das Hormon: Hat die Nebenniere tatsächlich eine Verteidigungsfunktion? Vol 3: Organon International BV, The Netherlands, 1951

Van den Berg DTW, De Jong W, De Kloet ER: Mineralocorticoid antagonist inhibits stress-induced blood pressure response after repeated daily warming. Am J Physiol **267**, E921-E926, 1994

Van den Buuse M, van Acker SA, Fluttert MF, de Kloet ER: Involvement of corticosterone in cardiovascular responses to an open-field novelty stressor in freely moving rats. Physiol Behav **75**, 207-215, 2002

Van der Lely AJ, Foeken K, van der Mast RC, Lamberts SW: Rapid reversal of acute psychosis in the Cushing syndrome with the cortisol-receptor antagonist mifepristone (RU 486). Ann Intern Med **114**,143-144, 1991

Van Eekelen JA, Jiang W, De Kloet ER, Bohn MC: Distribution of the mineralocorticoid and the glucocorticoid receptor mRNAs in the rat hippocampus. J Neurosci Res **21**, 88-94, 1988

Van Eekelen JA, Oitzl MS, De Kloet ER: Adrenocortical hyporesponsiveness and glucocorticoid feedback resistance in old male brown Norway rats. J Gerontol A Biol Sci Med Sci **50**, B83-B89, 1995

Van Haarst AD, Oitzl MS, de Kloet ER: Facilitation of feedback inhibition through blockade of glucocorticoid receptors in the hippocampus. Neurochem Res **22**, 1323-8, 1997

Van Haarst AD, Oitzl MS, Workel JO, de Kloet ER: Chronic brain glucocorticoid receptor blockade enhances the rise in circadian and stress-induced pituitary-adrenal activity. Endocrinology **137**, 4935-4943, 1996

Van Rossum EF, Koper JW, Huizenga NA, Uitterlinden AG, Janssen JA, Brinkmann AO, Grobbee DE, de Jong FH, van Duyn CM, Pols HA, Lamberts SW: A polymorphism in the glucocorticoid receptor gene, which decreases sensitivity to glucocorticoids in vivo, is associated with low insulin and cholesterol levels. Diabetes **51**, 3128-3134, 2002

Van Steensel B, van Binnendijk EP, Hornsby CD, van der Voort HT, Krozowski ZS, de Kloet ER, van Driel R: Partial colocalization of glucocorticoid and mineralocorticoid receptors in discrete compartments in nuclei of rat hippocampus neurons. J Cell Sci **109**, 787-792, 1996

Veenema AH, Meijer OC, de Kloet ER, Koolhaas JM, Bohus BG: Differences in basal and stress-induced HPA regulation of wild house mice selected for high and low aggression. Horm Behav **43**,197-204, 2003

Windle RJ, Wood SA, Lightman SL, Ingram CD: The pulsatile characteristics of the hypothalamo-pituitary-adrenal activity ion Lewis and Fisher 344 rats and its relationship to different stress responses. Endocrinology **139**, 4044-4052, 1998

Workel JO, Oitzl MS, Fluttert M, Lesscher H, Karssen A, de Kloet ER: Differential and age-dependent effects of maternal deprivation on the hypothalamic-pituitary-adrenal axis of Brown Norway rats from youth to senescence. J Neuroendocrinol **13**, 569-80, 2001

Yau JL, Noble J, Hibberd C, Rowe WB, Meaney MJ, Morris RG, Seckl JR: Chronic treatment with the antidepressant amitriptyline prevents impairments in water maze learning in aging rats. J Neurosci **22**, 1436-1442, 2002

Corresponding author: E. Ronald de Kloet, M.D.
Division of Medical Pharmacology
LACDR/LUMC, Leiden University
PO Box 9502
2300 RA Leiden
The Netherlands
Phone: +31-71-527.6210
Fax: +31-71-527.4715
e-mail: e.kloet@lacdr.leidenuniv.nl

CATECHOLAMINES AND STRESS

DAVID S. GOLDSTEIN

Clinical Neurocardiology Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, e-mail: goldsteind@ninds.nih.gov

The subject of catecholamines and stress has occupied researchers for many years and filled many books. A brief revew such as this cannot cover such a broad topic. Instead, provided here are a few concepts, reflecting somewhat different viewpoints from those in standard textbooks.

The first of these concepts is that there are three distinct peripheral catecholamine systems, each with different effectors, regulation, and roles. The three systems are the sympathetic nervous system, adrenomedullary hormonal system, and DOPA-dopamine autocrine/paracrine system. This contrasts with the traditional concept, promulated by WALTER B. CANNON, of a unitary sympathoadrenal system. It also contrasts with the notion of HANS SELYE that release of "adrenalines" characterizes the acute phase of what he called the "General Adaptation Syndrome."

Both these investigators held to the view that all forms of stress lead to the same stereotyped response. Indeed, in line with this "doctrine of nonspecificity," SELYE defined stress as the *nonspecific* response of the body to any demand imposed upon it [1]. According to a relatively new concept, however, stress responses have a primitive kind of specificity, with differential responses of the sympathetic nervous and adrenomedullary hormonal systems, depending on the type and intensity of the stressor as sensed by the organism and interpreted in light of experience [2].

Another concept in this paper that contrasts with CANNON's teachings is that instead of the sympathetic nervous system becoming active only in emergencies, tonic sympathetic nervous outflows to several vascular beds, organs, and glands maintain levels of a variety of monitored variables, both under resting conditions and in response to everyday challenges such as orthostasis, locomotion, the post-prandial state, and altered temperature.

This paper also notes a closer association between the hypothalamo-pituitary-adrenocortical system and adrenomedullary hormonal system in several forms of stress than between the sympathetic nervous and adrenomedullary hormonal systems (Table 1). In fainting, adrenomedullary activation with concurrent sympathoinhibition precedes and may precipitate the acute neurocirculatory collapse.

Finally, offered for consideration here is the notion that stress and distress can contribute to acute and chronic diseases, by worsening independent pathologic states and inducing "allostatic load."

These concepts may provide a theoretical basis for scientific integrative medicine in the post-genome era.

Three peripheral catecholamine systems

WALTER B. CANNON, extending CLAUDE BERNARD'S concept of the *milieu intérieur,* taught that coordinated body processes work toward the goal of an ideal set of steady-states, for which he coined the term, "homeostasis" [3-5]. CANNON's research was the first to document the role of adrenal secretion in rapid responses of the organism to threats to homeostasis [6,7].

CANNON taught that the adrenal gland and sympathetic nervous system functioned as a unit. Indeed, in 1939, he formally proposed epinephrine (adrenaline) not only as the active principle of the adrenal gland but also as the neurotransmitter of the sympathetic nervous system [8]. If this had proven to be the case, this would have confirmed

Table 1.
Hypothalamo-pituitary-adrenocortical (HPA), adrenomedullary hormonal system (AHS), and sympathetic nervous system (SNS) responses to different stressors

Condition	HPA	AHS	SNS	References
Active Escape/Avoidance in rats	+	+	++	75,79
Cardiac Arrest (or Bypass)	+++	++++	++	99-106
Cold Exposure, Hypothermia	+	++	++++	69-72
Cold Exposure, No Hypothermia	0	+	+++	29,37,64-68
Exercise	+	++	+++	65,82-85
Exercise to Exhaustion	++	+++	++++	84,86,87
Fainting	++	++++	0	44,50,51,89
Glucoprivation	+++	++++	+	29,58,59
Handling in rats	++	++	+	29,92,93
Hemorrhagic Hypotension	+++	+++	+	29,60-63
Hemorrhage, No Hypotension	+	+	++	29,60
Immobilization in rats	++++	++++	++++	29,90-92
Laboratory Mental Challenge	++	++	+	26,80,81
Pain	++	+++	++	5,29,73,74
Passive/Immobile Fear	++	+++	+	5,75-79
Public Performance	++	+++	+	26,85,88
Social Stress in rhesus monkeys	++	++	++	107
Surgery	+	+	++	94-98

Notes: Different intensities are indicated from 0 through ++++, based on the cited References, weighed equally. There is a generally closer association between AHS and HPA than between AHS and SNS responses.

the functional unity of the the sympathoadrenal system. In 1946, however, about a year after CANNON'S death, VON EULER correctly identified norepinephrine as the sympathetic neurotransmitter [9]. As discussed below, the notion of a unitary sympathoadrenal system continues in medical thinking [10-13], despite persuasive evidence for differential changes in sympathetic nervous and adrenomedullary hormonal activities not only with different forms of stress [13] but also as a function of variables such as aging and obesity [14,15].

Dopamine, the third member of the small family of endogenous catecholamines besides norepinephrine and epinephrine, functions in the brain as a neurotransmitter. Understanding of its functions in the periphery has lagged behind. At least in some organs, most notably the kidneys, dopamine seems to function neither as a neurotransmitter, released from putative dopaminergic nerves or co-released with norepinephrine from sympathetic nerves, nor as a hormone, released from the adrenal medulla along with epinephrine. Instead, dopamine production in the kidneys appears to depend mainly on uptake of its precursor, L-3,4-dihydroxyphenylalanine (L-DOPA), from the circulation, with conversion to dopamine by L-aromatic-amino-acid decarboxylase in proximal tubular - i.e., non-neuronal and non-chromaffin - cells [16,17]. Dopamine exiting the cells then appears to act as an autocrine/paracrine substance, promoting natriuresis by local inhibition of Na^{+}/K^{+} ATPase.

More of dopamine production and metabolism take place in the mesenteric organs than in the brain, sympathetic nerves, or adrenal chromaffin cells [18]. At least some of this production arises from tyrosine hydroxylase being expressed in non-neuronal cells such as gastric parietal, pancreatic acinar, and lamina propria cells [19,21]. It is possible that locally produced dopamine contributes to regulation of gastrointestinal motility or bicarbonate secretion.

Tonic activity of the sympathetic nervous system

According to CANNON rapid activation of homeostatic systems - especially of the "sym-

pathaodrenal system" - in emergencies would preserve the internal environment, by producing compensatory and anticipatory adjustments that would enhance the likelihood of survival. In the sheltered confines of a laboratory, however, with controlled temperature and *ad libitum* water, nutrients, and calories, mammals did not seem to require an intact sympathetic nervous system [22].

Compensatory activation of other vasoactive systems after destruction of the sympathetic nervous system helps to explain why many workers, including CANNON, erroneously concluded that the sympathetic nervous system acts only as an "emergency" system [23,24]. By now it is appreciated that even under resting conditions, pulse-synchronous bursts of skeletal muscle sympathetic nerve activity and plasma levels of norepinephrine are readily detectable, and norepinephrine continuously enters the venous drainage of most organs. Moreover, ganglion blockade abolishes skeletal sympathetic nerve traffic and markedly decreases plasma norepinephrine levels; and interference with ganglionic neurotransmission, destruction of sympathetic nerves or blockade of catecholamine receptors consistently decreases blood pressure.

It is also by now clear that activities of daily life, such as meal ingestion [25], public speaking [26], changing posture [27], and movement - i.e., not only emergencies - are associated with continual alterations in sympathetic nervous system outflows, maintaining appropriate blood flow to the brain, body temperature, delivery of metabolic fuel to body organs, and so forth. Each of these activities is associated with a somewhat different set of "normal" apparent steady-states, directed by the brain and determined by coordinated actions of a variety of effector systems. This principle leads directly to the concept of "allostasis," discussed below.

Specificity vs. non-specificity of responses to stressors

According to CANNON, whether the threat were exposure to cold, hemorrhage, hypoglycemia, or distressing emotional encounters, the response in these emergencies would be essentially the same [3,5].

SELYE introduced and popularized stress as a medical scientific idea. According to SELYE'S theory, "Stress is the nonspecific response of the body to any demand upon it [1]. Responses to stressors would have specific and nonspecific components, and he referred to only the nonspecific component as "stress." After removal of specific responses from consideration, a nonspecific syndrome would remain. Although nonspecific with respect to the inciting agents, the stress response itself was viewed to consist of a stereotyped pathological pattern, with enlargement of the adrenal glands, involution of the thymus gland (associated with atrophy of lymph nodes and inhibition of inflammatory responses), and peptic bleeding or ulceration. CHROUSOS and Gold [28] modified the doctrine of nonspecificity, by proposing that above a threshold intensity, any stressor would elicit the "stress syndrome." More than a half century elapsed before SELYE'S doctrine of nonspecificity underwent experimental testing, which failed to confirm it [29].

By now researchers have largely abandoned both CANNON'S and SELYE'S notions of stereotyped, nonspecific neuroendocrine responses regardless of the stressor. More modern theories view stress as a sensed threat to homeostasis [30,31], where the response has a degree of specificity, depending among other things on particular challenge to homeostasis and the organism's perception of the stressor and ability to cope with it [32].

A homeostatic definition of stress

Stress occurs when the organism perceives a disruption or a threat of disruption of homeostasis. Central to the present theory is that the body possesses numerous homeostatic comparators, which have been called "homeostats" [33]. Each homeostat compares information with a setpoint for responding, determined by a regulator. Homeostatic systems typically use multiple effectors to change values for the controlled variable. The loop is closed by monitoring changes in the levels of the controlled variable, via one or more monitored variables.

By analogy, in a home temperature control system, the thermostat plays a central role, by sensing

discrepancy between the setpoint, determined by a regulator, and the temperature, which produces differential bending of metal bands in the thermostat. This type of system is a classical example of regulation by negative feedback. Home temperature control systems always include multiple effectors. The redundancy comes at relatively little cost, compared with three advantages. The multiplicity extends the range of control of external temperatures where the internal temperature can be maintained; when a single effector fails to function, others are activated compensatorily, helping maintain the temperature at about the set level; and one can pattern the use of the effectors as appropriate to maximize economy and efficiency.

A tremendous array of homeostatic systems detect perturbations of monitored variables. In line with the home heating analogy, this even includes afferent information to the brain about cutaneous and blood temperature, leading to altered activities of cholinergic and noradrenergic nerve fibers in the skin that regulate sweating and vasomotor tone [34].

Principles of homeostatic system operation

Homeostatic systems operate according to a few principles, which, despite their simplicity, can explain complex physiological phenomena and help to resolve persistently controversial issues in the area of stress and disease. Homeostatic systems always include regulation by negative feedback. Increases in values of the monitored variable result in changes in effector activity that oppose and thereby "buffer" changes in that variable. This feedback regulation can be modulated at several levels and therefore can be quite complex.

Homeostatic systems generally use more than one effector, for the same reasons as home temperature control systems. Effector redundancy extends the ranges of control of monitored variables. It enables compensatory activation of alternative effectors, assuming no change in homeostat settings. Examples of compensatory activation in physiology include augmentation of sympathoneural responsiveness by adrenalectomy, hypophysectomy, or thyroidectomy [35-37]. Finally, effector redundancy introduces the potential for patterned effector responses. Patterning of neuroendocrine, physiological, and behavioral effectors increases the likelihood of adaptiveness to the particular challenge to homeostasis, providing another basis for natural selection to favor the evolution of systems with multiple effectors.

Different homeostats can regulate the activity of the same effector system. For instance, the osmostat and volustat share the vasopressin effector [38]. Blockade of afferent information to or interference with the function of a homeostat increases the variability of levels of the monitored variable. Thus, baroreceptor deafferentiation increases the variability of blood pressure, as does bilateral destruction of the nucleus of the solitary tract, the likely brainstem site of the arterial barostat [39].

Even a simple homeostatic reflex reflects stress, when a perceived discrepancy between a setpoint for a monitored variable and information about the actual level of that variable elicits compensatory responses to decrease the discrepancy. One way of looking at stress is as a condition where expectations, whether genetically programmed, established by prior learning, or deduced from circumstances, do not match the current or anticipated perceptions of the internal or external environment, and this discrepancy between what is obser-ved or sensed and what is expected or programmed elicits patterned, compensatory responses.

What is distress?

Distress is aversive to the organism, as evidenced by motivation for learning to escape or avoid the stressor. The homeostat theory does not assume an equivalence of noxiousness (i.e., negatively reinforcing properties) with production of pathological changes; that is, the theory does not assume that distress causes disease. In contrast, SELYE characterized distress as unpleasant or harmful [1], without separating these two very different characteristics. He never incorporated the relationship between distress and disease explicitly in his theory. As noted above, SELYE's theory emphasized the nonspecificity of the stress response, whereas according to the homostatic theory, the experience of distress responses depends on the character, intensity, and meaning of the stressor

as perceived by the organism and on the organism's perceived ability to cope with it. Distress responses, as all stress responses, have a "purpose," mitigating effects of a stressor in some way. This applies not only to neuroendocrine aspects of those responses (such as the glucose counterregulatory actions of pituitary-adrenocortical and adrenomedullary stimulation during insulin-induced hypoglycemia) but also to psychological aspects (such as conditioned aversive and instrumental avoidance learning). Distress responses evolved and probably continue to be expressed even in higher organisms, including humans who actually are only rarely exposed to truly "fight-or-flight" agonistic encounters, because of the importance of those responses in instinctive communication. SELYE'S theory did not consider the communication aspect of distress.

Allostasis and allostatic load

Levels of physiological activity required to reestablish or maintain homeostasis differ, depending on continually changing conditions in which the organism finds itself - e.g., running vs standing vs lying down. "Allostasis," a term used by STERLING and EYER in 1988 [40], refers to levels of activity required for the individual to "maintain stability through change" - i.e., to adapt [40-42]. In terms of the homeostatic theory, "allostasis" refers to the set of apparent steady-states maintained by multiple effectors. In the analogy of the home temperature control system, one can regulate temperature at different levels, by appropriate use of effectors. Among individuals, levels of glucose, blood pressure, body temperature, metabolism, and so forth can be held stable at different levels, with different patterns of effector activation.

Homeostat resetting redefines the conditions required to maintain homeostasis. Regulation around an altered apparent steady-state is the essence of allostasis. This would be analogous to a different thermostatic setting in the winter compared to the summer. A neuroendocrine example would be the hyperglycemia of exercise. Even in anticipation of the need for metabolic fuel, by activation of "central command," the blood glucose level increases to a new steady-state value. Resetting alters activities of multiple effector systems required to maintain allostasis, at least for short durations. During stress, short-term changes in homeostatic settings generally enhance the long-term well-being and survival of the organism. Responses during exercise provide an obvious example. When superimposed on a substrate of pathology, however, homeostatic resetting can cause harm. For instance, in the setting of ischemic heart disease, global or patterned increases in sympathetic outflows from homeostat resetting would increase cardiac work, the resulting imbalance between oxygen supply and demand precipitating angina pectoris, myocardial infarction, or sudden death.

"Allostatic load" [43] refers to effects of prolonged continuous or intermittent activation of effectors involved in allostasis. In the analogy of the home temperature control system, allostatic load would increase if a window or door were left open. In this situation, one or more effectors might be activated frequently or even continuously. An even more extreme example would be having the air conditioner and the furnace on at the same time, as is the case in an overheated apartment in the spring when there is a warm day before the boilers have been shut down. Continued use of the furnace and air conditioner in opposition to one another, an example of an inefficient "allostatic state," consumes fuel and contributes to wear-and-tear on both pieces of equipment. Long-term allostatic load - the wear and tear cost of adaptation -provides a conceptual basis for studying long-term health consequences of stress.

Stressor-specific responses of catecholamine systems

After adequately sensitive assay methods for plasma levels of norepinephrine and epinephrine became available, evidence rapidly accumulated for different noradrenergic vs. adrenergic responses in different situations [10,44-46]. A new concept began to emerge, in which norepinephrine levels, and thereby overall sympathetic nervous "activity", would play key roles in appropriate distribution of blood volume and homeostasis of

blood pressure (or blood delivery to the brain), such as during orthostasis, cold exposure, mild blood loss, locomotion, exercise, altered salt intake, and water immersion. Epinephrine levels, and thereby adrenomedullary hormonal system "activity," would respond to global or metabolic threats, such as hypoglycemia, hemorrhagic hypotension, exercise beyond an anerobic threshold, asphyxiation, emotional distress, and shock. Evidence also has accumulated for an association between norepinephrine and active escape, avoidance, or attack, and an association between epinephrine and passive, immobile fear. Table 1 provides some examples of different patterns of sympathetic nervous, adrenomedullary hormonal, and hypothalamo-pituitary-adrenocortical responses to different stressors.

Thus, in contrast with the doctrine of nonspecificity, according to the homeostatic theory of stress, activities of effector systems are coordinated in relatively specific patterns, including neuroendocrine patterns. These patterns serve different needs, and the sympathetic nervous and adrenomedullary hormonal systems play important roles in many of them. For instance, sympathetic nervous system activation predominates in response to orthostasis, moderate exercise, and exposure to cold, whereas adrenomedullary hormonal system activation predominates in response to glucoprivation and emotional distress (Table 1).

In terms of the body's thermostat, studies of humans exposed to cold or with mild core hypothermia have provided support for the notion of primitive specificity of neuroendocrine stress responses. Cold exposure increases plasma norepinephrine levels, with smaller increases in plasma epinephrine levels, consistent with sympathetic neuronal activation and relatively less adrenomedullary hormonal activation. Mild core hypothermia also increases antecubital venous levels of norepinephrine but not epinephrine [34]. Both norepinephrine and epinephrine levels in arterial plasma increase in this setting, but with larger norepinephrine responses. These findings make sense, in that one can maintain body temperature effectively by sympathetically-mediated cutaneous vasoconstriction, piloerection, and shivering. When these mechanisms give way, and core temperature falls, then high circulating epinephrine levels increase generation of calories [47], associated with the experience of distress, which motivates escape and avoidance, and augments norepinephrine release from sympathetic nerve terminals for a given amount of nerve traffic [48].

For each stress, neuroendocrine and physiological changes are coupled with behavioral changes. For instance, the regulation of total body water in humans depends on an interplay between behavior (the search for water and drinking), an internal experience or feeling (thirst), and the elicitation of a neurohumoral response pattern (in this case dominated by vasopressin, the antidiuretic hormone; and to a lesser extent angiotensin, a potent stimulator of drinking). Evoked changes in homeostat function often produce not only neuroendocrine and physiological effects but also behavioral responses; however, because of traditional boundaries among physiology, endocrinology, and psychology, interactions producing integrated patterns of response remain incompletely understood.

Medical and psychological consequences of stress and allostasis

Induction of a positive feedback loop in a homeostatic system evokes instability. An example would be renin-angiotensin-aldosterone system activation in congestive heart failure. Activation of this system increases sodium retention and vascular tone, leading to increased cardiac preload and afterload that worsen the congestive heart failure. Therefore, treatment with an angiotensin-converting-enzyme inhibitor or angiotensin II receptor blocker can successfully treat congestive heart failure [49].

Another example may be fainting reactions. Fainting is preceded by high circulating epinephrine levels and withdrawal of sympathetic vasoconstrictor tone [50,51]. This elicits skeletal muscle vasodilation, and total peripheral resistance to blood flow falls. If there were enough "shunting" of blood to the skeletal muscle, then blood flow to the brainstem might fall. The person would not feel "right." This could evoke more adrenomedullary secretion of epinephrine, and the consequent neurocircula-

tory positive feedback loop would lead to critical brainstem hypoperfusion and loss of consciousness within seconds to minutes.

In people who faint repeatedly, it is often the case that between episodes they do not feel normal. Patients who are susceptible to neurocardiogenic syncope often complain of chronic fatigue, headache, chest pain, orthostatic intolerance, difficulty concentrating, and heat intolerance, which can be debilitating. The patients also have tonic suppression of norepinephrine spillover from the heart [52]. In essence this may reflect consequences of long-term allostatic load, as discussed below.

Induction of a positive feedback loop "nested" in a larger system that includes negative feedback can lead to a new steady-state group of settings and values for monitored variables, rather than "explosion" of the system. For example, a distressing situation might elicit fear, resulting in release of norepinephrine in the brain and epinephrine in the periphery, both of which could augment vigilance behavior and heighten the experience of distress, resulting in greater fear [53]. The organism could enter an "escape mode," with a different set of homeostatic regulatory settings; however, there is a risk of the positive feedback loop leading to a behavioral "explosion", panic, or a pathophysiologic "explosion", pulmonary edema. The notion of induction of a nested positive feedback loop can also provide a model for developmental changes in adolescence, where stability would actually be abnormal, but there is a greater chance for both psychological and physiological disorders to emerge.

The homeostatic theory of stress and the concept of allostasis can help understand chronic as well as acute medical consequences of stress. Chronic activation of allostatic effectors in allostatic states increases allostatic load. For instance, chronic elevations in adrenomedullary and hypothalamic-pituitary-adrenocortical outflows may worsen insulin resistance, and chronic cardiac sympathetic activation may accelerate cardiovascular hypertrophy and development of heart failure [54].

Another application of the homeostatic idea to medical consequences of stress is in terms of the perceived ability to cope. As noted above, an organism experiences distress upon sensing that the effector responses will not be sufficient to restore or maintain allostasis. In contrast with distress, stress does not imply a conscious experience. For instance, even heavily sedated humans have substantial adrenomedullary stimulation in response to acute glucoprivation. Indeed, the larger adrenomedullary response to the same stressor in alert than in sedated humans might provide a measure of the distress. Distress instinctively elicits observable signs and pituitary-adrenocortical and adrenomedullary activation [2,32]. Via these neuroendocrine changes, distress could worsen pathophysiologic processes. For instance, because of adrenomedullary activation, in a patient with coronary artery stenosis, distress could elicit cardiovascular stimulation and produce an excess of myocardial oxygen consumption over supply, precipitating myocardial infarction or lethal ventricular arrhythmias. Moreover, long-term distress could augment both the risk of a mood disorder and the risk of worsening coronary disease.

Long-term physical or mental consequences of stress would depend on long-term effects of allostatic load. Prolonged, intensive activation of effector systems could exaggerate effects of intrinsic defects in any of them, just as increased air pressure in a tire could expand and eventually "blow out" a weakened area. It is not difficult to imagine that repeated or long-term stress or distress could lead to a medical or psychiatric "blow-out."

Maintenance of allostatic states requires energy. This requirement is perhaps clearest in allostasis of core temperature. In mammals, maintenance of a constant core temperature accounts for a substantial proportion of total body energy expenditure at rest. One may hypothesize that reducing allostatic load exerts beneficial health effects, just as one may hypothesize that excessive allostatic load exerts deleterious health effects. In the analogy of the home temperature control system, maintaining a temperature of 60 degrees Fahrenheit in the summer would require a great expenditure of energy and involve cooling systems being on continuously, whereas in the winter, maintaining the same temperature

would be energy-efficient. One can imagine that the likelihood of system breakdown would depend on the extent of long-term energy use by the effector systems.

Chronic effector system activation might alter the efficiency of the homeostatic system itself. For instance, chronic sympathetic nervous stimulation of the cardiovascular system could promote cardiovascular hypertrophy, "splinting" arterial baroreceptors in stiff blood vessel walls, in turn contributing to systolic hypertension and the risk of heart failure, kidney failure, and stroke.

Moreover, an inappropriately large adrenomedullary response to a stressor might exaggerate the experience of emotional distress [55]. Exaggerated distress responses might increase the risk of worsening an independent pathologic process, such as in panic-induced angina pectoris [56,57].

In summary, this essay reflects a merging of the homeostat theory of stress with the concept of allostatic load, in attempting to understand the relationships among stress, catecholamines, and disease. Until this conceptual merging, the homeostat theory did not lead easily to testable predictions about long-term effects of stress and distress; and the concept of allostatic load did not incorporate determinants of that load as sensed discrepancies between afferent information and setpoints for responding, leading to patterned alterations in activities of multiple effectors. Merging of the homeostat theory of stress with the notions of allostasis and allostatic load can provide a basis for explaining and predicting physical and psychiatric effects of acute and chronic stress.

Stress is an interdisciplinary topic, and understanding health consequences of stress requires an integrative approach. Research and ideas about stress must must move beyond considering only one effector system, such as the "sympathoadrenal system," and only one monitored variable, such as serum glucose levels, to incorporate multiple effectors and multiple homeostatic systems that are regulated in parallel. They must also move beyond the notion of a single set of ideal values for monitored variables – homeostasis – to incorporate dynamic changes in homeostatic settings - allostasis. Merging of the homeostatis definitions of stress and distress with the concept of allostasis should provide a better understanding of the roles of stress and distress, via catecholamine systems, in chronic diseases and also provide a conceptual basis for the further development of scientific integrative medicine.

References

1. Selye H: Stress without Distress. New York: New American Library; 1974.
2. Goldstein DS: Stress, Catecholamines, and Cardiovascular Disease. New York: Oxford University Press; 1995.
3. Cannon WB: The Wisdom of the Body. New York: W.W. Norton; 1939.
4. Cannon WB: Organization for physiological homeostasis. Physiol Rev 1929; **9**: 399-431, 1929
5. Cannon WB: Bodily Changes in Pain, Hunger, Fear and Rage. New York: D. Appleton & Co.; 1929.
6. Cannon WB, de la Paz D: Emotional stimulation of adrenal gland secretion. Am J Physiol **28:** 64-70, 1911
7. Cannon WB: The emergency function of the adrenal medulla in pain and in the major emotions. Am J Physiol **33:** 356-372, 1914
8. Cannon WB, Lissak K:. Evidence for adrenaline in adrenergic neurones. Am J Physiol **125:** 765-777, 1939
9. von Euler US: A specific sympathomimetic ergone in adrenergic nerve fibres (sympathin) and its relations to adrenaline and nor-adrenaline. Acta Physiol Scand **12:** 73-96, 1946
10. Cryer PE. Physiology and pathophysiology of the human sympathoadrenal neuroendocrine system. N Engl J Med **303:** 436-444, 1980
11. Shah SD, Tse TF, Clutter WE, Cryer PE: The human sympathochromaffin system. Am J Physiol **247:** E380-E384, 1984
12. Sofuoglu M, Nelson D, Babb DA, Hatsukami DK: Intravenous cocaine increases plasma epinephrine and norepinephrine in humans. Pharmacol Biochem Behav **68:** 455-459, 2001

13. KVETNANSKY R, PACAK K, FUKUHARA K, VISKUPIC E, HIREMAGALUR B, NANKOVA B, GOLDSTEIN, DS, SABBAN EL, KOPIN IJ: Sympathoadrenal system in stress. Interaction with the hypothalamic-pituitary-adrenocortical system. Ann NY Acad Sci **771:** 131-158, 1975
14. SEALS DR, ESLER MD. Human ageing and the sympathoadrenal system. J Physiol **528:** 407-417, 2000
15. DEL RIO G. Adrenomedullary function and its regulation in obesity. Int J Obes Relat Metab Disord **24 Suppl 2:** S89-S91, 2000
16. BROWN MJ, DOLLERY CT: A specific radioenzymatic assay for dihydroxyphenylalanine (DOPA). Plasma dopa may be the precursor of urine free dopamine. Br J Clin Pharmacol 11: 79-83, 1981
17. WOLFOVITZ E, GROSSMAN E, FOLIO CJ, KEISER HR, KOPIN IJ, GOLDSTEIN DS: Derivation of urinary dopamine from plasma dihydroxyphenylalanine in humans. Clin Sci (Colch) **84:** 549-557, 1993
18. EISENHOFER G, ANEMAN A, FRIBERG P, KEISER HR, KOPIN IJ, GOLDSTEIN DS: Substantial production of dopamine in the human gastrointestinal tract. J Clin Endocrinol Metab **42:**374-377, 1998
19. MEZEY E, EISENHOFER G, HANSSON S , HUNYADY B, HOFFMAN BJ: Dopamine produced by the stomach may act as a paracrine/autocrine hormone in the rat. Neuroendocrinology **67:** 336-348, 1998
20. MEZEY E, EISENHOFER G, HARTA G HANSSON S, GOULD L, HUNYADY B, HOFFMAN BJ: A novel nonneuronal catecholaminergic system: Exocrine pancreas synthesizes and releases dopamine. Proc Natl Acad Sci **93:** 10377-10382, 1996
21. MEZEY E, EISENHOFER G, HANSSON S, HARTA G, HOFFMAN BJ, GALLATZ K, PALKOVITS M, HUNYADY B. Non-neuronal dopamine in the gastrointestinal system. Clin Exp Pharmacol Physiol **26:** S14-S22, 1999
22. CANNON WB. The effects of progressive sympathectomy on blood pressure. Am J Physiol **97:** 592-595, 1931
23. GAUTHIER P, NADEAU R, DE CHAMPLAIN J. Acute and chronic cardiovascular effects of 6-hydroxydopamine in dogs. Circ Res **31:** 207-217, 1972
24. JULIEN C, KANDZA P, BARRES C, LO M, CERUTTI C, SASSARD J. Effects of sympathectomy on blood pressure and its variability in conscious rats. Am J Physiol **259:** H1337-H1342, 1990
25. PATEL JN, COPPACK SW, GOLDSTEIN DS, MILES JM, EISENHOFER G. Norepinephrine spillover from human adipose tissue before and after a 72-hour fast. J Clin Endocrinol Metab **87:** 3373-3377, 2002
26. GERRA G, ZAIMOVIC A, MASCETTI GG, GARDINI S, ZAMBELLI U, TIMPANO M, RAGGI MA, BRAMBILLA F. Neuroendocrine responses to experimentally-induced psychological stress in healthy humans. Psychoneuroendocrinology **26:** 91-107, 2001
27. LAKE CR, ZIEGLER MG, KOPIN IJ. Use of plasma norepinephrine for evaluation of sympathetic neuronal function in man. Life Sci **18:** 315-1325, 1976
28. CHROUSOS GP, GOLD PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. J Am Med Assoc **267:** 1244-1252, 1992
29. PACAK K, PALKOVITS M, YADID G, KVETNANSKY R, KOPIN IJ, GOLDSTEIN DS. Heterogeneous neurochemical responses to different stressors: A test of Selye's doctrine of nonspecificity. Am J Physiol **275:** R1247-R1255, 1998
30. MCEWEN B, STELLAR E. Stress and the individual. Mechanisms leading to disease. Arch Int Med **153:** 2093-2101, 1993
31. GOLDSTEIN DS, MCEWEN B. Allostasis, homeostats, and the nature of stress. Stress. **5:** 55-58, 2002
32. GOLDSTEIN DS. The Autonomic Nervous System in Health and Disease. New York, NY: Marcel Dekker, New York 2001
33. GOLDSTEIN DS. Stress as a scientific idea: A homeostatic theory of stress and distress. Homeostasis **4:** 177-215, 1995
34. FRANK SM, HIGGINS MS, FLEISHER LA, SITZMANN JV, RAFF H, BRESLOW MJ. Adrenergic, respiratory, and cardiovascular effects of core cooling in humans. Am J Physiol **272:** R557-R562, 1997
35. UDELSMAN R, GOLDSTEIN DS, LORIAUX DL, CHROUSOS GP. Catecholamine-glucocorticoid interactions during surgical stress. J Surg Res **43:** 539-545, 1987
36. GOLDSTEIN DS, GARTY M, BAGDY G, SZEMEREDI K, STERNBERG EM, LISTWAK S, DEKA-STAROSTA A, HOFFMAN A, CHANG PC, STULL R, GOLD PW, KOPIN IJ. Role of CRH in glucopenia-induced adrenomedullary activation in rats. J Neuroendocrinol **5:** 475-486, 1993.
37. FUKUHARA K, KVETNANSKY R, CIZZA G, PACAK K, OHARA H, GOLDSTEIN DS, KOPIN IJ: Interrelations between sympathoadrenal system and hypothalamo-pituitary-adrenocortical/thyroid systems in rats exposed to cold stress. J Neuroendocrinol **8:** 533-541, 1996
38. QUILLEN EW, JR., COWLEY AW, JR. Influence of volume changes on osmolality-vasopressin relationships in conscious dogs. Am J Physiol **244:** H73-H79, 1983
39. NATHAN MA, REIS DJ. Chronic labile hypertension produced by lesions of the nucleus
tractus solitarii in the cat. Circ Res **40:** 72-81, 1977
40. MCEWEN BS. Stress, adaptation, and disease. Allostasis and allostatic load. Ann NY Acad Sci. **840:** 33-44, 1998

41. SCHULKIN J, GOLD PW, MCEWEN BS. Induction of corticotropin-releasing hormone gene expression by glucocorticoids: implication for understanding the states of fear and anxiety and allostatic load. Psychoneuroendocrinology **23:** 219-243, 1998
42. MCEWEN BS. Allostasis and allostatic load: implications for neuropsychopharmacology. Neuropsychopharmacology **22:** 108-124, 2000
43. MCEWEN BS, STELLAR E. Stress and the individual. Mechanisms leading to disease. Arch Intern Med **153:** 2093-2101, 1993
44. ROBERTSON DA, JOHNSON GA, ROBERTSON RM, NIES AS, SHAND DG, OATES JA. Comparative assessment of stimuli that release neuronal and adrenomedullary catecholamines in man. Circulation **59:** 637-643, 1979
45. YOUNG JB, LANDSBERG L. Sympathoadrenal activity in fasting pregnant rats: Dissociation of adrenal medullary and sympathetic nervous system responses. J Clin Invest **64:** 109-116, 1979
46. YOUNG JB, ROSA RM, LANDSBERG L. Dissociation of sympathetic nervous system and adrenal medullary responses. AmJ Physiol **247:** E35-E40, 1984
47. STATEN MA, MATTHEWS DE, CRYER PE, BIER DM. Physiological increments in epinephrine stimulate metabolic rate in humans. Am J Physiol **253:** E322-E330, 1987
48. CHANG PC, GROSSMAN E, KOPIN IJ, GOLDSTEIN DS. On the existence of functional beta-adrenoceptors on vascular sympathetic nerve endings in the human forearm. J Hypertens **12:** 681-690, 1994
49. KLUGER J, CODY RJ, LARAGH JH. The contributions of sympathetic tone and the renin-angiotensin system to severe chronic congestive heart failure: response to specific inhibitors (prazosin and captopril). Am J Cardiol **49:** 1667-1674, 1982
50. MOSQUEDA-GARCIA R, FURLAN R, FERNANDEZ-VIOLANTE R, DESAI T, SNELL M, JARAI Z, ANANTHRAM V, ROBERTSON RM, ROBERTSON D. Sympathetic and baroreceptor reflex function in neurally mediated syncope evoked by tilt. J Clin Invest **99:** 2736-2744, 1997
51. GOLDSTEIN DS, HOLMES C, FRANK SM, NAQIBUDDIN M, DENDI R, SNADER S, CALKINS H. Sympathoadrenal imbalance before neurocardiogenic syncope. *Am. J. Cardiol.* 2003; 91:53-58.
52. GOLDSTEIN DS, HOLMES C, FRANK SM, DENDI R, CANNON RO, 3RD, SHARABI Y, ESLER MD, EISENHOFER G. Cardiac sympathetic dysautonomia in chronic orthostatic intolerance syndromes. *Circulation*. 2002; 106:2358-65.
53. ASTON-JONES G, RAJKOWSKI J, KUBIAK P, ALEXINSKY T. Locus coeruleus neurons in monkey are selectively activated by attended cues in a vigilance task. *J Neurosci.* 1994; 14:4467-4480.
54. RUNDQVIST B, ELAM M, BERGMANN-SVERRISDOTTIR Y, EISENHOFER G, FRIBERG P. Increased cardiac adrenergic drive precedes generalized sympathetic activation in human heart failure. *Circulation.* 1997; 95:169-175.
55. SCHACHTER S, SINGER J. Cognitive, social, and physiological determinants of emotional state. *Psychol Rev*. 1962; 69:379-399.
56. WILKINSON DJC, THOMPSON JM, LAMBERT GW, JENNINGS GL, SCHWARZ RG, JEFFREYS D, TURNER AG, ESLER MD. Sympathetic activity in patients with panic disorder at rest, under laboratory mental stress, and during panic attacks. *Arch Gen Psychiatry*. 1998; 55:511-520.
57. MANSOUR VM, WILKINSON DJ, JENNINGS GL, SCHWARZ RG, THOMPSON JM, ESLER MD. Panic disorder: coronary spasm as a basis for cardiac risk? *Med J Australia*. 1998; 168:390-392.
58. WATABE T, TANAKA K, KUMAGAE M, ITOH S, TAKEDA F, MORIO K, HASEGAWA M, HORIUCHI T, MIYABE S, SHIMIZU N. Hormonal responses to insulin-induced hypoglycemia in man. *J Clin Endocrinol Metab*. 1987; 65:1187-91.
59. TOSO CF, RODRIGUEZ RR, RENAULD AR, MARQUEZ AG, LINARES LM. Adrenocorticotrophic hormone, cortisol and catecholamine concentrations during insulin hypoglycaemia in dogs anaesthetized with thiopentone. *Can J Anaesth*. 1993; 40:1084-91.
60. SCHADT JC, LUDBROOK J. Hemodynamic and neurohumoral responses to acute hypovolemia in conscious mammals. *Am. J. Physiol.* 1991; 260:H305-H318.
61. BEREITER DA, ZAID AM, GANN DS. Effect of rate of hemorrhage on sympathoadrenal catecholamine release in cats. *Am J Physiol*. 1986; 250:E69-75.
62. DARLINGTON DN, SHINSAKO J, DALLMAN MF. Responses of ACTH, epinephrine, norepinephrine, and cardiovascular system to hemorrhage. *Am J Physiol*. 1986; 251:H612-8.
63. GRASSLER J, JEZOVA D, KVETNANSKY R, SCHEUCH DW. Hormonal responses to hemorrhage and their relationship to individual hemorrhagic shock susceptibility. *Endocrinol Exp*. 1990; 24:105-16.
64. FRANK SM, HIGGINS MS, BRESLOW MJ, FLEISHER LA, GORMAN RB, SITZMANN JV, RAFF H, BEATTIE C. The catecholamine, cortisol, and hemodynamic responses to mild perioperative hypothermia. A randomized clinical trial. *Anesthesiology*. 1995; 82:83-93.

65. Graham TE, Sathasivam P, MacNaughton KW. Influence of cold, exercise, and caffeine on catecholamines and metabolism in men. *J.Appl.Physiol.* 1991; 70:2052-2058.
66. Marino F, Sockler JM, Fry JM. Thermoregulatory, metabolic and sympathoadrenal responses to repeated brief exposure to cold. *Scand.J.Clin.Lab.Invest.* 1998; 58:537-545.
67. Wittert GA, Or HK, Livesey JH, Richards AM, Donald RA, Espiner EA. Vasopressin, corticotrophin-releasing factor, and pituitary adrenal responses to acute cold stress in normal humans. *J. Clin. Endocrinol. Metab.* 1992; 75:750-755.
68. Marino F, Sockler JM, Fry JM. Thermoregulatory, metabolic and sympathoadrenal responses to repeated brief exposure to cold. *Scand J Clin Lab Invest*. 1998; 58:537-45.
69. Gibbs DM. Inhibition of corticotropin release during hypothermia: the role of corticotropin-releasing factor, vasopressin, and oxytocin. *Endocrinology*. 1985; 116:723-7.
70. Frank SM, Cattaneo CG, Wieneke-Brady MB, El-Rahmany H, Gupta N, Lima JA, Goldstein DS. Threshold for adrenomedullary activation and increased cardiac work during mild core hypothermia. *Clin. Sci.* 2002; 102:119-125.
71. Hirvonen J, Huttunen P. Increased urinary concentration of catecholamines in hypothermia deaths. *J Forensic Sci*. 1982; 27:264-71.
72. Chi OZ, Choi YK, Lee DI, Kim YS, Lee I. Intraoperative mild hypothermia does not increase the plasma concentration of stress hormones during neurosurgery. *Can J Anaesth*. 2001; 48:815-8.
73. Harden RN, Duc TA, Williams TR, Coley D, Cate JC, Gracely RH. Norepinephrine and epinephrine levels in affected versus unaffected limbs in sympathetically maintained pain. *Clinical Journal of Pain*. 1994; 10:324-330.
74. Brand HS, Gortzak RA, Palmer-Bouva CC, Abraham RE, Abraham-Inpijn L. Cardiovascular and neuroendocrine responses during acute stress induced by different types of dental treatment. *Int Dent J*. 1995; 45:45-8.
75. de Boer SF, Slangen JL, Van der Gugten J. Plasma catecholamine and corticosterone levels during active and passive shock-prod avoidance behavior in rats: effects of chlordiazepoxide. *Physiol. Behav.* 1990; 47:1089-1098.
76. Nijsen MJ, Croiset G, Diamant M, Stam R, Kamphuis PJ, Bruijnzeel A, de Wied D, Wiegant VM. Endogenous corticotropin-releasing hormone inhibits conditioned-fear-induced vagal activation in the rat. *Eur J Pharmacol*. 2000; 389:89-98.
77. Pitman DL, Natelson BH, Ottenweller JE, McCarty R, Pritzel T, Tapp WN. Effects of exposure to stressors of varying predictability on adrenal function in rats. *Behav Neurosci*. 1995; 109:767-76.
78. Pitman DL, Natelson BH, Ottenweller JE. Classical aversive conditioning of catecholamine and corticosterone responses. *Integr Physiol Behav Sci*. 1992; 27:13-22.
79. Korte SM, Bouws GA, Koolhaas JM, Bohus B. Neuroendocrine and behavioral responses during conditioned active and passive behavior in the defensive burying/probe avoidance paradigm: effects of ipsapirone. *Physiol Behav*. 1992; 52:355-61.
80. Costa A, Martignoni E, Blandini F, Petraglia F, Genazzani AR, Nappi G. Effects of etoperidone on sympathetic and pituitary-adrenal responses to diverse stressors in humans. *Clin Neuropharmacol*. 1993; 16:127-38.
81. Yoshiuchi K, Nomura S, Ando K, Ohtake T, Shimosawa T, Kumano H, Kuboki T, Suematsu H, Fujita T. Hemodynamic and endocrine responsiveness to mental arithmetic task and mirror drawing test in patients with essential hypertension. *Am J Hypertens*. 1997; 10:243-9.
82. Deuster PA, Chrousos GP, Luger A, DeBolt JE, Bernier LL, Trostmann UH, Kyle SB, Montgomery LC, Loriaux DL. Hormonal and metabolic responses of untrained, moderately trained, and highly trained men to three exercise intensities. *Metabolism*. 1989; 38:141-8.
83. Nagata S, Takeda F, Kurosawa M, Mima K, Hiraga A, Kai M, Taya K. Plasma adrenocorticotropin, cortisol and catecholamines response to various exercises. *Equine Vet J Suppl*. 1999; 30:570-4.
84. Schwarz L, Kindermann W. Beta-endorphin, adrenocorticotropic hormone, cortisol and catecholamines during aerobic and anaerobic exercise. *Eur J Appl Physiol Occup Physiol*. 1990; 61:165-71.
85. Dimsdale JE, Moss J. Plasma catecholamines in stress and exercise. *JAMA*. 1980; 243:340-342.
86. Oleshansky MA, Zoltick JM, Herman RH, Mougey EH, Meyerhoff JL. The influence of fitness on neuroendocrine responses to exhaustive treadmill exercise. *Eur J Appl Physiol Occup Physiol*. 1990; 59:405-10.
87. Casa DJ, Maresh CM, Armstrong LE, Kavouras SA, Herrera-Soto JA, Hacker Jr FT, Scheett TP, Stoppani J. Intravenous versus oral rehydration during a brief period: stress hormone responses to subsequent exhaustive exercise in the heat. *Int J Sport Nutr Exerc Metab*. 2000; 10:361-74.
88. Malarkey WB, Lipkus IM, Cacioppo JT. The dissociation of catecholamine and hypothalamic-pituitary-adrenal responses to daily stressors using dexamethasone. *J Clin Endocrinol Metab*. 1995; 80:2458-63.

89. Jardine DL, Melton IC, Crozier IG, Bennett SI, Donald RA, Ikram H. Neurohormonal response to head-up tilt and its role in vasovagal syncope. *Am J Cardiol*. 1997; 79:1302-6.
90. Jezova D, Ochedalski T, Glickman M, Kiss A, Aguilera G. Central corticotropin-releasing hormone receptors modulate hypothalamic-pituitary-adrenocortical and sympathoadrenal activity during stress. *Neuroscience*. 1999; 94:797-802.
91. Tjurmina OA, Armando I, Saavedra JM, Goldstein DS, Murphy DL. Exaggerated adrenomedullary response to immobilization in mice with targeted disruption of the serotonin transporter gene. *Endocrinology*. 2002; 143:4520-6.
92. Kvetnansky R, Goldstein DS, Weise VK, Holmes C, Szemeredi K, Bagdy G, Kopin IJ. Effects of handling or immobilization on plasma levels of 3,4-dihydroxyphenylalanine, catecholamines, and metabolites in rats. *Journal of Neurochemistry*. 1992; 58:2296-2302.
93. Dobrakovova M, Kvetnansky R, Oprsalova Z, Jezova D. Specificity of the effect of repeated handling on sympathetic-adrenomedullary and pituitary-adrenocortical activity in rats. *Psychoneuroendocrinology*. 1993; 18:163-74.
94. Donald RA, Perry EG, Wittert GA, Chapman M, Livesey JH, Ellis MJ, Evans MJ, Yandle T, Espiner EA. The plasma ACTH, AVP, CRH and catecholamine responses to conventional and laparoscopic cholecystectomy. *Clin Endocrinol (Oxf)*. 1993; 38:609-15.
95. Kudoh A, Ishihara H, Matsuki A. Response to surgical stress in elderly patients and Alzheimer's disease. *Can J Anaesth*. 1999; 46:247-52.
96. Friedrich M, Rixecker D, Friedrich G. Evaluation of stress-related hormones after surgery. *Clin Exp Obstet Gynecol*. 1999; 26:71-5.
97. Nguyen NT, Goldman CD, Ho HS, Gosselin RC, Singh A, Wolfe BM. Systemic stress response after laparoscopic and open gastric bypass. *J Am Coll Surg*. 2002; 194:557-66; discussion 566-7.
98. Udelsman R, Norton JA, Jelenich SE, Goldstein DS, Linehan WM, Loriaux DL, Chrousos GP. Responses of the hypothalamic-pituitary-adrenal and renin-angiotensin axes and the sympathetic system during controlled surgical and anesthetic stress. *J. Clin. Endocrinol. Metab.* 1987; 64:986-994.
99. O'Leary MJ, Timmins AC, Appleby JN, Medbak S, Grossman AB, Nathan AW, Hinds CJ. Dissociation of pituitary-adrenal and catecholamine activation after induced cardiac arrest and defibrillation. *Br. J. Anaesth.* 1999; 82:271-273.
100. Little RA, Frayn KN, Randall PE, Yates DW. Plasma catecholamines and cardiac arrest. *Lancet*. 1985; ii:509-510.
101. Foley PJ, Tacker WA, Wortsman J, Frank S, Cryer PE. Plasma catecholamine and serum cortisol responses to experimental cardiac arrest in dogs. *Am. J. Physiol.* 1987; 253:E283-E289.
102. Wortsman J, Frank S, Cryer PE. Adrenomedullary response to maximal stress in humans. *Am.J.Med.* 1984; 77:779-784.
103. O'Leary MJ, Timmins AC, Appleby JN, Medbak S, Grossman AB, Nathan AW, Hinds CJ. Dissociation of pituitary-adrenal and catecholamine activation after induced cardiac arrest and defibrillation. *Br J Anaesth*. 1999; 82:271-3.
104. Lindner KH, Haak T, Keller A, Bothner U, Lurie KG. Release of endogenous vasopressors during and after cardiopulmonary resuscitation. *Heart*. 1996; 75:145-50.
105. Schultz CH, Rivers EP, Feldkamp CS, Goad EG, Smithline HA, Martin GB, Fath JJ, Wortsman J, Nowak RM. A characterization of hypothalamic-pituitary-adrenal axis function during and after human cardiac arrest. *Crit Care Med*. 1993; 21:1339-47.
106. Lindner KH, Strohmenger HU, Ensinger H, Hetzel WD, Ahnefeld FW, Georgieff M. Stress hormone response during and after cardiopulmonary resuscitation. *Anesthesiology*. 1992; 77:662-8.
107. Habib KE, Weld KP, Rice KC, Pushkas J, Champoux M, Listwak S, Webster EL, Atkinson AJ, Schulkin J, Contoreggi C, Chrousos GP, McCann SM, Suomi SJ, Higley JD, Gold PW. Oral administration of a corticotropin-releasing hormone receptor antagonist significantly attenuates behavioral, neuroendocrine, and autonomic responses to stress in primates. *Proc Natl Acad Sci U S A*. 2000; 97:6079-84.

Corresponding author: David S. Goldstein, M.D.
NINDS, NIH
Building 10 Room 6N252
10 Center Drive, MSC-1620
Bethesda, Maryland 20892-1620
Phone: (301) 496-2103
Fax: (301) 402-0180
E-mail: goldsteind@ninds.nih.gov

EIGHTH SYMPOSIUM

ON CATECHOLAMINES

AND OTHER NEUROTRANSMITTERS

IN STRESS

SMOLENICE CASTLE, SLOVAKIA

JUNE 28 – JULY 3, 2003

A B S T R A C T S

Organized by:

INSTITUTE OF EXPERIMENTAL ENDOCRINOLOGY
Centre of Excellence of European Union
Slovak Academy of Sciences
Bratislava, Slovakia

NATIONAL INSTITUTE OF CHILD HEALTH
AND HUMAN DEVELOPMENT
National Institutes of Health
Bethesda, Maryland, USA

HONORARY SYMPOSIUM CHAIRMEN

AXELROD J.
KOPIN I. J.
National Institutes of Health, Bethesda, Maryland, USA

SYMPOSIUM CHAIRMAN

KVETŇANSKÝ R.
Institute of Experimental Endocrinology, SAS, Bratislava, Slovakia

SECRETARY GENERAL

PACAK K.
National Institute of Child Health and Development, NIH, Bethesda, MD, USA

EXECUTIVE SECRETARIES

AGUILERA G.
National Institutes of Child Health and Human Development, NIH, Bethesda, MD, USA
GOLDSTEIN D.S.
National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland, USA
JEŽOVÁ D.
Institute of Experimental Endocrinology, SAS, Bratislava, Slovakia
SABBAN E.L.
New York Medical College, Valhalla, New York, USA

INTERNATIONAL ADVISORY COMMITTEE

ARMANDO I., USA
BUCKENDAHL P., USA
BURROWS G., Australia
CHROUSOS G. P., USA
DALLMAN M. F., USA
De KLOET R., Netherlands
De SOUZA E., USA
GOLD P. W., USA
HELLHAMMER D., Germany
HOLSBOER F., Germany
LIGHTMAN S. L., UK
MAJZOUB J. A., USA
MAKARA G. B., Hungary
McEWEN B. S., USA
MEANEY M. J., Canada
NAGATSU T., Japan
NEMEROFF Ch. B., USA
OLIVER C., France
PALKOVITS M., Hungary
PLOTSKY P. M., USA
SAITO H., Japan
SAWCHENKO P. E., USA
STANFORD C. S., UK
SUDAKOV K. V., Russia
SYKOVA E., Czech Republic
TANK W., USA
TIGRANIAN R. A., Russia
TILDERS F., Netherlands
UNGER T., Germany
VIZI S. E., Hungary
WEINSTOCK M., Israel
WONG D. L., USA

LOCAL ORGANIZING COMMITTEE CHAIRMAN

KRIŽANOVÁ O.
Institute of Experimental Endocrinology, SAS, Bratislava, Slovakia

MAILING ADDRESS OF THE SECRETARIAT:

Richard Kvetňanský
Institute of Experimental Endocrinology
Slovak Academy of Sciences
Vlárska 3
833 06 BRATISLAVA
SLOVAK REPUBLIC

Tel: (+4212) 5477 4101
Fax: (+4212) 5477 4908
E-mail: ueenkvet@savba.sk
Url: http://www.stress.sav.sk

EFFECT OF IMMOBILIZATION ON IN VITRO TRH RELEASE FROM BRAIN SEPTUM IN WILD-TYPE (WT) AND CRH KNOCK-OUT (CRH-KO) MICE

ADAMEOVA A., KUBOVCAKOVA L., JAMAL B., BACOVA Z., KVETNANSKY R., KISS A., STRBAK V. *Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia*

There is considerable evidence linking alcohol consumption and sedation and TRH in the brain septum. We have shown that ethanol in clinically relevant concentrations can induce TRH release from the septum by a mechanism involving neuronal swelling. Corticotropin-releasing hormone deficient mice serve as an interesting model to understand the role of CRH in the regulation of different neuroendocrine systems. The aim of this study was to compare TRH system in brain tissue at basal and stress conditions in CRH-KO mice and their WT mates. Experimental mice were decapitated immediately or 3 h after the single and repeated (seven times for 2 h daily) immobilization stress. Brain septum was immediately withdrawn and incubated *in vitro* to measure basal, ethanol and hyposmosis stimulated TRH release. Ethanol in isosmotic medium or hyposmotic medium stimulated TRH release from mice septum explants from both WT and CRH-KO mice. The response was disturbed immediately after immobilization and recovered 3 h later. Recovery was less complete after repeated immobilization. Immobilization stress affects TRH system in brain septum. Inborn absence of CRH does not affect septal TRH and its response to ethanol and stress. *The work was supported by project 2/3191/23 of Slovak Grant Agency (VEGA).*

Keywords: TRH, septum, immobilization stress, CRH, mice

VASOPRESSIN V1B RECEPTOR SIGNALING AND REGULATION AND STRESS ADAPTATION

AGUILERA G., RABADAN-DIEHL C., VOLPI S. *Section on Endocrine Physiology, National Institute of Child Health and Human Development, NIH, Bethesda, MD, USA*

Adaptation of the hypothalamic pituitary adrenal (HPA) axis to stress involves upregulation of pituitary V1b vasopressin receptors (V1bR) through transcriptional and translational mechanisms. GAGA repeats located near the transcription start point are essential for transcriptional activation of the receptor. Increases in binding of pituitary nuclear proteins to these GAGA repeats during stress, suggest the involvement of this mechanism on the physiological regulation of V1bR transcription. In addition, two mechanisms mediating stimulation and inhibition of V1bR mRNA translation were identified in the 5'untranslated region (5'UTR). Upstream open reading frames in the 5'UTR of the V1b receptor mRNA repress translation of the major ORF encoding the V1b receptor, in part through translation of a 38 amino acid peptide encoded by the proximal upstream ORF. While upstream ORFs could account for low basal translational activity of the V1b receptor, an internal ribosome entry site (IRES) activates V1bR translation. Stimulation of IRES activity through protein kinase C- and PI3 kinase-mediated pathways results in V1bR mRNA translation increasing V1bR protein levels in the pituitary. Facilitation of receptor coupling though increases in Gq/11 content may also contribute to the adaptation of pituitary corticotroph responsiveness during stress. The involvement of transcriptional and post-transcriptional events provides multiple loci of response to facilitate plasticity of regulation of the number of pituitary vasopressin receptors according to physiological demand.

Keywords: vasopressin, V1b receptor, transcription, translation, signaling

TRANSDUCTION OF ADRENAL CELLS BY ADENOVIRAL VECTORS: IMPLICATIONS FOR STRESS RESPONSE, NATURAL INFECTIONS AND GENE THERAPY

ALESCI S., CHROUSOS G.P., PACAK K. *Pediatric and Reproductive Endocrinology Branch, NICHD, NIH, Bethesda, MD, USA*

Recombinant adenoviral vectors (RAVs) are effective in transferring foreign genes to a variety of cells and tissues. However, during gene transfer, these vectors may alter the cell function and its local environment. The adrenal gland is a major target for adenoviruses. Nevertheless, little information is currently available on adenoviral gene transfer to adrenal cells. Therefore, we investigated the effects of *ex vivo* transduction with RAVs on bovine adrenocortical cells (BACCs) morphology, ultrastructure, proliferation and basal and stimulated hormone release. Transduction of BACCs by an E1/E3-deleted RAV, engineered to express green fluorescent protein (GFP), was highly efficient, as documented by fluorescent microscopy. However, it was accompanied by nuclear and mitochondrial alterations, increased cell proliferation and basal steroidogenesis, and decreased cell response to ACTH. Interestingly, neither purified viral capsid, nor two different E4-deleted RAVs, interfered with BACCs steroidogenesis. Intact adrenal function and appropriate steroid balance are crucial for adaptation to stress. Thus, the clinical implications of these findings should be evaluated in patients with severe adenoviral infections or undergoing gene therapy (GT) with RAVs. At the same time, the highly efficient RAV-mediated transgene expression in BACCs suggests that appropriately engineered RAVs may be suitable for GT of various adrenal disorders. We are currently extending our studies to bovine adrenal chromaffin cells and pheochromocytoma cells.

Keywords: adrenal, adenovirus, stress, gene therapy

ANGIOTENSIN II AT1 RECEPTOR BLOCKADE DECREASES BRAIN ARTERY INFLAMMATION IN A STRESS-PRONE RAT STRAIN

ANDO H., JEZOVA M., ZHOU J., SAAVEDRA J.M. *Section on Pharmacology, Division of Intramural Research Programs, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA*

The Spontaneously Hypertensive Rats (SHR) are a genetically hypertensive strain with exaggerated central cardiovascular responses to stress. We hypothesize altered brain mechanisms of control of the stress reaction in these animals. In SHR, the brain angiotensin II (Ang II) system is chronically stimulated, resulting in brain arterial remodeling, with increased growth, decreased lumen, decreased compliance and arterial inflammation, a pathology paralleling the development of hypertension and the enhanced response to stress. Long-term inhibition of brain Ang II AT1 receptors reverses the pathological arterial remodeling and arterial inflammation in SHR, as revealed by normalization of arterial structure, decreased expression of the pro-inflammatory adhesion molecule ICAM-1, decreased macrophage infiltration, reversal of the decreased eNOS expression in the endothelium of brain microvessels, and reversal of the increased iNOS expression in arterial adventitia. Antagonists of brain AT1 receptors prevent stress-related pathology, in part by protecting regional blood flow. We speculate that normalization of brain arterial function through control of a hyperactive Ang II system not only protects against brain ischemia but also renders the brain less vulnerable to stress.

Keywords: cerebrovascular inflammation, AT1 receptors, brain angiotensin II, NO, stress treatment

ANGIOTENSIN II REGULATES THE STRESS REACTION AT PERIPHERAL AND CENTRAL SITES

ARMANDO I., JEZOVA M., BREGONZIO C., BAIARDI G., SAAVEDRA J.M. *Section on Pharmacology, Division of Intramural Research Programs, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA*

Important sites for angiotensin II (Ang II) control of the stress reaction are located in adrenal medulla, paraventricular nucleus, and higher cortical centers. In the adrenal medulla, AT_1 and AT_2 receptors regulate basal catecholamine synthesis controlling the TH transcription factor Fra-2. AT_1 receptor stimulation additionally regulates TH transcription through participation of pCREB and ERK2. During stress, AT_1 receptor blockade substantially decreases adrenomedullary catecholamine release. Thus, Ang II regulates both the basal adrenomedullary catecholamine tone and its response to stress. In the brain, AT_1 receptors control CRH formation and release. AT_1 receptor blockade resets the HPA axis tone and makes animals less vulnerable and responsive to stress. When stress occurs under AT_1 receptor blockade, the release of ACTH, corticosterone and vasopressin is substantially decreased. The prevention of stress-induced cortical CRH_1 receptor decrease, and the anti-anxiety effects of AT_1 antagonists suggest a modulation of the cognitive response to stress. This explains how selective AT_1 receptor inhibition, by controlling the most important components of the stress reaction, could be advantageously utilized as a preventive and therapeutic anti-stress intervention.

Keywords: transcription factors, Fra-2, AT_1 receptors, AT_2 receptors, CRH receptors

LONG-TERM EFFECTS OF A SINGLE EXPOSURE TO IMMOBILIZATION ON THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS: NEUROBIOLOGICAL MECHANISMS

ARMARIO A. *Institute of Neuroscience and Animal Physiology Unit, Faculty of Sciences, Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain*

A single previous exposure to severe stressors such as immobilization (IMO) results in long-lasting reduction of the responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis and some other physiological variables to the homotypic, but not to a heterotypic stressor. Such desensitization process matures over time (days to weeks), with no additional exposure to the stressor, affecting firstly the release of peripheral HPA hormones and later to the transcriptional activity of the CRF gene in the paraventricular nucleus of the hypothalamus (PVN). We have characterized the brain areas sensitive to the previous experience with IMO using the induction of the immediately early gene c-fos and the putative neurochemical mechanisms involved blocking some neurotransmitter systems before the first exposure to the stressor. Our results indicate that a few limbic brain areas are sensitive to previous experience and these areas may send inputs to lower areas such as the PVN and the locus coeruleus. Long-lasting effects of a single exposure to IMO have been difficult to block with pharmacological tools effective in other emotional learning paradigms, perhaps due to the triggering of parallel redundant brain pathways. Taking into account the resistance of the phenomenon to be pharmacologically blocked and the evidence that IMO is a very severe stressor, the possibility remains that IMO may be an animal model of post-traumatic stress disorder.

Keywords: immobilization, HPA axis, paraventricular hypothalamic nucleus, c-fos

FIGHT, FLIGHT, FORBEARANCE AND FORTITUDE: THE SPECTRUM OF EFFECTS OF THE CATECHOLAMINES AND THEIR COUSINS

ARUN C.P. *Department of Urology, Lincoln County Hospital, England*

Description of catecholamine effects in acute stress as the 'fight or flight' response is only half the picture. We explore other implications of the effects of catecholamines and functionally related substances in the early stress response. With mild stress, and compensated shock, the effect is to produce vasoconstriction in the organ systems that are not crucial to survival and thus help in facilitating recovery by promoting forbearance. With severe shock however, vasoconstriction promotes organ failure and a quick demise. In this action, vasoconstriction does to the body what apoptosis does to the individual cell. Since in nature, predation, sepsis and major trauma are common ways of dying, such an effect avoids prolonged suffering to the animal. It would appear that the spectrum of fight, flight, forbearance and fortitude to a threat allows a preprogrammed response to a spectrum of scenarios from a good chance of survival to an utterly hopeless one. Modern techniques of resuscitation implicitly recognise this sequence by promoting the importance of the 'golden hour' in resuscitation. The present theory offers a fresh perspective on the actions of the catecholamines and their cousins in acute stress.

Keywords: acute stress, critical phenomena, fight or flight, catecholamines

LONG-TERM ANGIOTENSIN II AT_1 RECEPTOR BLOCKADE REDUCES STRESS-INDUCED RELEASE OF CATECHOLAMINES, GLUCOCORTICOIDS AND VASOPRESSIN

BAIARDI G., BREGONZIO C., JEZOVA M., ARMANDO I., SAAVEDRA J.M. *Section on Pharmacology, Division of Intramural Research Programs, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA*

We have recently reported that a two-week pretreatment with an angiotensin II (Ang II) AT_1 antagonist prevented the adrenomedullary and hormonal response to acute isolation stress (Armando et al., Endocrinology, 2001). We now report that AT_1 receptor blockade retains its capacity to blunt the response to isolation stress over a very long period of treatment. Spontaneously Hypertensive Rats (SHR) and their Wistar Kyoto normotensive controls (WKY) were treated with the AT_1 receptor antagonist candesartan, 10 mg/kg/day, administered orally in their drinking water, from 8-weeks of age throughout their lifespan, and were submitted to 24 hour isolation stress at different times during the treatment. The AT_1 antagonist inhibited epinephrine release in SHR during the first three months, corticosterone release in SHR and WKY during ten months, and vasopressin release and urinary excretion of dihydroxyphenylglycol in SHR during eighteen months of treatment. We conclude that the blockade of the stress response by the AT_1 receptor antagonist is long lasting, differs between stress-prone SHR and WKY, and that specific components of the stress response react differently to AT_1 receptor blockade.

Keywords: isolation stress, angiotensin AT_1 receptors, stress, catecholamines, vasopressin

PRENATAL IMMUNE CHALLENGE AFFECTS GROWTH, BEHAVIOR, AND BRAIN DOPAMINE IN THE OFFSPRING

BAKOS J., DUNCKO R., PIRNIK Z., MAKATSORI A., KISS A., JEZOVA D. *Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia*

It is known that the development and plasticity of neuroendocrine system can be affected by many factors and that adverse events during prenatal period can result in long lasting changes in the adulthood. This study was aimed to evaluate possible consequences of chronic inflammation in pregnancy for the offspring. Chronic inflammation was simulated by treatment with increasing doses of lipopolysaccharide (LPS) to dams on days 15-19 of pregnancy. Attempts were made to prevent possible negative alterations by keeping animals in enriched environment (EE). Maternal exposure to LPS resulted in a significant reduction of body weight of male offspring during the weaning period. This difference remained till the age of 63 days in controls but not in animals rearing in EE. Content of dopamine in the nucleus accumbens was found to be lower in prenatally stressed adult males. Furthermore, prenatal exposition to maternal immune challenge in females was associated with lower locomotor activity in elevated plus maze and increased number of skips in the beam walking test. Hormonal assays revealed no differences in ACTH and corticosterone concentrations with regard to prenatal treatment, however both groups kept in EE showed augmented levels of corticosterone as well as enlarged adrenals. Thus, immune activation during pregnancy may induce long-term changes in brain catecholamines and behavior, but it is not harmful to basal hormone secretion in the offspring. *This study was supported by grants of EC ICA1-CT-2000-70008 and VEGA 2/2007.*

Keywords: prenatal stress, endotoxin, behavior, growth, dopamine

IN VIVO ADRENERGIC REGULATION OF LIPOLYSIS AND PLASMA LEPTIN LEVELS IN PATIENTS WITH ANOREXIA NERVOSA DURING EXERCISE

BARTAK V.[1,2], DOSTALOVA I.[1,2], NEDVIDKOVA J.[1], VYBIRAL S.[2], PACAK K.[3] [1]*Institute of Endocrinology, Prague,* [2]*Charles University, Faculty of Science, Prague, Czech Republic,* [3]*Pediatric and Reproductive Endocrinology Branch, NICHD, NIH, USA*

Anorexia nervosa (AN) is a psychiatric disease characterized by severe malnutrition and loss of body fat. Sympathetic nervous system (SNS) is the main regulator of adipose tissue. The present study was focused on adrenergic regulation of lipolysis and on plasma levels of adipocyte-derived hormone leptin in patients with AN during exercise. *In vivo* microdialysis (CMA, Sweden) of the subcutaneous adipose tissue was used for the assessment of norepinephrine (NE) and dihydroxyphenylalanine (DOPA) concentrations in interstitial fluid, obtained from 10 patients with AN and 10 controls. HPLC was used for measurement of NE and DOPA. Plasma leptin was measured with high sensitive RIA. Aerobic exercise at 1,5 $W.kg^{-1}$ lean body mass was used for stimulation of SNS. Baseline and exercise-induced extracellular NE and DOPA levels were significantly higher compared to controls. Baseline and exercise-induced plasma leptin levels were significantly lower in patients with AN than in controls. The present results suggest that patients with AN have higher baseline and exercise-induced adipose tissue SNS activity, but lower plasma leptin levels. *Supported by Grant GACR 303/00/1555.*

Keywords: anorexia nervosa, microdialysis, exercise, norepinephrine, leptin

IMMOBILIZATION STRESS INDUCED INCREASE IN PLASMA CATECHOLAMINE LEVELS IS INHIBITED BY A PROLACTOLIBERIN (SALSOLINOL) ADMINISTRATION

BODNAR I.[1], MRAVEC B.[1], KUBOVCAKOVA L.[1], FEKETE M.I.K.[2], NAGY G.M.[2], KVETNANSKY R.[1] [1]*Institute of Experimental Endocrinology, SAS, Bratislava, Slovakia,* [2]*Neuroendocrine Research Laboratory, Semmelweis University, Budapest, Hungary*

Catecholamines (CA), prominent mediators of sympathoadrenal system, are involved in regulation of homeostasis of the organism especially during stress. Exposure to different stressors caused a huge increase in both plasma CA and prolactin (PRL) levels. We have recently found that a dopamine derived compound, salsolinol, produced by the neuro-intermediate lobe of the pituitary gland as well as by the hypothalamus, can selectively release PRL from the anterior lobe. Based on the similarity of CA and PRL response to stressors, we have investigated whether salsolinol plays also a role in the immobilization stress (IMO) induced CA release. We have performed a single one-hour IMO and measured the changes in plasma CA levels of male rats with salsolinol and saline treatment before and during exposure to IMO. We have found that salsolinol can inhibit IMO induced CA release without having any influence on corticosterone secretion. In summary, our results suggest that salsolinol is not only a releasing factor in stress-induced PRL release, but is also a potent inhibitor of CA release during IMO stress. *Supported by the EU Center of Excellence Support (ICA1-CT-2000-70008), VEGA 2/2090 and ETT 277/2001.*

Keywords: salsolinol, immobilization stress, catecholamines

ANGIOTENSIN II AT_1 RECEPTOR BLOCKADE PREVENTS GASTRIC ULCERS DURING COLD RESTRAINT STRESS

BREGONZIO C., ARMANDO I., ANDO H., JEZOVA M., BAIARDI G., SAAVEDRA J.M. *Section on Pharmacology, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA*

Stress reduces gastric blood flow and produces acute gastric ulcers. We studied the role of Angiotensin II (Ang II) on gastric blood flow and gastric ulceration during stress. Spontaneously hypertensive rats were pretreated for 14 days with the AT_1 receptor antagonist candesartan before cold-restraint stress. AT_1 blockade increased gastric blood flow 40-50%, prevented gastric ulcer formation by 70-80%, reduced the increase in adrenomedullary epinephrine and TH mRNA without preventing the stress-induced increase in adrenal corticosterone, decreased the stress-induced expression of necrosis factor α (TNF-α), adhesion protein ICAM-1 in arterial endothelium and neutrophil infiltration in the gastric mucosa, and decreased PGE_2 content. AT_1 receptor blockers prevent stress-induced ulcerations by a combination of gastric blood flow protection, decreased sympathoadrenal activation, anti-inflammatory effects with reduction in TNF-α, and ICAM-1 expression leading to reduced neutrophil infiltration, while maintaining the protective glucocorticoid effects and PGE_2 release. Ang II has a crucial role, through stimulation of AT_1 receptors, in the production and progression of stress-induced gastric injury, and AT_1 receptor antagonists could be of therapeutic benefit.

Keywords: stress ulcers, inflammation, angiotensin II receptors, AT_1 receptor antagonists

STRESS IS ASSOCIATED WITH INHIBITION OF THE TYPE I IODOTHYRONINE 5´-DEIODINASE ACTIVITY IN RAT LIVER

BRTKO J., MACEJOVA D., KNOPP J., KVETNANSKY R. *Institute of Experimental Endocrinology, SAS, Bratislava, Slovak Republic*

Type I iodothyronine deiodinase (5´-DI), one of three deiodinase isoenzymes, generates the thyromimetically active hormone 3,5,3´-triiodothyronine (T3) by reductive monodeiodination of the phenolic ring of L-thyroxine (T4). The present study was undertaken in order to compare the effects of cold stress (4 ^{0}C) alone or in combination with immobilization stress (IMO), insulin treatment (5 IU/kg, i.p.) or 2-deoxy-D-glucose (2DG)-induced intracellular glucopenia on the activity of 5´-DI in rat liver. Cold stress either for 24 h or 28 days when compared to non-stressed group of rats significantly reduced ($p < 0.001$) the activity of 5´-DI in liver. In comparison with cold stressed rats for 28 days, an additional decrease in 5´-DI activity was observed when those rats underwent 1 x IMO in cold for 2 h ($p < 0.001$) or insulin treatment ($p < 0.05$). Significant decrease in 5´-DI activity has been found also in rats maintained at room temperature that underwent a single 1 x IMO for 2 h ($p < 0.001$) or insulin treatment ($p < 0.01$) when compared to non-stressed animals. In comparison with non-stressed rats, no significant change of the 5´-DI activity was observed after 2DG application (500 mg/kg ip) at room temperature.

In conclusion, cold stress and/or 1 x IMO, insulin treatment or 1 x IMO at room temperature markedly affect reductive monodeiodination of T4, and thus reduce concentration of biologically active T3 in liver.

This work has been supported by the grants of VEGA No. 2/2070/22 and No. 2/2090/22 and the Centre of Excellence grant No. EC ICA1-CT-2000-70008.

Keywords: iodothyronine-5´-deiodinase activity, cold stress, immobilization, glucopenia, rat liver

STRESS-INDUCED SENSITIZATION TO STIMULATING EFFECTS OF D-AMPHETAMINE: CHANGES IN MRNA NMDA R1, R2A AND R2B IN RAT BRAIN

CANCELA L., PACCHIONI A., PEREZ M., BUSSOLINO D. *Dpto. Farmacologia, Fac. Cs. Quimicas, UNC, Cordoba, Argentina*

Previous studies from our laboratory have shown that acute and chronic repeated stress induces sensitization to the stimulating properties of psychostimulants. However, this does not seem to be the case when the influence of different restraint stress regimes is assessed on drug rewarding effects. The aim of the present work was to study the effects in levels of mRNA encoding N-methyl-D-aspartate (NMDA) receptor subunit NR1, NR2A and NR2B after acute and chronic restraint stress, inducing sensitization to effects of d-amphetamine (d-amph) in mesocorticolimbic areas. Wistar male rats (250–330 g) were submitted to zero, one or seven daily 2h-restraint sessions. Twenty-four hours later, locomotor activity was measured during 2 h following d-amph (0.5 mg/kg i.p.) and then, the gene expression of NMDA receptor subunits was measured by quantitative *in situ* hybridization. Compared to no stress groups, either saline or d-amph in acute stress groups produced an up-regulation in NR1 gene expression in all regions analyzed (PrL cortex, IL cortex, NAcc core and shell, CPu). In chronic stress groups, the increase following saline was reversed in most of the areas analyzed meanwhile that following d-amph it was maintained elevated in some areas (Nacc core, PrL cortex and CPu). In addition, in chronic stress groups an increase in NR2B/NR1 was observed following d-amph, when compared to that observed in acute stress groups. These results show that acute and chronic stress alters NR1, and NR2B gene expression in these brain regions, and that the changes depend upon the brain region examined and the type of restraint stress regime. Thus, the differential modulation of stress could depend on the limbic and motor areas, which are primarily affected by acute, or chronic stress exposure.

Keywords: stress, sensitization, d-amphetamine, NMDAR subunits, gene expression

DOES ORTHOSTATIC STRESS INFLUENCE NEUROENDOCRINE RESPONSE TO SUBSEQUENT HYPOGLYCEMIA IN HUMANS?

CERVENAKOVA Z., PENESOVA A., KOSKA J., KVETNANSKY R., JEZOVA D., HUCKOVA M., VIGAS M., MACHO L.
Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia

Neuroendocrine response to stress stimuli is influenced by previous stimuli of different nature. The aim of the study was to test whether antecedent orthostatic stress may affect neuroendocrine response to subsequent hypoglycemia. A group of 12 (6 men, 6 women) nonobese, healthy volunteers aged 19 – 27 years (mean 24 ± 0.8) participated in the study in two sessions: controlled insulin-induced hypoglycemia to 2.7 mmol/l for 15 min either with or without antecedent orthostatic stress (30 min of 60° head-up tilt before insulin administration). Orthostatic stress caused a significant decrease in plasma volume (-9.6%; $p<0.001$) and a significant increase in plasma renin activity, aldosterone, norepinephrine ($p<0.01$) and ACTH concentrations ($p<0.05$) in all subjects. Epinephrine response to hypoglycemia was diminished in women in comparison to men ($p<0.001$) but unaffected by antecedent orthostatic stress. Hypoglycemia failed to activate ACTH release after its response to orthostatic stress. ACTH response to hypoglycemia without previous orthostatic stress was evident only in men in contrast to women ($p<0.05$). Conclusions: Epinephrine and ACTH responses to hypoglycemia are diminished in women. Except ACTH, the neuroendocrine response to mild hypoglycemia is not affected by previous orthostatic stress in healthy subjects. In the case of ACTH, the first stress stimulus is consequential for the subsequent response of this hormone, probably due to negative feedback effects. *This study was supported by the grant of European Commission (ICA1-CT-2000-70008).*

Keywords: orthostatic stress, hypoglycemia, catecholamines, ACTH, humans

THE NEUROENDOCRINOLOGY OF THE HUMAN STRESS RESPONSE

CHROUSOS G.P. *PREB/NICHD, National Institutes of Health, Bethesda, MD, USA*

The main components of the stress system are the CRH and Locus Ceruleus-Norepinephrine (LC/NE)-Autonomic systems and their peripheral effectors, the pituitary-adrenal axis, and the limbs of the autonomic system. The CRH and LC/NE systems stimulate arousal and attention. CRH inhibits appetite and activates thermogenesis via the catecholaminergic system and plays an important role in inhibiting GnRH secretion during stress, while, via somatostatin, it also inhibits GH, TRH and TSH secretion. These functions depend on positive catecholaminergic input. Glucocorticoids simultaneously inhibit the CRH, LC/NE and β-endorphin systems and stimulate the mesocorticolimbic dopaminergic system. In addition, they directly inhibit pituitary gonadotropin, GH and TSH secretion. They also have direct as well as insulin-mediated effects on adipose tissue (insulin resistance, dyslipidemia and hypertension). Central CRH, via glucocorticoids and catecholamines, inhibits the inflammatory reaction, while directly secreted by peripheral nerves stimulates local inflammation. Antalarmin, a novel CRH receptor type 1 antagonist, decreases the activity of the HPA axis and suppresses neurogenic inflammation. Chronic administration of antalarmin is not associated with glucocorticoid deficiency. These data suggest that such antagonists may be useful in human pathologic states, such as melancholic depression and chronic anxiety, associated with chronic hyperactivity of the stress system, along with predictable behavioral, neuroendocrine, metabolic and immune changes, based on the interrelations outlined above. Conversely, it is needed potentiators of CRH secretion/action to treat atypical depression, postpartum depression and the fibromyalgia/chronic fatigue syndromes, all characterized by low HPA axis and LC/NE activity.

Keywords: stress, CRH, catecholamines, CRH antagonists, antalarmin

INTERACTION BETWEEN SUBSTANCE P AND SEROTONIN IN THE BRAIN: EFFECT OF STRESS

CULMAN J.[1], ZHAO Y.[1], TAUER U.[1], RUPNIAK N.M.J.[2] [1]*Institute of Pharmacology, Christian-Albrechts University of Kiel, Germany and* [2]*Merck Sharp Dohme Neuroscience Research Centre, Harlow, Essex, United Kingdom*

Interaction between the substance P (SP)- and serotonin (5-HT) systems in the brain participates in the initiation and regulation of central stress responses. 5-HT represents a very potent activator of the hypothalamo-pituitary-adrenal axis (HPA). In rats, acute immobilization (IMO) stress increased the turnover of 5-HT in the hypothalamus and the dorsal raphe nucleus, where 5-HT cell bodies are located. Stimulation of forebrain NK_1 (substance P) receptors induced by intracerebroventricular (i.c.v.) injection of SP activates the sympathoadrenal system and the release of oxytocin, but inhibits the release of ACTH. A SP-induced blockade in 5-HT release from serotonergic terminals in the paraventricular nucleus accounts for the inhibition of ACTH release. Interaction between the 5-HT and SP systems upon stress were also studied in NK1 receptor knock-out mice or after pharmacological blockade of central NK_1 receptors by the selective, non-peptide NK_1 receptor antagonist, RP 67580. Following acute IMO of mice for 15 min, 5-HT and 5-hydroxyindolacetic acid concentrations increased in the hypothalamus but decreased in the hippocampus in the brain stem. Only discrete changes in the accumulation of 5-HT synthesis precursor, 5-hydroxytryptophan, were detected. Neither genetic disruption of the NK_1 receptor nor its pharmacological blockade affected the metabolism of 5-HT in brain areas of mice exposed to acute IMO stress. Our results demonstrate that, 1) the serotoninergic system in the brain participates in the SP-induced inhibition of the HPA, and 2) genetic disruption or pharmacological blockade of the NK_1 receptor does not substantially affect 5-HT metabolism in the brain during acute stress.

Keywords: substance P, serotonin, adrenocorticotropin, brain, stress

EFFECT OF COLD EXPOSURE ON SERUM DBH AND BROWN ADIPOSE TISSUE MAO ACTIVITIES IN HYPOTHYROID T3 AND T4 TREATED RATS

CVIJIC G., PETROVIC N., DJORDJEVIC J., DAVIDOVIC V., PETROVIC V.M. *Faculty of Biology, University of Belgrade, Serbia and Montenegro*

In small mammals responding to cold stress, the increased activity of sympathetic nervous system (SNS) is accompanied with changes in the interscapular brown tissue (IBAT) thermogenic capacity. Under the same conditions thyroid hormones also affect IBAT termogenic capacity. As the indicator of the SNS function intensity the activity of serum dopamine-ß-hydroxylase (DBH) and IBAT monoamine oxidase (MAO) were examined in male Wistar rats chemically thyroidectomized by methimasole (HT), treated with replacement doses of T3 and T4, and exposed to cold (4°C-24h). Serum DBH and IBAT MAO activities of HT rats were higher as compared to controls irrespective of the ambient temperature (AT). If HT rats were treated with T3 and T4, serum DBH activity was decreased ($p<0.01$) in respect to those of HT regardless of AT but significantly increased in controls exposed to cold ($p<0.001$). In T3 and T4 HT animals IBAT MAO activity remained at the same high level as in the HT ones, regardless of AT. However, a 24 h cold exposure induced the significant increment of IBAT MAO activity in all the treated groups except in controls. In conclusion, IBAT MAO and serum DBH activities respond to cold depending on both SNS and thyroid status.

Keywords: cold, thyroid, IBAT, DBH, MAO

TWO FEEDBACK EFFECTS OF CORTICOSTERONE ON BRAIN: DIRECT AND INDIRECT

DALLMAN M.F., AKANA S.F., LA FLEUR S.E., PECORARO N., HOUSHYAR H., GOMEZ F., MANALO S. *Department of Physiology, University of California of San Francisco, San Francisco, CA, USA*

Acutely, glucocorticoids act to inhibit stress-induced CRF and ACTH secretion, and they do this through their actions in brain and anterior pituitary (canonical feedback). However, with chronic stress, glucocorticoid feedback inhibition of ACTH secretion changes markedly. Chronically stressed rats characteristically exhibit facilitated ACTH responses to acute, novel stressors. Moreover, studies in adrenalectomized rats replaced with corticosterone showed that corticosterone concentrations in the high circadian range (~120-160 ng/ml) are required to exhibit facilitation of ACTH responses after chronic stress. Infusion of corticosterone intracerebroventricularly into adrenalectomized rats increases basal ACTH and tends to increase CRF, and also allows facilitation of ACTH responses to repeated restraint. Therefore, with chronic stressors, corticosterone appears to act in brain in an excitatory, rather than inhibitory fashion. We believe that under conditions of chronic stress, there is still an indirect glucocorticoid feedback that is mediated through the effects of the steroid on metabolism. Increased energy stores feedback on brain to inhibit hypothalamic CRF and decrease the expression of dopamine-β-hydroxylase in the locus coeruleus. These changes would be expected to decrease the level of discomfort and anxiety induced by chronic stress. Moreover, central neural actions of glucocorticoids abet the peripheral effects of the steroids by increasing the salience and ingestion of pleasurable foods.

Keywords: glucocorticoids, feedback, chronic stress, comfort food

CHANGES IN ACTIVITY OF NEUROTRANSMITTER SYSTEMS IN PATIENTS WITH WOUNDS TO THE HEART IN EARLY POSTOPERATIVE PERIOD

DAVYDOVA N.A, NIKITINA O.V., DORFMAN A.G. *SCESMP MH RF, SRC EH in the honour of N.V. Sklifasofsky, Moscow, Russia*

The activity of sympatho-adrenal, serotonin-, histamine- and cholinergic systems in 69 patients with stab and cut wounds to the heart that can be divided into 3 groups (1st wounds to the right ventricle of heart, 2nd wounds to the left ventricle of heart, and 3rd isolated wounds to pericardium) was studied for 3 postoperative days. The patients were also assigned to different groups, depending on haemorrhage: 500 ml, 1000 ml, 1500 ml and 2000 ml. On the first postoperative day the activity of serotonin- and cholinergic systems were found increased, as well as the hormonal activity of the sympatho-adrenal system. The mediator activity of the sympatho-adrenal system was decreased, with further increase at later stages. These changes in the activity of sympatho-adrenal system influenced the hemodynamics differently depending on the wound localization. In blood loss of more than 1500 ml, alongside with the increase in the hormonal activity of the sympatho-adrenal system, its mediator activity increased as well. This increase took place in parallel with the decrease of blood serotonin level and cholinergic system activation. At the third postoperative day the activity of neurotransmitter system was not normalized. The study results may be useful to optimise the intensive care.

Keywords: heart, haemorrhage, ventricle, pericardium, wounds

DIFFERENTIAL EFFECTS OF GLUCOCORTICOIDS ON THE DOPAMINERGIC REWARD SYSTEM IN TWO GENETICALLY DISTINCT MOUSE STRAINS

DE JONG I.E.M., OITZL M.S., DE KLOET E.R. *Div.of Medical Pharmacology, Leiden/Amsterdam Center for Drug Research, Einsteinweg 55, P.O. Box 9502, 2300 RA Leiden, The Netherlands*

Corticosteroid hormones secreted during stress act on brain dopamine and modulate vulnerability to drugs. This study investigates the role of glucocorticoids in interaction with genetic background for the sensitivity of mice to cocaine. Glucocorticoid levels are modulated by 1) exposing mice to rats, a stressor that alters reactivity of the hypothalamo-pituitary-adrenal (HPA) axis and thus corticosteroid production, and 2) surgical manipulation of the HPA axis. Genetic differences are examined by using two mouse strains (C57Bl/6 and DBA2) that display a natural difference in vulnerability to drugs. Our studies have revealed differential sensitivity of these strains to the locomotor-enhancing effects of cocaine. Rat stress consists of intermittent exposure to rats during two weeks. The surgical approach involves removal of the adrenals, primary source of corticosteroids. Drug response is quantified by measuring the locomotor response to cocaine and sensitisation after repeated drug exposure. It was shown that modulation of the HPA axis differentially affects drug response in C57Bl/6 and DBA2 mice. These results indicate that environmental factors interact with genetic background to determine individual vulnerability to drugs of abuse. *Supported by NWO-INSERM 985-10-014.*

Keywords: corticosteroids, dopamine, genes, drugs, cocaine

HORMONES AND THE STRESSED BRAIN

DE KLOET E.R. *Department of Medical Pharmacology, Leiden University, The Netherlands*

The stress system orchestrates body and brain responses to the environment in two modes of operation. The immediate response mode driven by corticotropin releasing hormone (CRH) organises via CRH-1 receptors the behavioural, sympathetic and hypothalamic-pituitary-adrenal (HPA) responses to a stressor. In the other - slower - mode urocortin-mediated CRH-2 receptor activation facilitates behavioural adaptation. Corticosteroid hormones secreted by the adrenal cortex are implicated in both modes through their high affinity type 1 (MR) and lower affinity type 2 (GR) receptors. MR controls the threshold or sensitivity of the fast CRH-1 driven stress system important for maintenance of homeostasis, while GR facilitates its recovery. GR also promotes storage of energy resources and storage of information to prepare for future events. The balance in the two stress system modes is thought to be essential for cell homeostasis, mental performance and health. Imbalance induced by gene modification or exposure to a stressor changes specific neural signalling pathways underlying psychic domains of cognition and emotion, anxiety and aggression. This Yin - Yang stress concept may be helpful in experiments designed to understand the mechanistic underpinning of cortisol-induced stress-related disease such as i.e. severe forms of depression.

Keywords: stress, behaviour, corticosteroids, brain, genes

DIFFERENT EFFECT OF NOVEL STRESSORS ON SYMPATHOADRENAL SYSTEM ACTIVATION IN RATS EXPOSED TO LONG-TERM IMMOBILIZATION

DRONJAK S.[1], JEZOVA D.[2], KVETNANSKY R.[2] [1]*Institute of ''Vinca'', Belgrade, Serbia and Montenegro and* [2]*Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic*

Activation of the sympathoadrenal system, evaluated by plasma levels of adrenaline (A) and noradrenaline (NA), after the exposure of rats to various stressors is well documented. Response of rats exposed long-term to homotypic stressor and then exposed once to heterotypic novel stressors is, however, poorly understood. In the present study we examined changes in plasma levels of catecholamines (CA) and corticosterone (COTR) after a single (2 h) and long-term repeated immobilization (41 times, 2 h daily), as well as in rats adapted to long-term immobilization exposed once to novel stressors: cold, insulin or 2DG administration. Long-term immobilized rats exposed to insulin or 2DG showed significant elevation of plasma CA and CORT levels in comparison to the administration to control rats. Exposure of long-term immobilized and control rats to cold stress increased plasma NA and CORT, whereas plasma A was not significantly changed. The exposure of long-term immobilized rats to a further single immobilization (2 h) still increased plasma CA levels, but in naive control rats, the single immobilization produced more pronounced increases. These data suggest that rats exposed to homotypic long-term immobilization are able to respond to heterotypic stressors by higher activation of the sympathoadrenal system, as compared to the control, previously unstressed rats. Reduced plasma CA and CORT levels in long-term immobilized rats exposed to homotypic stressor are most probably due to an adaptation at the level of brain regulatory centers. *Supported by ICA1-CT-2000-70008, VEGA 2/2090 and Slovak US grant 002/2001.*

Keywords: catecholamines, corticosterone, immobilization, novel stressors

EFFECT OF CHRONIC EMOTIONAL STRESS ON HABITUATION PROCESSES IN OPEN FIELD IN ADULT RATS

DUBOVICKY M.[1], JEZOVA D.[2] [1]*Institute of Experimental Pharmacology,* [2]*Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia*

In our previous study, repeated stress in neonatal period has been found to slow down habituation in open field in adult rats (1). Objective of the present study was to investigate how chronic stress can affect habituation processes in the open field when occurring in the adulthood. Animals were exposed to one week immobilization on metal boards followed by one week hypokinesis. After termination of the stress procedure rats were tested in the open field (once daily in six-min sessions for four consecutive days). Immediately after the last open field test, animals were decapitated. Rapidity of between- as well as within-session habituation was found to be lower. However, this lowering failed to be statistically significant compared to controls. On the other hand, time latency to step down from an elevated platform was significantly increased in stress-exposed animals. Four days after the last stressful event CRH mRNA level in the paraventricular nucleus were significantly increased indicating a long-term activation of the HPA axis. The results suggest that in contrast to neonatal stress exposure, chronic emotional stress in adult rats does not represent a risk factor for alteration of habituation processes. *This study was supported by grants of VEGA 2/2053, 2/2007 and by EC ICA1-CT-2000-70008.*

1. Dubovicky et al., Pharmacol. Biochem. Behav. 64, 681-686, 1999.

Keywords: emotional stress, habituation, CRH, anxiety, rat

ANXIETY IN RELATION TO BEHAVIOURAL AND NEUROENDOCRINE CHANGES DURING MENTAL STRESS IN HEALTHY HUMANS

DUNCKO R.[1], MAKATSORI A.[1], MONCEK F.[1], LODER I.[3], JAKUBEK M.[4], FICKOVA E.[2], JEZOVA D.[1] [1]*Inst. Exp. Endocrinology,* [2]*Inst. Exp. Psychology, SAS, Bratislava, Slovakia,* [3]*Inst. Adaptive and Spaceflight Physiol., Graz, Austria,* [4]*Dep. Psychol., Faculty of Philosophy, Comenius Univ., Bratislava, Slovakia*

Anxiety is an emotional state believed to be related to stress response. However, only limited information is available on the links between anxiosity as a trait and neuroendocrine aspects of stress. In the present study the stress response was investigated in healthy male volunteers using a modified version of the Trier social stress test (TSST). The volunteers were selected according to their performance in the State and Trait Anxiety Inventory (STAI). Only subjects with anxiosity score over 45 and below 36 were included. The exposure to the TSST was associated with significant cardiovascular and neuroendocrine activation. The stress related rise in concentrations of ACTH, cortisol, prolactin, adrenaline and noradrenaline in plasma was significantly lower in subjects with high anxiosity in comparison to that in non-anxious subjects. Stress exposure was associated with a rise in skin conductance responses, which was less pronounced in anxious subjects. On the other hand, the stress-induced rise in heart rate was significantly higher in anxious subjects. As to the psychological parameters, high anxiosity was associated with low hardiness, low expression of problem oriented and high expression of emotion oriented stress coping strategies. The present data indicate, that high anxiosity is associated with blunted neuroendocrine activation during psychosocial stress. *The study was supported by grants of EC ICA1-CT-2000-70008 and VEGA 2/2007.*

Keywords: mental stress, HPA axis, catecholamines, anxiety, humans

BRAIN CIRCUITS INVOLVED IN CRH AND NORADRENALINE INTERACTIONS IN STRESS

DUNN A.J., SWIERGIEL A.H., PALAMARCHOUK V. *Department of Pharmacology and Therapeutics, Louisiana State University Health Sciences Center, Shreveport, Louisiana, USA*

Both CRF- and noradrenaline (NA)-containing neurons in the brain are activated during stress, and both have been implicated in behavioural responses. NA neurons in the brain stem can stimulate CRF neurons in the hypothalamic paraventricular nucleus (PVN) to activate the HPA axis, and may also act on other CRF neurons. PVN CRF neurons project to the area of the locus coeruleus (LC) and CRF injected into the LC affects the electrophysiological activity of LC-NA neurons. These reciprocal interactions may create a "feed forward loop". It has been postulated that such a loop may be sensitized in clinical depression. Neurochemical studies indicate that CRF applied *icv* or locally activates the LC-NA system, and microdialysis and chronoamperometric measurements indicate increased NA release. However, chronoamperometry indicates a significant delay in NA release, suggesting that the CRF-NA connections are indirect. Behavioral studies in the multicompartment chamber and defensive withdrawal suggest that CRF acts via β1-adrenergic receptors. On the other hand, α1-adrenergic agonists may act via CRF. Unfortunately, the locations of none of the relevant receptors are known. Thus studies employing local injections of agonists and antagonists may reveal the details of the circuitry.

Keywords: corticotropin-releasing factor, noradrenaline, locus coeruleus, chronoamperometry

LEAKY CATECHOLAMINE STORES: UNDUE WASTE OR A STRESS RESPONSE COPING MECHANISM?

EISENHOFER G., GOLDSTEIN D.S., KOPIN I.J. *National Institutes of Health, Bethesda, Maryland, USA*

Turnover of catecholamines, representing the constant loss and replenishment of neurotransmitter by synthesis, is usually considered to be driven exclusively by catecholamine release. This is incorrect. An important contribution of intraneuronal metabolism of norepinephrine (NE) to turnover, and dependence of this on leakage of NE from vesicular storage granules, was originally proposed by Kopin in 1964. Several years later Maas and colleagues concluded that at least 75% of NE turnover in man is due to intraneuronal metabolism without prior release at sympathetic nerve endings. More recently it was shown in the resting human heart that 18% of NE turnover is due to extraneuronal uptake and metabolism or loss of the transmitter to the circulation, 12% is due to intraneuronal metabolism after reuptake, and 70% is due to intraneuronal metabolism of NE leaking from storage vesicles. Thus, contrary to usual depictions, vesicular stores of catecholamines do not exist in a static state simply waiting for a signal for exocytotic release. Rather, these stores exist in a highly dynamic equilibrium with the surrounding cytoplasm, with passive outward leakage of amines counterbalanced by inward active transport under the control of vesicular monoamine transporters. The large contribution of leakage to catecholamine turnover may seem inconsistent with cellular economy. In fact, this contribution provides an important mechanism for "gearing down" the requirement for increases in catecholamine synthesis to match increases in catecholamine release, and thereby provides sympathetic nerves with a capacity for a more extended range of sustainable release rates than would otherwise be possible.

Keywords: catecholamine turnover, metabolism, release, synthesis, vesicular leakage

THE SYMPATHETIC-IMMUNE INTERFACE: FUNDAMENTAL AND CLINICAL ASPECTS

ELENKOV I.J., KVETNANSKY R.[1], HASHIRAMOTO A., BAKALOV V.K., ROVENSKY J.[1], JEZOVA D.[1], FLEISHER T., GOLDSTEIN D., WILDER R.L., CHROUSOS G.P., GOLD P.W. *National Institute of Mental Health, NIH, Bethesda, USA and* [1]*Institute of Experimental Endocrinology, SAS, Bratislava, Slovak Republic*

Norepinephrine, ATP and adenosine, released in the vicinity of immunocompetent cells and the adrenomedullary hormone epinephrine affect lymphocyte traffic, circulation and the activity of lymphoid cells. They inhibit the production of interleukin (IL-12), tumor necrosis factor TNF-α and interferon (IFN)-γ, whereas they stimulate the production of IL-10 and transforming growth factor (TGF)-β. Thus, systemically, sympathetic neurotransmitters may induce a T helper (Th) 2 shift, whereas neuropeptide Y may further amplify this effect. In certain local responses, however, catecholamines may boost, transiently, innate immunity through induction of IL-1, IL-6, IL-8 and TNF-a production, while ATP via the P2X7 receptor may activate the post-translational processing of IL-1β and IL-18. In contrast to autoimmune-resistant F344 rat, the highly autoimmune-prone LEW rat has pronounced hypo-responsiveness of the sympathetic-adrenomedullary system to different stressors. Our data also indicate that two opposite neuroendocrine-innate immune phenotypes exist in the human population that are analogous to that observed in LEW and F344 inbred rat strains. These phenomena could be associated with the susceptibility of the organism to certain immune-mediated common human diseases and/or they may participate in the pathogenesis of autoimmunity and atherosclerosis.

Keywords: sympathetic nervous system, cytokines, Th1 cells, Th2 cells, inflammation

CARDIAC SYMPATHETIC NERVE BIOLOGY IN PANIC DISORDER

ESLER M.D. *Baker Heart Research Institute, Melbourne, Australia*

Panic disorder serves as a clinical model for testing whether mental stress can cause heart disease. Our own cardiological management of panic disorder provides case material of triggered arrhythmias (atrial fibrillation, ventricular tachycardia), recurrent emergency room attendances with angina and electrocardiogram ischemia, and documented coronary artery spasm, in some cases complicated by coronary thrombosis. We have used internal jugular venous sampling and measurement of overflowing lipophilic brain monoamine metabolites to quantify brain noradrenaline and serotonin turnover in untreated patients. We find a marked increase in brain serotonin turnover, but normal noradrenaline turnover. Application of radiotracer catecholamine kinetics and clinical microneurography methodology suggests there is a genetic predisposition to panic disorder, which involves faulty neuronal noradrenaline uptake, possibly sensitising the heart to symptom generation. Sympathetic activation importantly mediates cardiac risk. There are huge sympathetic bursts in the MSNA neurogram and large increases in cardiac noradrenaline spillover during panic attacks, accompanied by surges of adrenal medullary adrenaline secretion. There is continuous adrenaline cotransmission in cardiac sympathetic nerves of panic sufferers, perhaps attributable to adrenaline loading by uptake from plasma during panic attacks. The sympathetic cotransmitter, neuropeptide Y (NPY), is released from the cardiac sympathetics during panic attacks, an intriguing finding given that NPY can cause coronary artery spasm.

Keywords: mental stress, heart risk, neuropeptide Y, sympathetic cotransmission

GLUCOCORTICOID RECEPTOR (GR) AND BETA-ADRENOCEPTORS (β-ARS) IN EPIDIDYMAL ADIPOSE TISSUE FROM STRESSED RATS

FARIAS-SILVA E., SANTOS I.N., AMARAL M.E., GRASSI-KASSISSE D.M., SPADARI-BRATFISCH R.C. *Department of Physiology and Biophysics, Institute of Biology, State University of Campinas. Sao Paulo, Brazil*

We have demonstrated that adipocytes isolated from epididimal adipose tissue (EAT) of foot shock stressed rats were supersensitive to isoprenaline and subsensitive to norepinephrine, and suggested that these alterations were dependent on stress induced increase in plasma corticosterone levels. Aim: To test if in EAT from foot shock stressed rats the GR and β-ARs protein expression were altered. Methods: EAT were isolated from control (CO) or stressed (ST) male Wistar rats. Rats of stress group were submitted to one daily foot shock sessions (30 min duration, 120 pulses, 1.0 mA, 1.0 s, random intervals of 5-25 s) for three days. EAT was isolated and homogenized for Western blotting of GR and β-ARs. Results: Foot shock stress decreased GR, β_1-AR and β_3-AR protein levels, but increased β_2-AR. Conclusions: Our results confirm that the supersensitivity to isoprenaline and subsensitive to norepinephrine previously reported are associated with an increase of β_2-AR and a decrease of β_1-AR and β_3-AR. These alterations on proteins expression are modulated by high corticosterone levels, which did also down-regulate its own receptor. *Financial Support: FAPESP.*

Keywords: β-adrenoceptors, foot shock stress, glucocorticoid receptor, adipose tissue, rats

MECHANISMS UNDERLYING GASTROPROTECTIVE ACTION OF GLUCOCORTICOIDS RELEASED IN RESPONSE TO ULCEROGENIC STRESS FACTORS

FILARETOVA L.P., BAGAEVA T.R., PODVIGINA T.T., TANAKA A., TAKEUCHI K. *Pavlov Institute of Physiology, Russian Academy of Sciences, St-Petersburg, Russia and Kyoto Pharmaceutical University, Kyoto, Japan*

Our previous results demonstrate gastroprotective but not ulcerogenic action of glucocorticoids released in response to ulcerogenic stress factors. In the present study the possible mechanisms underlying the gastroprotective action of glucocorticoids were investigated. The effects of the deficiency of glucocorticoid production followed by corticosterone replacement on the gastric mucosal blood flow, microvascular permeability, hypermotility, gastric secretion (acid, pepsin and mucus), blood glucose levels as well as gastric lesions were studied 3-4 h after the onset of ulcerogenic stimuli. The contribution of glucocorticoids in the healing process of gastric lesions was also estimated. The deficiency in glucocorticoid production significantly potentiated the functional disorders induced by ulcerogenic stimuli: a decrease in blood glucose level, in gastric mucosal blood flow, in mucus production and an increase in gastric motility and in microvascular permeability; and as result aggravated the formation of gastric lesions and then delayed their healing. The changes observed were prevented by supplementation of corticosterone at a dose mimicking a stress-induced corticosterone rise, whereas the preventive effect of corticosterone was attenuated by RU-38486, a glucocorticoid receptor antagonist. We conclude that gastroprotective action of glucocorticoids may be provided by their beneficial influence on glucose homeostasis and healing processes, attenuate effect on enhanced gastric motility and microvascular permeability as well as maintaining the gastric mucosal blood flow and the mucus production.

Keywords: stress, glucocorticoids, gastric lesion, gastroprotection, prostaglandins

AGE-DEPENDENT ALTERATIONS IN EXPRESSION OF DRUG METABOLIZING ENZYMES IN THE MOUSE LIVER DURING STRESS

FILIPENKO M.L., MIKHAILOVA O.N., GULYAEVA L.F. *Novosibirsk Institute of Bioorganic Chemistry, Novosibirsk, Russia*

The role of stress in the regulation of enzymatic systems involved in the biotransformation of xenobiotics, as well as endogenous substrates in the liver was investigated using two-hour immobilization stress as a model. Adult (3 months of age) and aged (26 months) C3H/a male mice were used. Cytochrome P450 1a1 and 1a2 (Cyp1a1 and Cyp1a2), Glutathione S-transferase M1 (GSTM1), Aryl Hydrocarbon Receptor (AHR), Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT) and Catechol-*O*-methyltransferase (COMT) mRNA levels in the mouse liver were measured by a semi-quantitative RT-PCR method. Experiments revealed the effect of immobilization stress on the expression of Cyp1a2, AHR, GSTM1 and COMT genes in adult animals. No influence of stress on Cyp1a1 and ARNT was detected, perhaps due to very low constitutive mRNA levels. Stress increased the level of ARNT mRNA expression in aged animals. As far as all the other genes studied are concerned, our results clearly demonstrate the attenuation, or even the lack of response, to the stress in aged animals. This fact may play an important role in age-related pathology and disease. It is apparent that the biological mechanisms underlying effect of stress is a complex subject, which needs to be further elucidated. In conclusion, age-associate changes were revealed in the enzyme systems that are vital to metabolism by the body. Stress modulated these systems significantly, but stress response was mostly absent during ageing. Our experimental approach could provide a framework for understanding and analysis of drug metabolizing enzyme regulation in the liver under the influence of environmental factors and physiology.

Keywords: aging, immobilization stress, liver, catechol-O-methyltransferase

MAPPING OF QUANTITATIVE TRAIT LOCI THAT INFLUENCE PLASMA CATECHOLAMINE RESPONSE TO STRESS

GASPERIKOVA D.[1], WESTON K.[2], KOVACS P.[3], KVETNANSKY R.[1], DIXON R.[2], THOMSON J.R.[4], SEBOKOVA E.[1], SAMANI N.J.[2], KLIMES I.[1] [1]*Institute of Experimental Endocrinology, SAS, Bratislava, Slovakia,* [2]*Division of Cardiology, Department of Medicine, University of Leicester, UK,* [3]*Phoenix Epidemiology and Clinical Research Branch, NIDDK, NIH, Phoenix, AZ, USA*

Stress is one of the major risk factors for cardiovascular diseases. At least a part of its detrimental effects is mediated via activation of the sympathoadrenal system (SAS). There is variability in the SAS response to stress although the extent to which this is genetically regulated is unclear. Some rodent models, including the hereditary hypertriglyceridemic (hHTg) rat, are hyperresponsive to stress. We investigated whether quantitative trait loci (QTL) that affect the plasma catecholamine response to stress in the hHTg rat could be identified. 189 F2 rats derived from a hHTg x Brown Norway cross were phenotyped for plasma noradrenaline and adrenaline levels in resting state and in response to an immobilization stress. Responses were assessed early (20 min) and late (120 min) after the application of the stressor. A genome-scan was conducted using 153 microsatellite markers. Two QTLs (peak lod scores of 4.17 and 3.52 respectively) influencing both the early and late plasma NA response to stress were found on chromosome 10. Interestingly, the QTLs had no effect on plasma adrenaline response to the stressor. These findings provide the first evidence for a genetic determination of a specific sympathetic nervous system response to stress.

Keywords: catecholamine, stress, quantitative trait loci, hereditary hypertriglyceridemic rats

OXIDATIVE STRESS TO DOPAMINERGIC NEURONS AS MODELS OF PARKINSON´S DISEASE

GILLE G.[1], HUNG S.-T.[2], REICHMANN H.[1], RAUSCH W.-D.[2] [1]*Department of Neurology, Technical University, Dresden, Germany;* [2]*Institute of Medicinal Chemistry, Veterinary University, Vienna, Austria*

Objective: Neuroprotective agents to dopaminergic neurons exposed to oxidative stress. *Methods:* Primary dopaminergic cultures were treated with MPP^+, L-DOPA or rotenone together with dopamine agonists and coenzyme Q_{10}. Cell viability, dopamine uptake, and lactate were determined. Striatal slice cultures of adult mice were subjected to MPP^+ treatment and coenzmye Q_{10}. *Results:* The number of dopaminergic neurons in primary culture is markedly reduced by treatment with MPP^+, rotenone and, to a lesser extent, by L-DOPA. Clinically relevant dopamine receptor agonists (D1, D2 and D3) improved the survival of dopaminergic neurons (protection ranging between 20-50%). In striatal cultures, coenzyme Q_{10} protected tyrosine hydroxylase, respiratory chain complexes and hexokinase from loss in activity after MPP^+-induced degeneration up to 100%. *Conclusion:* Though clinical effectivity is still under debate, cellular models contribute to an understanding of neuroprotection.

Keywords: dopaminergic system, agonists, neuroprotection

FUNCTIONAL NEUROIMAGING OF SYMPATHETIC INNERVATION OF THE HEART

GOLDSTEIN D.S. *Clinical Neurocardiology Section, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland, USA*

Many concepts about acute and chronic effects of stress depend on alterations in sympathetic nerves supplying the heart. Physiologic, pharmacologic, and neurochemical approaches have been used to evaluate cardiac sympathetic function. This lecture describes a fourth approach, based on nuclear scanning to visualize cardiac sympathetic innervation and function, and relationships between the neuroimaging findings and those from other approaches. Multiple system atrophy with orthostatic hypotension (formerly the Shy-Drager syndrome) features normal cardiac sympathetic innervation and normal entry of norepinephrine into the coronary sinus (cardiac norepinephrine spillover), in marked contrast to Parkinson's disease with orthostatic hypotension, which seems invariably to feature neuroimaging and neurochemical evidence for loss of cardiac sympathetic nerves. This striking difference may have important implications for understanding the etiology of Parkinson's disease. By analysis of curves relating myocardial radioactivity with time (time-activity curves) after injection of a sympathoneural imaging agent, it is possible also to obtain information about cardiac sympathetic function. Abnormal time-activity curves are seen in common disorders such as heart failure and diabetic neuropathy and provide an independent, adverse prognostic index. Analogous abnormalities might help explain increased cardiovascular risk in psychiatric disorders such as melancholic depression.

Keywords: sympathoadrenal imaging, heart, cardiac sympathetic function, NE spillover

CHANGES IN GENE EXPRESSION OF THE PHENYLETHANOLAMINE N-METHYLTRANSFERASE IN TRANSPLANTED HUMAN HEART

GONCALVESOVA E.[1], MICUTKOVA L.[2], MRAVEC B.[2], KSINANTOVA L.[2], KRIZANOVA O.[2], FABIAN J.[1], KVETNANSKY R.[2]
[1]*Slovak Institute of Cardiovascular Diseases, Bratislava, Slovakia and* [2]*Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia*

Heart transplantation (HTx) is an accepted treatment for precisely defined patients with chronic congestive heart failure. As a result of procedure, the graft is completely denervated. In our study we focused on the catecholamine pathway, i.e. production of adrenaline, which is known to have positive chronotropic and inotropic effect on the heart. In 14 patients (0–10 years after HTx) mRNA levels of the phenylethanolamine N-methyltransferase (PNMT), the enzyme catalyzing adrenaline synthesis in myocardial tissue, were determined. Samples of myocardium were obtained from the right ventricle at regular routine endomyocardial biopsy performed for diagnosis of the graft rejection. Results were correlated with selected clinical parameters – heart rate, heart rate variability, blood pressure, graft systolic function, and presence of the rejection. We observed that the PNMT mRNA was significantly higher during the first two years as compared to further period after HTx. Also, decrease of the average heart rate and increase of the heart rate variability was documented. Levels of the PNMT mRNA seem not to correlate with blood pressure, left ventricular systolic function at rest, and rejection. Thus, gradual decrease of the heart rate and an increase in the heart rate variability after HTx is considered as a sign of cardiac graft reinervation. We can speculate that PNMT transcription in myocardium after HTx reflects autonomous sympathicotrophy. Decrease in the PNMT gene expression with years after HTx could be a consequence of the reinervation.

Keywords: human heart, transplantation, PNMT

AUTONOMIC NERVOUS SYSTEM AND RESISTANCE OF CARDIOVASCULAR FUNCTION UNDER EMOTIONAL STRESS: EFFECT OF CHEMICAL FACTORS

GORBUNOVA A.V. *P.K.Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences, Moscow, Russia*

The aim of the present study was to evaluate individual adaptation reaction (by biogenic amines content and their metabolic enzymes, proteins and RNA content) of the nodose ganglion of the vagus nerve, the stellate, superior cervical ganglion and brain nuclei involved in central mechanisms of maintaining arterial pressure under emotional stress of different duration. Emotional stress was induced in immobilized rabbits by simultaneous aperiodic stimulation of the hypothalamus and skin. Also August and Wistar rats with different behavioral characteristics in "open field" test were immobilized during 38 and 48 hours with the rest. Present data showed that metabolic activities of the stellate and superior cervical sympathetic ganglia were correlated with the regulation of arterial blood pressure during the stressful procedure. Changes in the nodose ganglion were found in rabbits that did not maintain blood pressure. It has been revealed that the biogenic amines metabolism level in the brain nuclei supports the stability of cardio-vascular function. Effect of angiotensin-II, neurotropin, delta-sleep-inducing peptide on resistance of animals to emotional stress realized through biogenic amines metabolism. Resistance was connected with increasing of activity of stress-realizing noradrenergic system in a number of brain structures don't accompany exhaustion of neurons; in turn the activity of the stress-limiting dopaminergic, serotoninergic, adrenergic systems could have modulatory effects on the noradrenergic system.

Key words: brain, biogenic amines, arterial blood pressure, emotional stress

CHANGES IN THE CONCENTRATIONS OF TETRAHYDROBIOPTERIN, A COFACTOR OF TYROSINE HYDROXYLASE, IN BLOOD UNDER PHYSICAL STRESS AND IN DEPRESSION

HASHIMOTO R.[1], NAGATSU T.[2], OHTA T.[3], MIZUTANI M.[3], OMURA I.[4] [1]*Aichi Medical University, Aichi,* [2]*Fujita Health University, Aichi,* [3]*Nagoya University, Nagoya,* [4]*Nagoya City University, Nagoya, Japan*

Tetrahydrobiopterin (BH4) is an essential cofactor of pteridine-dependent monooxygenases, and is rich in sympathetic nerves and in the adrenal medulla as the cofactor of tyrosine hydroxylase (TH) for biosynthesis of noradrenaline and adrenaline. We investigated whether BH4 is also released into blood with noradrenaline and adrenaline under physical stress in humans. Sixty normal volunteers (thirty (15-29 years-old) young subjects; thirty (40-59 years-old) middle-aged subjects) were subjected to physical exercise by an ergometer. Total biopterin (B) and BH4 concentrations in plasma were measured by HPLC/fluorometry. The total B and BH4 concentrations in plasma before exercise were similar between the young and middle-aged groups. After severe exercise total B and BH4 concentrations were increased in both young and middle-aged groups up to about 150% with similar ratio of the concentration of BH4 to that of total B before and after exercise; approximately 0.75. There was a tendency that the increased total B and BH4 concentrations continued longer in the middle-aged group than those in the young group. These results are in contrast to our previous report that patients with monopolar or dipolar depression had higher total B concentration and lower ratio of the concentration of BH4 to that of total B than normal controls. BH4 may be increasedly released from the sympathetic nerves under severe physical stress and in depression, but not only the rate of release but also the oxidation rate of BH4 to B may be increased in depression.

Keywords: tetrahydrobiopterin, blood, physical stress, normal volunteers, depression

DISSOCIATION OF PSYCHOLOGICAL, ADRENOCORTICAL, AND ADRENOMEDULLARY REACTIVITY TO STRESS

HELLHAMMER D.H., FRIES E., WUEST S., BUSKE-KIRSCHBAUM A., SCHOMMER N. *Department of Psychobiology, Trier University, Trier, Germany*

We commonly believe that the physiological stress response is determined by cognitive, emotional, and coping processes. Lacey (1967), however, already addressed the ”co-variation problem”, referring to missing co-variation of the psychological and autonomic stress response. In a re-analysis of many studies from this laboratory, we could confirm his findings for the Trier Social Stress Test (TSST). We could further extend these findings to different endocrine and immune measures, all suggesting a missing co-variation of the psychological and physiological stress response. Clearly, subjective psychological state measures are unrelated to the bodily response to stress. However, genetic and psychological trait measures may predict differences in physiological stress reactivity. Some co-variation among physiological stress measures was observed. Repeated exposure to the TSST resulted in a quick habituation of adrenocortical reactivity, while the adrenomedullary stress response remained unchanged. We also demonstrate evidence that different patterns of the adrenocortical stress response exist in patients with stress related disorders. The clinical relevance of these finding will be discussed.

Keywords: co-variation, stress response, adrenal cortex, adrenal medulla

LIMBIC-NEUROENDOCRINE INTEGRATION AND THE STRESS RESPONSE

HERMAN J.P., MUELLER N.K., CHOI D.C., FIGUEIREDO H.F. *University of Cincinnati, College of Medicine, Cincinnati, OH, USA*

GABA and glutamate play a major role in central integration of hypothalamo-pituitary-adrenocortical (HPA) stress responses. Recent work in our group has focused on mechanisms whereby GABAergic and glutamatergic circuits interact with parvocellular paraventricular nucleus neurons controlling the HPA axis. GABAergic neurons in the bed nucleus of the stria terminalis, preoptic area and hypothalamus can directly inhibit paraventricular nucleus outflow and thereby reduce ACTH secretion. These local neurons are in turn controlled by descending information from limbic forebrain structures, including glutamatergic neurons of the ventral subiculum and prefrontal cortex and GABAergic cells from the amygdala. Lesion studies indicate that the ventral subiculum and prefrontal cortex are involved in inhibition of HPA axis responses to psychogenic stimuli, whereas the amygdala is positioned to enhance hormone secretion by way of GABA-GABA disinhibitory connections. Thus, it appears the psychogenic responses to stress are gated by discrete sets of GABAergic neurons in the basal forebrain and hypothalamus. As such, these neurons are positioned to summate limbic inputs into net inhibitory tone on the PVN, and may thus play a major role in HPA dysfunction seen in affective disease states and aging.

Keywords: paraventricular nucleus, HPA axis, glucocorticoids, GABA, glutamate

MYCOBACTERIUM TUBERCULOSIS INFECTION RECRUITS CATECHOLAMINERGIC NEURONS IN A TEMPORALLY, TOPOGRAPHICALLY ORGANISED MANNER

HOLLIS J.H.[1], GOOSEN K.D.[1], HERNANDEZ-PANDO R.[2], OROZCO H.[2], ROOK G.A.W.[3], LIGHTMAN S.L.[1], LOWRY C.A.[1] [1]*University of Bristol, Bristol, UK.* [2]*Department of Pathology, Instituto Nacional de la Nutricion 'Salvador Zubiran', Mexico City, Mexico.* [3]*Department of Bacteriology, Royal Free and University College Medical School, London, UK*

Brainstem catecholaminergic (CA) systems modulate neuroendocrine responses to acute peripheral inflammation; their role in neuroendocrine responses to chronic peripheral inflammation is not well understood. Chronic infection with M. tuberculosis results in a robust, prolonged activation of the hypothalamo-pituitary-adrenal (HPA) axis. We hypothesised that the brainstem CA neurons would be recruited in a temporally, topographically organised manner which reflected neuroendocrine responses known to occur following M. tuberculosis infection. Male, Balb/c mice were infected with virulent M. tuberculosis H37Rv (1X106 viable mycobacteria, i.t.), and at various time points post-infection (12h, 24h, 3d) were perfused in preparation for immunohistochemistry for detection of c-Fos and tyrosine hydroxylase. Compared to controls, infection with M. tuberculosis significantly increased the number of c-Fos-positive CA neurons of: 1) the A2 noradrenergic (NA) group at 12h, 2) the C1 adrenergic (A) group at 12h, 3) the A1/C1 NA/A group at 12h and 24h, 4) A2/C2 NA/A group at 24h, 5) the rostral A1 NA group at 24h, and 6) the caudal A1 NA group at 3d. *Supported by the Neuroendocrinology Charitable Trust, UK*

Keywords: catecholamine, brainstem, tuberculosis, chronic, inflammation

EFFECT OF ACUTE STRESS AND GENDER ON ISATIN IN RAT PLASMA AND TISSUES

IGOSHEVA N., MATTA S., GLOVER V. *The University of Saratov, Saratov, Russia, The Institute of Reproductive and Developmental Biology, Imperial College, London, UK*

Isatin is an endogenous indole present in mammalian tissues and fluids. The substance was discovered as a component of the endogenous monoamine oxidase inhibitory activity, tribulin. The most potent action of isatin, *in vitro,* is the inhibition of atrial natriuretic peptide receptor binding and G protein-mediated intracellular signaling. Previous findings have suggested that isatin may be involved in stress. However, the functional significance of isatin in the physiology of stress is not yet clearly established. The present study was thus designed to analyse the effects of both acute stress and gender on isatin levels in rat plasma and tissues using a new HPLC-UV based method for its detection. Sprague-Dawley rats of both genders were used. Immobilized rats were subjected to intermittent sound stimuli for 15 minutes. Under basal conditions, female rats had a significantly higher isatin concentration in the brain than males (46.8 ± 7.9 ng/ml and 32.1 ± 13.1 ng/ml, respectively, $p < 0.05$); levels in the heart and serum, although somewhat higher in females, were not significantly different between the genders. In stressed rats of both genders serum corticosterone (CS) levels were considerably higher than in the control groups, confirming the efficacy of the stress paradigm. Males showed a greater stress-induced rise in serum CS than females. Acute stress resulted in a significant increase ($p<0.05$) in isatin levels in male serum, heart and brain, and in female serum and heart. The percentage increases were greater in males than in females in all three sources. Brain isatin levels did not correlate with that in the serum or heart in either control or stressed animals. Thus, isatin rose in response to acute stress, and the effects were gender and tissue specific. This consolidates previous evidence that isatin has a role in stress.

Keywords: Isatin, stress, gender, corticosterone, rat

REPEATED STRESS-INDUCED STIMULATION OF CATECHOLAMINE RESPONSE IS NOT FOLLOWED BY ALTERED IMMUNE CELLS REDISTRIBUTION IN MEN

IMRICH R.[1], TIBENSKA E.[2], KOSKA J.[1], KSINANTOVA L.[1], KVETNANSKY R.[1], BLAZICEK P.[3] , VIGAS M.[1] *Institute of Experimental Endocrinology, SAS [1]; Department of Clinical Immunology, Pediatric University, Bratislava, Slovakia [2]; Hospital of the Ministry of Defense [3], Bratislava, Slovakia*

Stress response is considered an important factor participating in modulation of immune responses. Neuroendocrine hormones including catecholamines affect the immune cells redistribution crucially involved in the processes of the cell-mediated immunity. Our study was aimed to evaluate the effect of repeated stress-induced elevation of catecholamines on immune cell redistribution and expression of adhesive molecules in longitudinal investigation. Therefore, we assessed the responses of epinephrine (E), norepinephrine (NE); the changes in lymphocytes subpopulations as well as the percentage of CD11a+, CD11b+, CD62L+ lymphocytes to treadmill exercise of intensity equal 80 % of individual's $VO_{2\,MAX}$ lasting 20 min before and after 6 week of endurance training (T) in 10 healthy males. The exercise test led to a significant elevation of E and NE levels, absolute numbers of leucocytes, granulocytes, monocytes, CD3+, CD4+, CD8+, CD16+, CD19+ lymphocytes, percentage of CD11a+, CD11b+ and to a decrease of CD62L+ lymphocytes before as well as after T. The changes in all measured immune parameters after T were comparable to those before T. In conclusion, repeated stress-induced E and NE secretion was not associated with altered immune cells redistribution during a response to exercise test of submaximal intensity.

Keywords: exercise, lymphocyte subsets, norepinephrine, epinephrine, adhesion molecules

IN VIVO PROTEOMICS: VALIDATION OF RECEPTOR BINDING RADIOTRACERS IN STRESS USING SMALL ANIMAL IMAGING

JAGODA E.M.[1], CONTOREGGI C.[2], KIESEWETTER D.O.[1], LANG L.[1], RICE K.C.[3], ECKELMAN W.C.[1] [1]*PET Department, Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, MD,* [2]*Imaging Branch, IRP, NIDA, National Institutes of Health, DHHS, Baltimore, MD, USA,* [3]*National Institutes of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA*

Many receptor systems are implicated in stress and depression. Muscarinic cholinergic receptors, serotonin receptors, and corticotrophin-releasing hormone (CRH) receptor has also been shown to play a role. We have developed new radioligands for the cholinergic and CRH systems and have validated them as selective receptor binding radiotracers. In mice, rats, monkeys, and humans, 3-(3-(3-fluoropropyl)thio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine(FP-TZTP) displayed regional brain distribution consistent with M2 receptor concentrations. A significant decrease of [^{18}F]FP-TZTP uptake was observed in all brain regions examined for the M2 KO vs WT mice, but not for M4 KO, M3 KO, and M1 KO vs WT mice. In vitro, specific binding of a CRH receptor antagonist [^{76}Br]MJL 1-109-2 was proven by blocking with 10^{-6} M o-CRH or sauvagine and by 10^{-8} M MJL 1-109-2. This is the first non-peptide radiotracer that combines high affinity and appropriate lipophilicity and hence has the potential to be used for positron emission tomography (PET) imaging studies. Both are candidates for monitoring the effect of stress on receptor binding.

Keywords: muscarinic receptors, serotonin receptors, CRH receptors, stress

THE DIFFERENCES IN MITOCHONDRIAL FUNCTION AND WILD TYPE MICE

JAROSCAKOVA I.[1], SCHILD L.[2], WOLF G.[1] AND KEILHOFF G.[1] [1]*Institute for Medical Neurobiology,* [2]*Institute for Clinical Chemistry, Medical Faculty, Otto-von-Guericke University, Magdeburg, Germany*

Nitric oxide (NO) is one of the most popular and important biological molecules of recent years. This biomodulator and neurotransmitter is generated from arginine by a nitric oxide synthase (NOS), which is one of the most regulated enzymes in biology. There are three major classes of NOS: neuronal (nNOS), endothelial form (eNOS), and the inducible enzyme (iNOS). The role of NO in stress is still not clearly defined. Recent studies indicate that NO has a neurodegenerative potential performed by extensive release of glutamate after trauma and apoptosis triggered, amongst others by blocking of mitochondrial respiratory chain. Therefore we investigated the potential effect of NOS deficiency on activities of the mitochondrial respiratory chain complexes in various tissues. We focused our interest on complexes I, II and III of the respiratory chain. Differences between wild type (WT) and nNOS deficient (KO) mice were investigated. Our results show that there are statistically significant differences between WT and KO mice in activities of monitored respiratory chain enzymes: succinate cytochrome c oxidoreductase and NADH cytochrome c oxidoreductase. In KO mice, brain cortex, heart, dorsal root ganglia and liver mentioned enzyme activities are significantly reduced, whereas in kidney, muscle and spinal cord are almost unaffected. Our results indicate that there are NOS-dependent differences in the activity of succinate cytochrome c oxidoreductase, which forms complex II and III of the mitochondrial respiratory chain. The obtained data support the hypothesis that the presence of NO affects the mitochondrial respiration.

Keywords: neuronal NOS, KO mice, mitochondria, respiratory chain

PSYCHOSOCIAL STRESS, ANXIETY AND DIETARY AMINO ACIDS IN HEALTHY MEN

JEZOVA D.[1], DUNCKO R.[1], SMRIGA M.[2], MORINAGA Y.[2], MONCEK F.[1], MAKATSORI A.[1] [1]*Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia,* [2]*Ajinomoto Co., Inc., Life Sci. Lab., Kawasaki, Japan*

Emotional and psychosocial stressors are dominant in stressful life conditions and it is not easy to simulate them under laboratory settings. Appropriate models are standardized psychosocial laboratory stressors based on public speech, such as Trier social stress test (TSST). Altered stress responsiveness has been related to the development and course of mood and anxiety disorders, but the limited results obtained in humans are equivocal. A traditional view is to look for treatments, which reduce the stress response thought to be exaggerated. We have examined selected groups of male mentally and physically healthy volunteers at the upper and lower limits of the normal range of the scale in the State and Trait Anxiety Inventory (STAI-x2). Changes in blood pressure during a modified TSST were similar in both groups, while the rise in heart rate was enhanced in anxious subjects. In contrast, plasma cortisol, prolactin ACTH, and catecholamines during TSST were not enhanced, but significantly lower in anxious than in non-anxious subjects. Since a prolonged dietary treatment with L-lysine and L-arginine reduced stress-enhanced anxiety in laboratory animals, we have tested the clinical effects of these amino acids (3g/d each, 10 days) in anxious subjects. Treatment with amino acids resulted in increased responses of salivary cortisol, plasma cortisol, ACTH and catecholamines during TSST, while changes in blood pressure remained unaffected. Thus, an adequate rather than reduced hormonal response to mental stress seems to be needed and the traditional view should be reevaluated. *The study was supported by grants of EC ICA1-CT-2000-70008 and VEGA 2/2007.*

Keywords: HPA axis, catecholamines, anxiety, amino acids, humans

A PANICOGENIC STIMULUS (ACUTE HYPERCAPNIA) SELECTIVELY ACTIVATES BRAINSTEM NORADRENERGIC AND ADRENERGIC NUCLEI

JOHNSON P.L.[1], HOLLIS J.H.[1], MORATALLA R.[2], LIGHTMAN S.L.[1], LOWRY C.A.[1 1]*University Research Centre for Neuroendocrinology, University of Bristol, Bristol, UK.* [2]*Instituto Cajal, Madrid, Spain*

Acute hypercapnia (>15% CO2), in humans, increases sympatho-adrenomedullary (SAM) and hypothalamo-pituitary-adrenal (HPA) axis output, and elicits panic-like symptoms. Since 1) evidence suggests that elevated CO2 is detected centrally within brainstem noradrenergic (NA) and adrenergic (A) nuclei, and 2) glucocorticoids may alter the excitability of CO2-sensitive NA and A systems, we determined the effect of adrenalectomy (ADX) on responsiveness of NA and A nuclei to acute normoxic hypercapnia using immunohistochemical staining for c-Fos and tyrosine hydroxylase. Adult male SD rats were group housed for 3 days following either nonsurgical control, sham ADX or ADX + corticosterone replacement. Two rats were randomly placed in either a normoxic, or hypercapnic (20% CO2) chamber. After 5 minutes of gas infusion, cages were opened and 5 minutes later rats were returned to their homecages. Hypercapnia significantly increased c-Fos in the C1 A nuclei (-11.6, -11.8, -11.96 mm bregma) in all surgical groups, whereas in the A6 NA nuclei (-9.8, -10.04, -10.3 mm bregma) and A1/C1 nuclei (-13.68, -13.8 mm bregma) it occurred in the intact and sham ADX rats only. Although CO2 had no effect in ADX rats there was a trend for increased c-Fos expression in NA nuclei (A6, A2, A1/C1) in normocapnic controls, which could be a novel environment effect. Neither hypercapnia nor ADX had any effect on A7, A5, A2 or C3 nuclei. *Supported by the Wellcome Trust, BBSRC, and Neuroendocrinology Charitable Trust.*

Keywords: panic, adrenalectomy, hypercapnia, catecholamine, brainstem

EFFECT OF 3-DAY BED REST ON PLASMA CATECHOLAMINE RESPONSES TO ENVIRONMENTAL STRESSORS DEPENDS ON PREVIOUS HABITUAL ACTIVITY

KACIUBA-USCILKO H.[1], MIKULSKI T.[1], NAZAR K.[1], SMORAWINSKI J.[2], ADRIAN J.[2 1]*Medical Research Centre, Polish Academy of Sciences, Warsaw and* [2] *Academy of Physical Education, Poznan, Poland*

The effect of bed rest (BR) on the sympathetic nervous system (SNS) is not fully recognized. We hypothesized that the effect is most pronounced in physically active subjects. Thus, basal plasma norepinephrine [NE] and epinephrine [E] and catecholamine responses to physiological stimuli were compared before and after 3 days of horizontal BR in subjects differing in habitual activity. Eleven untrained men, 8 endurance and 10 power/strength athletes were submitted to oral glucose tolerance test (OGTT) and orthostatic stand test (OST). Other 12 untrained men, 10 endurance and 10 power/strength athletes underwent cold pressor test (CPT) and maximal exercise test (ET). After BR basal [NE] was decreased only in athletes ($p<0.01$). Increases in catecholamines during OGTT were diminished ($p<0.05$), particularly in endurance athletes. Plasma [NE] response to standing was diminished in the whole group ($p<0.05$). BR did not affect the increases in catecholamines induced by CPT and ET. Basal sympathetic activity is diminished after 3 days of BR in physically active men. Although catecholamine responses to OGTT and OST were reduced the general reactivity and sensitivity of SNS were not attenuated since catecholamine responses to the most (exercise) and least (CPT) powerful stimuli were unchanged.

Keywords: catecholamines, bed rest, physiological stimuli, athletes, sedentary subjects

PROTEIN SYNTHESIS IN THE AXON AND PRESYNAPTIC NERVE TERMINAL: NEW INSIGHTS INTO THE BIOLOGY OF THE NEURON

KAPLAN B.B., GIOIO A.E. *Laboratory of Molecular Biology, NIMH/NIH, Bethesda, MD, USA*

During the past few years, it has become well-established that the distal structural/functional domains of the neuron do indeed contain numerous mRNAs. However, there is still a paucity of information on the composition, and function of these unique mRNA populations. In this presentation, the evidence to support the hypothesis that protein synthesis occurs in microcompartments in the neuron to include the axon and presynaptic nerve terminal will be reviewed. The studies to be described employ the squid giant axon and photoreceptor neuron as model invertebrate motor and sensory systems, respectively. Initial cell-free translation studies and molecular hybridization analysis established that the giant axon contained a heterogeneous population of polyadenylated mRNAs. The application of differential mRNA display methodology greatly facilitated the isolation and identification of several of these mRNAs which encode: cytoskeletal proteins, translation factors, various nuclear-encoded mitochondrial mRNAs, and several novel mRNA species. The axonal localization of several of these mRNAs was unequivocally established by in situ hybridization histochemistry and their relative distribution in the axon and parental cell bodies defined by quantitative RT-PCR methodology. Evidence derived from a proteomics experimental approach and electron spectroscopic imaging techniques further substantiated the presence of active polyribosomes in squid axons and nerve terminals. The role that local protein synthesis might play in the mammalian nervous system, and in the neuronal response to stress will be discussed.

Keywords: mRNA, local protein synthesis, axon, nerve terminal

CORTICOTROPIN RELEASING HORMONE (CRH): ENDOCRINE/ IMMUNE INTERACTIONS

KARALIS K., VENIHAKI M., VANVLERKEN L., GAY J., PACAK K.[1], CHANDRAS C. *Children's Hospital, Harvard Medical School, Boston MA and [1]NIH, Bethesda, MD, USA*

CRH has dual effects on the immune/inflammatory response, i.e. indirect antiinflammatory of hypothalamic CRH via stimulation of glucocorticoid release and direct proinflammatory of peripherally expressed CRH, most likely via induction of NF-kB DNA-binding activity and downregulation of IkB expression. Endotoxemia, and its extreme presentation sepsis, are associated with high mortality. Endotoxemia activates the hypothalamic-pituitary-adrenal (HPA) axis and the catecholaminergic system via the significantly increased levels of circulating cytokine and other proinflammatory mediators. To further understand the contribution of CRH on endotoxemia and sepsis, we administered LPS to CRH-deficient (*Crh-/-*) and *Crh+/+* mice. Our findings revealed delayed corticosterone rise in the *Crh-/-* mice, together with very elevated plasma TNFα and chemokines, such as MIP-1 and MIP-2, at the very early stages of endotoxemia. Interestingly, the susceptibility of the *Crh-/-* mice to a moderate dose of LPS (120μg/mouse) was increased, as shown by increased shivering, cold skin, restricted moving and severe vasoconstriction as early as 12 hrs after LPS administration and 100% mortality rate by 24 hrs of exposure to LPS, as opposed to almost 0% in the *Crh+/+* mice. We have previously shown altered epinephrine secretion during inflammation in *Crh-/-* mice, which masked the proinflammatory effects of CRH deficiency. Based on the above and the critical role of catecholamines in sepsis, we suggest the possibility that the LPS susceptibility in states of genetic CRH deficiency is related to altered catecholamine secretion. The role of CRH deficiency independently of glucocorticoid insufficiency in this process is under investigation.

Keywords: corticotropin releasing hormone, sepsis, glucocorticoid, TNFa, epinephrine

SPATIAL PERFORMANCE AND CORTICOSTEROID RECEPTOR STATUS IN THE 21-DAY RESTRAINT STRESS PARADIGM

KITRAKI E.[1], KREMMYDA O.[1], YOULATOS D.[2], KITTAS C.[1] [1]*Athens University Medical School,* [2]*University of Thessaloniki, Greece*

Twenty-one days of restraint stress has been shown to affect hippocampal plasticity, neurogenesis and spatial memory. Hippocampal glucocorticoid (GR) and mineralocorticoid receptors (MR) are main mediators of both stress response and learning/memory processes. Based on the above we studied the performance of male and female rats on a hippocampal-dependent spatial task following 21 days of restraint, in relation to the stress-induced GR and MR status in their hippocampi. Reduced GR immunostaining was detected in the dentate gyrus and CA1 area of stressed males, which also performed worse than the controls on Morris water maze. In contrast, stressed females didn't show significant changes in hippocampal GR status, while their memory score in the task was improved. In addition, stressed females showed increased MR immunostaining in the CA3 area, known to be the most affected by stress in males. The observed sexually dimorphic effects of 21-day restraint in spatial learning and memory may be associated to the corticosteroid receptor status of the hippocampus following stress.

Keywords: chronic restraint, corticosteroid receptors, hippocampus, spatial memory, gender

MECHANISMS THAT CONSTRAIN THE ACTIVITY OF THE HYPOTHALAMO-PITUITARY-ADRENOCORTICAL ACTIVITY

KOVACS K.J. AND BALI B. *Laboratory of Molecular Neuroendocrinology, Institute of Experimental Medicine, Budapest, Hungary*

There are neuronal and hormonal (negative feedback) mechanisms that constrain the basal and stress-induced activity of the HPA axis. Both mechanisms may involve limbic components that are relayed through local GABA and glutamatergic interneurons in the near surrounding of the hypothalamic paraventricular nucleus (PVH). To assess the functional impact of local inhibitory GABAergic cell population on the synaptic and transcriptional activity of parvocellular neurons, we followed the expression of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) genes along with the activation marker c-fos in response to the blockade of GABA-A receptors. We analyzed the effect of GABA-A receptor antagonist bicuculline in organotypic cultures of hypothalamic slices. These preparations preserve the cytoarchitecture of CRH-synthesizing cell population and the local network, while remote connections originating from limbic areas are missing. Suspension of the GABAergic tone *in vitro* upregulated CRH and AVP expression and induced c-Fos-ir in the PVH, while bicuculline microinjections *in vivo* into the PVH region resulted in a selective activation of AVP hnRNA and different pattern of c-Fos expression. These results reveal an intrinsic GABAergic circuit in the microenvironment of the PVH that by itself, without limbic contribution, impinge a tonic inhibitory influence on the CRH neurons. In vivo, remote inputs are superimposed on this local circuit allowing differential transcriptional regulation.

Keywords: CRH, AVP, gene expression, GABA, organotypic culture

REPEATED IMMOBILIZATION STRESS DECREASES mRNA AND PROTEIN LEVELS OF THE TYPE 1 AND 2 IP_3 RECEPTORS IN RAT HEART

KREPSOVA K.[1], MICUTKOVA L.[2] , NOVOTOVA M.[1], KUBOVCAKOVA L.[2], KVETNANSKY R.[2], KRIZANOVA O.[1] [1]*Institute of Molecular Physiology and Genetics and* [2]*Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia*

Stress is one of the major contributors to the development of cardiovascular disorders and psychiatric illnesses. Immobilization stress belongs to severe stressors and is known to activate several calcium transport systems. The aim of this work was to determine, whether repeated immobilization stress for seven times changes mRNA and protein levels of the type 1 and 2 IP_3 receptors in cardiac tissue. Rats were immobilized for 7 days, two hours daily. After repeated immobilization, no significant morphological changes in the heart were observed compared to hearts of the control group of rats. Gene expression was determined with reverse transcription and subsequent Real-Time PCR, using SYBR Green fluorescent dye. Protein levels were determined by Western blot and subsequent hybridization with primary antibody against IP_3 receptors. Contrary to single immobilization, repeated immobilization decreased a gene expression of the type 1 and 2 IP_3 receptors, and also the protein levels of IP_3 receptors. Although the physiological relevance of our observations remains to be elucidated, we propose that decrease in IP_3 receptors may have an impact on the development of the pathological state of the heart. *Supported by grants VEGA 2/3008, VEGA 2/3189 and APVT-51-013802.*

Keywords: inositol-1,4,5-trisphosphate receptors, rat heart, immobilization stress

INOSITOL 1,4,5-TRISPHOSPHATE RECEPTORS IN THE HEART ARE DIFFERENTLY MODULATED BY THE STRESS COMPARED TO OTHER TISSUES

KRIZANOVA O.[1], MICUTKOVA L.[2], KREPSOVA K.[1], HUDECOVA S.[1], BRTKO J.[2], PALKOVITS M.[3], KVETNANSKY R.[2] [1]*Institute of Molecular Physiology and Genetics and* [2]*Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia,* [3]*Semmelweis University, Budapest, Hungary*

Inositol 1,4,5-trisphosphate receptors (IP_3 receptors) are intracellular calcium channels, releasing calcium from the sarcoplasmic reticulum. In the heart, IP_3 receptors of type 1 and 2 predominate in atria, although they occur also in ventricles, as determined by real-time PCR and Western blot analysis. These receptors are regulated at the transcription level by cfos, glucocorticoid responsive element and retinoic acid. Single immobilization stress increases mRNA and/or protein levels of the type 1 and 2 IP_3 receptor. However, in stellate ganglia, which innervate the heart, no changes in the mRNA of the type 1 IP_3 receptors were observed after single immobilization stress. In renal medulla, a moderate decrease in both, mRNA and protein levels of IP_3 receptors was observed after single immobilization exposure. After repeated immobilization, mRNA and protein levels of the type 1 and 2 IP_3 receptors decreased significantly in all tested tissues. Our results point to different processing of the single stress in different tissues, while repeated stress results in rapid and significant decrease of the IP_3 receptors, which can have an impact in development of some cardiovascular diseases. *This work was supported by grants VEGA 2/3008, VEGA 2/2090 and APVT 51-013802.*

Keywords: IP_3 receptors, immobilization stress, heart, gene expression

EFFECT OF SPACE FLIGHT AND HEAD-DOWN BEDREST ON NEUROENDOCRINE RESPONSE TO METABOLIC STRESS IN PHYSICALLY FIT SUBJECTS

KSINANTOVA L., KOSKA J., VIGAS M., MACHO L., KVETNANSKY R. *Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic*

The aim of this study was evaluate the association of plasma epinephrine-EPI and norepinephrine-NE responses to insulin induced hypoglycemia (ITT) 3 weeks before the space flight (SF), on the 5th day of SF, on the 2nd and 16th days after the landing in the first Slovak astronaut, and before and on the 5th day of prolonged subsequent head-down (-6°) bed rest (BR) in 15 military aircraft pilots. Insulin hypoglycemia was induced by i.v. administration of 0.1 IU/kg BW insulin (Actrapid HM) in bolus. Insulin administration led to a comparable hypoglycemia in pre-flight, in-flight conditions and before and after bed rest. ITT led to a pronounced increase in EPI levels and moderate increase in NE in pre-flight studies. However, an evidently reduced EPI response was found after insulin administration during SF and during BR. Thus, during the real microgravity in SF and simulated microgravity in BR, ITT activates the adrenomedullary system to less extent than at conditions of the Earth gravitation. Post-flight changes in EPI, NE did not differ from those of pre-flight since SF was relatively short (8 days) and the readaptation to Earth gravitation was fast. It seems, that an increased blood flow in brain might be responsible for the reduced EPI response to insulin. Responses to ITT in physically fit subjects indicate the stimulus specificity of deconditioning effect of 5 days bed rest on stress response. *The study was supported by the grant of VEGA 2/2090, 2/3150 and by European EC ICA1-CT-2000-70008.*

Keywords: catecholamines, space flight, bed rest, ITT, metabolic stress

CATECHOLAMINE SYNTHESIZING ENZYMES AND THEIR MODULATION BY IMMOBILIZATION STRESS IN KNOCK-OUT MICE

KUBOVCAKOVA L.[1], KRIZANOVA O.[1], SABBAN E.L.[2], MAJZOUB J.[3], VIETOR I.[4], WAGNER E.F.[4], KVETNANSKY R.[1]
[1]*Institute of Experimental Endocrinology, SAS, Bratislava, Slovak Republic,* [2]*NY Medical College, Valhalla, NY,* [3]*Harvard Medical School, Boston, MA, USA,* [4]*I.M.P., Vienna, Austria*

Corticotropin-releasing hormone knock-out mice (CRH KO) and c-fos knock-out mice (c-fos KO) can serve as interesting models for studying the mechanisms involved in the response of the hypotalamic-pituitary-adrenal axis to stress. In our work we focused on: 1. investigation of the changes in tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH) and phenylethanolamine N-methyl transferase (PNMT) gene expression and protein level in adrenal medulla (AM) of immobilized CRH KO and c-fos KO mice, 2. comparison of PNMT gene expression in the spleen of wild-type (WT) and c-fos KO mice. Levels of TH, DBH and PNMT mRNA were determined by RT-PCR, the amount of corresponding proteins was determined by Western blot analysis. Unlike TH and DBH mRNA levels, AM PNMT mRNA was significantly decreased after single and also repeated immobilization in CRH KO mice compared to WT mice. Single immobilization significantly increased adrenomedullary TH, DBH and PNMT mRNA levels in both, c-fos KO and WT mice compared to unstressed controls. In the spleen we revealed a reduction in immobilization induced PNMT mRNA levels in c-fos KO female mice. Our data indicate that CRH deficiency can influence PNMT mRNA level in AM during stress. On the other hand, c-Fos probably does not play a crucial role in TH, DBH and PNMT gene expression in AM under the stress conditions. *Supported by EU Grant ICA1-CT-2000-70008, VEGA 2/2090 and Slovak-US Grant 002/2001.*

Keywords: TH, DBH, PNMT, knock-out mice, immobilization stress

GLUCOCORTICOID RECEPTOR GENE VARIANTS HAVE AN IMPACT ON ADRENOCORTICAL RESPONSES TO PSYCHOSOCIAL STRESS

KUMSTA R.[1], VAN ROSSUM E.F.C.[2], FEDERENKO I.S.[1], KOPER J.W.[2], HELLHAMMER, D.H.[1], WUST S.[1] [1]*Department of Psychobiology, University of Trier, Germany,* [2]*Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands*

Recent evidence suggests that polymorphisms of the glucocorticoid receptor (GR) gene may be an important source of the large interindividual variation of hypothalamus-pituitary-adrenal axis activity. In the present investigation 112 young healthy males were studied in order to estimate the impact of three GR gene polymorphisms (*Bcl*I RFLP, N363S, ER22/23EK) on cortisol and ACTH responses to psychosocial stress and pharmacological stimulation. Compared to subjects with the rather common allele pattern '*Bcl*I CC & N363S AA' (n=36), carriers of the 363S allele (n=10) showed a significantly increased salivary cortisol response to the 'Trier Social Stress Test (TSST)', while the *Bcl*I genotype GG (n=18) was associated with a diminished cortisol response. Likewise, in 363S carriers a trend towards higher ACTH responses to the TSST was observed compared to *Bcl*I GG subjects. After ingestion of 0.5 mg dexamethasone a trend towards enhanced cortisol suppression in 363S carriers was detected. The administration of 1µg $ACTH_{1\ 24}$ showed no significant difference in cortisol reactions between genotypes. This is the first report documenting an impact of GR gene polymorphisms on Cortisol responses to psychosocial stress. One could speculate that these genetic variants contribute to the individual vulnerability for HPA related clinical states.

Keywords: GR gene polymorphisms, psychosocial stress, cortisol

QUANTITATIVE EVALUATION OF CATECHOLAMINE ENZYMES GENE EXPRESSION IN ADRENAL MEDULLA AND SYMPATHETIC GANGLIA OF STRESSED RATS

KVETNANSKY R., MICUTKOVA L., RYCHKOVA N., KUBOVCAKOVA L., MRAVEC B., FILIPENKO M.[1], SABBAN E.L.[2], KRIZANOVA O. *Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic,* [1]*IBCH, Novosibirsk, Russia,* [2]*New York Medical College, Valhalla, NY, USA*

Stress-induced mRNA levels of tyrosine hydroxylase (TH), dopamine-ß-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT) have been expressing as relative arbitrary units compared to control group. The aim of this study was to quantify basal and stress-induced levels of TH, DBH and PNMT mRNAs in rat adrenal medulla (AM) and stellate ganglia (SG) by RT-competitive PCR using corresponding competitors of known concentration. In rats stressed by immobilization (IMO) once for two hours mRNAs were determined in various intervals after the end of stress stimulus. In SG, concentration of TH mRNA was 17 amol/µg total RNA, which is approximately 30-times lower than in AM. The concentration of DBH mRNA was 2600 amol/µg total RNA, which is about 150-times more than TH mRNA in SG but only two-times less than DBH mRNA in AM in which PNMT mRNA is present in the highest concentration. After a single 2-hour IMO the peak elevation of TH and DBH mRNA concentration in SG occurred 24 h after termination of the stress stimulus, when their AM concentrations including PNMT mRNA were already at control values. Repeated IMO (7 days, 2 h daily) did not produce further increase in the mRNA levels compared to adapted control. Levels of TH protein were significantly changed only after repeated IMO. Thus, our data show for the first time the exact concentrations of TH, DBH and PNMT mRNA in SG and AM of rats under control and stress conditions. *Supported by EU Grant ICA1-CT-2000-70008, VEGA (2-2090) and Slovak-US Grant (002/2001).*

Keywords: CA enzymes, mRNA quantification, immobilization, stellate ganglia, adrenal medulla

REGULATION OF CARDIAC PHENYLETHANOLAMINE-N-METHYLTRANSFERASE GENE EXPRESSION IN RAT DURING STRESS

KVETNANSKY R., MICUTKOVA L., PALKOVITS M.[1], SABBAN E.L.[2], KRIZANOVA O. *Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic,* [1]*Semmelweis University, Budapest, Hungary,* [2]*New York Medical College, Valhalla, N.Y., USA*

Recently we have described the existence of PNMT mRNA in the heart of adult rats. The aim of this study was to determine distribution of the PNMT mRNA in the heart and to examine whether the gene expression of this enzyme is affected by immobilization (IMO) stress in a time-dependent manner. PNMT mRNA levels were detected in all seven parts of the heart studied (atria, atrial ganglia, ventricles, septum) with the highest levels in the left atrium and its ganglionic part. Both Southern blot and sequencing verified the specificity of PNMT detected by RT-PCR. Single IMO for 2 hours increased gene expression of PNMT, as determined by both RT-PCR and Real-Time PCR in right and left atria, ventricles and septum. Surprisingly, the ganglionic parts of atria did not respond to stress stimulation. Peak levels of PNMT mRNA were found in the interval of 3 h after the IMO terminated, and also 24 h after the first or sixth IMO. In atria, the effect of IMO was clearly modulated by glucocorticoids, since adrenalectomy prevented the increase in PNMT mRNA levels. In ventricles, adrenalectomy did not affect the IMO-induced increases in mRNA and therefore the PNMT gene expression in cardiac ventricles might be regulated by other factors. Thus, our data have shown that at least two mechanisms exist in the regulation of cardiac PNMT gene expression. The stress-induced increase in atria is dependent on the presence of glucocorticoids, however in ventricles another mechanism is involved. *Supported by EU Grant ICA1-CT-2000-70008, VEGA (2-2090) and Slovak-US Grant (002/2001).*

Keywords: PNMT mRNA, heart atria and ventricles, immobilization stress, glucocorticoids

NEUROPEPTIDES, NEUROTRANSMITTERS, AND EMOTIONALITY-DEPENDENT STRESS COPING

LANDGRAF R. *Max Plack Institute of Psychiatry, Munich, Germany*

Behavioral, neurochemical and molecular-genetic correlates of extremes in inborn emotionality were investigated in Wistar rats, bred for either high (HAB) or low (LAB) anxiety-related behavior. In a variety of behavioral tests, HAB animals show signs of a hyper-reactive hypothalamo-pituitary-adrenocortical axis and prefer passive coping strategies upon stressor exposure, indicative of depression-like behavior. The clinically relevant co-morbidity between trait anxiety and depression is the result of the long-lasting breeding, as it is not shown by animals arbitrarily selected from a purchased population of Wistar rats. Interestingly, HAB rats compared to LAB rats over-express and over-release the neuropeptide vasopressin, but not CRH and oxytocin, in their hypothalamic PVN. This phenomenon is due to single nucleotide polymorphisms (SNPs) in the promoter of the vasopressin gene, giving rise to reduced binding of the inhibitory transcription factor CBF-A and, consequently, to an increased expression of intra-PVN vasopressin. These SNPs, which are detectable in HAB but not LAB animals, seem to be more critical in determining levels of AVP, trait anxiety and stress coping than the synaptic input of potential neurotransmitters to the PVN of HAB versus LAB animals. Indeed, bilateral administration of a combined vasopressin V1a/b receptor antagonist into the PVN by inverse microdialysis in freely behaving HABs resulted in a shift towards active stress coping, further suggesting the vasopressin system to be critically involved in emotionality and stress coping.

Keywords: anxiety, polymorphisms, stress coping, vasopressin, HPA axis

CARDIOVASCULAR RESPONSES TO STRESS AFTER CAROTID BARORECEPTOR DENERVATION IN HUMANS

LENDERS J.W.M., TIMMERS H. *Dept. of Internal Medicine, University Medical Centre Nijmegen St. Radboud, Nijmegen, The Netherlands*

Iatrogenic bilateral denervation of carotid sinus baroreceptors may occur as a complication of carotid body tumor resection (CBR) and radiation therapy of the neck (RAD). The resulting syndrome of baroreflex failure is characterized by paroxysms of severe hypertension and tachycardia which are ascribed to a limited blood pressure buffering capacity in response to emotional and physical stimuli The aim was to investigate the long-term effects of CBR and RAD on baroreflex hemodynamic control. Measurements were carried out in 8 CBR patients, 12 RAD patients and 15 healthy controls. Studies included assessment of cardiovascular responses to physical and emotional stressors (blood pressure, heart rate and MSNA responses to cold pressor test and mental arithmetic) as well as under daily life conditions (ambulatory blood pressure measurements).

At baseline, plasma catecholamine levels were similar in all groups. Despite a ~50% lower baroreflex sensitivity in both CBR and RAD patients as compared to controls, increases in blood pressure, heart rate and MSNA in response to cold pressor test and mental arithmetic did not differ between groups. Ambulatory blood pressure variability, however, was significantly increased in CBR patients. Chronic attenuation of baroreflex sensitivity due to iatrogenic baroreceptor trauma may result in increased blood pressure variability. However, unopposed sympathetic activation in response to physical and emotional stress, as in the acute phase of baroreflex failure, is absent.

Keywords: baroreflex, denervation, afferent, muscle sympathetic nerve activity, stress

STRESS AND RESTENOSIS AFTER ANGIOPLASTY: IS NEUROPEPTIDE Y A RISK FACTOR?

LI L., ABE K., LEE E.W., ZUKOWSKA Z. *Department of Physiology and Biophysics, Georgetown University, Washington D.C., USA*

Atherosclerosis is a progressive process leading to occlusion of arteries and resulting in ischemic vascular diseases. In spite of identification of multiple risk factors, it remains poorly manageable. Balloon angioplasty re-canalizes occluded arteries but in half of the patients results in restenosis with neointima and thrombus. This is believed to result from growth factor release from injured vessels and platelet aggregation. The role of sympathetic nerves remains unclear, although they are trophic, and stress, which activates them, exacerbates cardiovascular events. Neuropeptide Y (NPY) is a sympathetic vasoconstrictor co-transmitter acting via multiple G-protein coupled receptors (Y1-Y5Rs). NPY is also angiogenic and mitogenic for vascular smooth muscle cells, and expressed in endothelium and platelets, although variably. In rats, carotid artery angioplasty up-regulates vascular NPY, Y1 and Y5Rs, and markedly increases platelet NPY content. Angioplasty in rats, in the presence of elevated platelet NPY by a depot pellet, or in mice over-expressing platelet NPY (SV129, but not non-expressing C57/BL) – results in vessel occlusion with an atherosclerotic-like lesion. These effects are fully mimicked by cold stress (2hr/day/14days), which occludes rat carotid artery with neointima and thrombus. Both NPY- and stress-induced restenosis are completely prevented by a specific Y1R antagonist. This is the first direct evidence that stress may be a risk factor for restenosis by releasing NPY.

Keywords: neuropeptide Y, stress, restenosis, platelets, neointima

EFFECTS OF STRESS AND STRESS-RELATED NEUROPEPTIDES ON TOPOGRAPHICALLY ORGANISED SUBSETS OF SEROTONERGIC NEURONES

LOWRY C.A. *University Research Centre for Neuroendocrinology, University of Bristol, UK*

Serotonergic systems play an important and generalised role in regulation of sleep-wake states and behavioural arousal. Recent *in vivo* electrophysiological recording studies in behaving animals suggest, however, that several different subtypes of serotonergic neurones with unique behavioural correlates exist within the brainstem raphe nuclei, raising the possibility that topographically organised subpopulations of serotonergic neurones may have unique functional properties. We have shown that the stress-related and anxiogenic neuropeptide, corticotropin-releasing factor (CRF) can stimulate the *in vitro* neuronal firing rates of topographically organised subpopulations of serotonergic neurones within the dorsal raphe nucleus. These findings are consistent with a wealth of behavioural studies suggesting that serotonergic systems within the dorsal raphe nucleus are involved both in the modulation of ongoing anxiety-related behaviour and in behavioural sensitisation, a process whereby anxiety- and fear-related behavioural responses are sensitised for a period of up to 24-48 hours. The dorsomedial subdivision of the dorsal raphe nucleus, particularly its middle and caudal aspects, has attracted considerable attention as a region that may play a critical role in the regulation of acute and chronic anxiety states. Future studies aimed at characterisation of the molecular and cellular properties of topographically organised subpopulations of serotonergic neurones are likely to lead to major advances in our understanding of the role of serotonergic systems in stress-related physiology and behaviour.

Keywords: serotonin, 5-hydroxytryptamine, median raphe nucleus, central nucleus of the amygdala

EFFECTS OF REAL AND SIMULATED MICROGRAVITY ON RESPONSE OF SYMPATHOADRENAL SYSTEM TO VARIOUS STRESS STIMULI

MACHO L.[1], KOSKA J.[1], KSINANTOVA L.[1], VIGAS M.[1], BLAZICEK P.[2], NOSKOV V.B.[3], GRIGORIEV A.I.[3], PACAK K.[4], KVETNANSKY R.[1] [1]*Institute of Experimental Endocrinology, SAS, Bratislava, Slovakia,* [2]*Hospital of Ministry of Defense, Bratislava, Slovakia,* [3]*Instit. Biomed. Problems, RAS, Moscow, Russia,* [4]*NIH, Bethesda, USA.*

Changes in plasma levels of epinephrine (EPI) and norepinephrine (NE) were investigated in humans exposed to physical exercise (WL), to psychic stressor (mental arithmetic test, MAT) and to oral glucose administration (oGTT) before and during stay in microgravity (real space flight, SF) or in simulated microgravity (head down bed rest, HDBR). Chronic cannula inserted into cubital vein and special appliance Plasma-03 were used for blood collection, plasma separation and freezing of samples during SF. Plasma EPI, NE, DHPG and DOPA levels were measured by HPLC method. Basal plasma EPI, NE, DHPG and DOPA levels were found within the range of control values during SF. Preflight WL produced high increase in plasma NE and moderate elevation of plasma EPI, DHPG and DOPA levels. Exaggerated exercise induced increases in plasma NE, DHPG, EPI and DOPA levels were demonstrated in real microgravity. A return to pre-flight responses of sympathoadrenal system was seen after the landing. Plasma EPI, NE and DHPG response to MAT were relatively small but were increased during SF. During the oGTT plasma EPI levels were slightly reduced in microgravity. Similarly as in SF, WL in HDBR was followed by significantly exaggerated responses of plasma catecholamines. These results showed that both somatic and psychic stressors are able to induce an increased activation of sympathoadrenal system during SF or simulated microgravity in HDBR.

Keywords: catecholamines, work-load, psychic stress, space flight, bed rest

CRH-RELATED HYPOTHALAMIC PEPTIDES: ENDOCRINE, BEHAVIORAL AND AUTONOMIC EFFECTS

MAJZOUB J.A., VENIHAKI M., JEONG K.H., WENINGER S.C., KARALIS K. *Children's Hospital, Harvard Medical School, Boston, MA, USA*

The mammalian family of corticotropin-releasing hormone (CRH)-related peptides now consists of four members: CRH (also known as corticotropin-releasing factor [CRF]), as well as urocortin (UCN) I through III. Following its discovery, CRH was hypothesized to broadly integrate endocrine, behavioral, and autonomic responses to stress. In that regard, we have found that CRH, via stimulation of adrenal glucocorticoid synthesis, is required for epinephrine secretion from the adrenal medulla, and further that epinephrine activates the innate immune system as part of the "fight or flight" stress response. However, as the CRH-related family of neuropeptides has grown, and with the ability to create animal models with targeted deletion of specific genes, it is possible that some of the actions previously ascribed to CRH are actually carried out by other members of the CRH-related peptide family. For example, although CRH-deficient mice are markedly deficient in their endocrine stress response, we have found that they appear to have a normal behavioral response to stress. On the other hand, UCN I-deficient mice have altered behavioral stress responses, but normal endocrine responses to stress. Given the neuroanatomic distribution of UCN I, it is possible that it might mediate some of the autonomic functions attributed to CRH. We have found that UcnIII may have a role in attenuating behavioral stress responses, but probably is not involved in endocrine responses to stress. It is likely that CRH and UCN I - UcnIII are paralogs derived from a common ancestral gene. As such, they probably share some common regulatory properties, which may govern coordinated regulation among them. This may be a basis for their collaboration in hormonal, behavioral, and autonomic domains to create an integrated stress response.

Keywords: CRH, urocortin, behavior, catecholamine, innate immune system

DIABETES MELLITUS AND MORPHINE ADDICTION AS CHRONIC STRESS: THE ROLE OF VASOPRESSIN

MAKARA G.B., ZELENA D., MERGL ZS., BARNA I. *Institute of Experimental Medicine, Budapest, Hungary*

HPA activation occurs in chronic stress in spite of the corticosteroid feedback signal. A shift to vasopressin (VP) rather than CRH in the PVN may explain how chronic stress maintains high corticosteroid levels. Repeated restraint in VP-deficient Brattleboro rats suggested that VP does not have an important role in chronic stress. When streptozotocin-diabetes mellitus or a high dose morphine treatment is used for two weeks, the body weight loss, adrenal hypertrophy, thymus involution and elevation of baseline plasma corticosterone were similar in the male di/+ and di/di Brattleboro rats. This suggests that VP either is not important for chronic stress-induced changes or a mechanism compensates for the long-term absence of VP. The rapid ACTH response to a single restraint or the response to morphine withdrawal was also slightly inhibited in chronically stressed VP-deficient rats. The hormonal response to an acute stress in the chronically stressed rat may partially depend on VP mediation. VP may not have an essential role in the tonic stimulation of the HPA axis in chronic stress.

Keywords: Brattleboro, streptozotocin, ACTH, corticosterone, POMC

NEUROENDOCRINE RESPONSES AND NON-VERBAL BEHAVIOR DURING MENTAL STRESS ARE MODULATED BY LAMOTRIGINE

MAKATSORI A.[1], DUNCKO R.[1], LODER I.[3], MONCEK F.[1], KATINA S.[2], JEZOVA D.[1] [1]*Institute of Experimental Endocrinology, SAS, Bratislava, Slovakia,* [2]*Faculty of Mathematics, Physics and CS, Comenius University, Bratislava, Slovakia,* [3]*Inst. Adaptive and Spaceflight Physiology, Graz, Austria*

Despite the fact that animal studies have shown the involvement of glutamate neurotransmission in the stress response, human studies using glutamate antagonists are very limited. To test the hypothesis that glutamate participates in the stress response in humans, a single dose of lamotrigine (300 mg per os) was administered. A modified version of Trier Social Stress Test (TSST) was applied in a placebo controlled double blind study in 19 young healthy males. Five hours after drug administration a 15 min talk on a particular topic was given in front of an unknown audience preceded by a 15 min preparation. Data were obtained from the video recorded speech, cardiovascular monitoring, as well as blood and saliva sampling. The stressor significantly increased cardiovascular responses, ACTH, plasma and salivary cortisol, prolactin and catecholamine release. Lamotrigine significantly inhibited diastolic blood pressure, growth hormone and salivary cortisol increases during TSST. In contrast, it potentiated plasma renin activity and aldosterone responses. Non-verbal behavior analysis revealed correlation between catecholamines and submissive or flight behavior in the control group while between catecholamines and displacement behavior after lamotrigine administration. These data indicate an involvement of glutamate in the control of growth hormone and cortisol release, while the effects on other hormones and non-verbal behavior observed seem to be related to other actions of lamotrigine. *This study was supported by grants of EC ICA1-CT-2000-70008 and VEGA 2/ 2007.*

Keywords: glutamate, lamotrigine, mental stress, non-verbal behavior, hormones

DEVELOPMENT OF ENANTIOSELECTIVE IMMUNOASSAYS FOR FREE PLASMA METANEPHRINES

MANZ B., BOOLTINK E., KUPER M., FISCHER-BRÜGGE U. *Labor Centrum, Nordhorn, Germany*

The development of enantioselective radio and enzyme immunoassays for L-normetanephrine and L-metanephrine are described. Prior to the immunoassay, the protein matrix of the EDTA plasma samples is removed by acid precipitation followed by derivatization of the L-metanephrines to N-acyl-L-metanephrines. For the EIA, N-acyl-L-normetanephrine and N-acyl-L-metanephrine, respectively, are bound to the surface of microtiter plates. Acylated L-metanephrines from the sample and solid phase bound N-acyl-L-normetanephrine or N-acyl-L-metanephrine compete for a fixed number of rabbit anti-N-acylnormetanephrine or anti-N-acylmetanephrine antibody binding sites. When the system is in equilibrium, free antigens and free antigen-antiserum complexes are removed by washing. The antibodies bound to the respective solid phase N-acyl-L-normetanephrine or N-acyl-L-metanephrine are detected by a mouse anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The RIA´s are conventional double antibody tests using the above rabbit antisera and specific ^{125}I-N-acyl-L-metanephrine tracers. Chiral recognition of the L- enantiomers was observed not only for the native molecules but for all N-acyl derivatives tested. The cross-reactivity to the correspondend D-enantiomers was always < 1%. The detection limits were found to be approximately 0.04 pmol/l (7.5 pg/ml) for L-metanephrine and 0.08 pmol/l (15 pg/ml) for L- normetanephrine in RIA and EIA.

Keywords: EDTA plasma, metanephrines, enantioselective, RIA, EIA

ADRENOMEDULLIN AS A STRESS-RELATED HORMONE

MARTINEZ A., CUTTITTA, F. *Cell and Cancer Biology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA*

Adrenomedullin (AM) is a 52 amino acid peptide hormone that has a broad distribution throughout the organism and participates in a variety of physiological and pathological conditions. The peptide has an amidated carboxy end and an internal 6 amino acid ring structure in the middle of the molecule. Both features are needed for receptor recognition and elevation of intracellular cAMP levels. The receptor for AM has been identified recently and encompasses a seven transmembrane domain G-protein linked receptor known as CRLR, a chaperone single transmembrane domain protein called RAMP, and an intracellular protein named RCP. Although AM and its receptor complex are found throughout the body, they are well represented in the hypothalamic-pituitary-adrenal axis and regulate its physiology at all levels, including secretion regulation of arginine-vasopresin, oxytocin, ACTH, aldosterone and catecholamines. AM circulating levels change when experimental animals are exposed to different stressors such as cold, restrain, water deprivation, hypoxia/ischemia, and even the stress associated with birth. In addition, AM is elevated in the plasma of patients suffering disease stress in ailments such as cancer, cardiovascular diseases, sepsis, or following strenuous exercise. In most of these cases AM has a protective role and may constitute a defense mechanism against the damage caused by stress.

Keywords: adrenomedullin, HPA axis, hormone regulation, disease stress, cellular stress

MODULATION OF CATECHOLAMINE SYNTHESIZING ENZYMES IN THE RAT HEART AND STELLATE GANGLIA BY REPEATED IMMOBILIZATION STRESS

MICUTKOVA L.[1], KREPSOVA K.[2], SABBAN E.L.[3], KRIZANOVA O.[1,2], KVETNANSKY R.[1] [1]*Institute of Experimental Endocrinology,* [2]*Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia and* [3]*New York Medical College, Valhalla, NY, USA*

Stress is one of the major risk factors involved in the increased incidence of a number of common life-threatening disorders, predominantly of the cardiovascular origin. Aim of the present study was to establish a gene expression (mRNA levels) of the tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT) in a rat heart and stellate ganglia under control conditions and immobilization stress. In the process of repeated immobilization, rats were immobilized for 7 days, two hours daily and subsequently decapitated 3 hours after the last immobilization. Gene expression was determined by seminested PCR, or Real-Time PCR (using SYBR Green I fluorescent dye). Quantification of mRNA levels of these enzymes in stellate ganglia was performed by RT-competitive-PCR. In both, the rat heart and stellate ganglia we identified mRNA of all three enzymes: TH, DBH and PNMT. Repeated immobilization significantly increased PNMT mRNA levels in the heart and also TH and DBH mRNA levels in stellate ganglia compared to unstressed controls. However, no further increase was observed compared to group of rats immobilized once and decapitated three hours afterwards. Observed elevation in the gene expression of catecholamine biosynthetic enzymes in heart and stellate ganglia may be involved in the increased risk of cardiovascular diseases with stress. *Supported by grants VEGA 2/2090, Slovak-American Scientific-Technical Grant 002/2001and European EC grant ICA1-CT-2000-70008.*

Keywords: TH, DBH, PNMT, rat heart and stellate ganglia, immobilization stress

NEURAL PATHWAYS INVOLVED IN SEROTONERGIC ACTIVATION OF THE HPA AXIS

MIKKELSEN J.D., HAY-SCHMIDT A., KISS A.[1] *Dept. Molecular Anatomy and Physiology, Neurosearch A/S, Dept Anatomy, University of Copenhagen, and* [1]*Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia*

The HPA axis can be stimulated by serotonin via both 5-HT_{1A} and 5-HT_{2A} receptors. This study was undertaken to localize the neurons affected by either a 5-HT_{1A} receptor (8-OH-DPAT) or 5-HT_{2A} receptor (DOI) agonist that project directly to the PVN. Cholera Toxin subunit B was iontophoretically injected into the PVN, and rats were subsequently administered with a single dose of either 8-OH-DPAT (1 mg/kg) or DOI (2.5 mg/kg) sixty minutes before fixation. The fixed brains were processed immunocytochemically for the simultaneous detection of the tracer and Fos. In animals administered with 8-OH-DPAT and with correct placement of tracer, a marked induction of Fos in projecting neurons were observed in the parastriatal nucleus of the bed nucleus, in the hypothalamic arcuate, ventromedial, dorsomedial, lateral, and anterior hypothalamic nuclei/areas, as well as the parabrachial nucleus ipsilateral to the injection site. In contrast, DOI activated Fos in the PVN, but not in any of the first-order projecting neurons. These findings are consistent with the hypothesis that 5-HT_{1A} and 5-HT_{2A} receptors regulate PVN neurons via distinct pathways. These results are discussed in relation to the neural pathways mediating stress- and serotonin-dependent activation and/or inhibition of the HPA axis.

Keywords: serotonin, receptors, neural tracing, paraventricular nucleus, pathways

EFFECT OF STRESS AT BIRTH ON CORTISOL RESPONSE IN INFANCY

MILLER N., FISK N., MODI N., GLOVER V. *Imperial College London, UK*

Animal studies show that perinatal exposure to high cortisol levels can result in permanent programming of the hypothalamic-pituitary-adrenal (HPA) response. Our hypothesis was that cortisol levels in cord blood, which vary by type of delivery, may influence long-term HPA responses. Arterial cord blood was collected at delivery from consenting primiparous women who were de livered normally, by assisted delivery, or by elective or emergency caesarean. At 2 months, infant salivary cortisol was measured pre and post routine vaccination. Cord artery cortisol differed significantly at birth between the 4 delivery groups.(Kruskal-Wallis, $p < 0.0001$). Cord blood cortisol was correlated with the cortisol response to vaccination at 2 months ($r = 0.23$, $p<0.05$, $n = 76$). Those in the most and least stressed groups at birth had markedly different cortisol responses at 2 months ($p = 0.017$ Student's t-test). Because babies have different cortisol exposure at birth depending on mode of delivery, and this correlates with cortisol response to vaccination at 2 months, we conclude that stressful delivery may exert a programming effect on the HPA axis, at least until the age of 2 months.

Keywords: cortisol, delivery, birth, stress, programming

EFFECT OF ENRICHED ENVIRONMENT ON STRESS RESPONSE INDUCED BY IMMUNE CHALLENGE

MLYNARIK M.[1], JOHANSSON B.B.[2], JEZOVA D.[1] [1]*Institute of Experimental Endocrinology, SAS, Bratislava, Slovakia,* [2]*Wallenberg Neuroscience Center, Lund University, Sweden*

Housing of animals in enriched environment (EE) has many positive effects on brain structure and function and can facilitate recovery from various brain injuries. The purpose of this study was to evaluate whether signs of stress response induced by repeated immune challenge could be reduced by enriched rearing as well as to determine the effect of EE on some physiological and molecular parameters. Male 65 days old Wistar rats were kept in standard (SC) or in EE cages for 5 weeks. Immune challenge was performed by Escherichia coli lipopolysaccharide (LPS) injected repeatedly (i.p.) in increasing doses (10, 20, 40 mg/kg) once daily for five consecutive days. Controls were injected with saline. Two hours after the last injection blood samples and adrenals were collected for hormone measurements. Glutamate receptor gene expression was evaluated to characterize the model of enriched housing. EE resulted in an increase in adrenal weights and enhanced expression of AMPA Glur1 receptor subunit in the hippocampus. No effects of LPS on adrenal and thymus weights and glutamate receptor mRNA levels in the hippocampus were noticed. LPS induced robust increase in plasma as well as adrenal level of corticosterone and transient decrease in body weight in SC but not in EE kept rats. Thus, vulnerability to some negative effects of repeated immune challenge may be modified by environmental conditions associated with changes in brain plasticity. *This study was supported by grants of EC ICA-CT-2000-70008 and VEGA 2/2007.*

Keywords: glutamate receptor, corticosterone, enriched environment, LPS, immune challenge

ENVIRONMENTAL INFLUENCES ON STRESS RELATED SYSTEMS

MONCEK F., DUNCKO R., JEZOVA D. *Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia*

Many studies have pointed out positive effects of enriched environment (EE) on brain plasticity and function, which include increase in adult neurogenesis, improved learning, problem solving ability and better indices of emotionality. We hypothesized that rats kept in EE would show faster adaptation of pituitary-adrenocortical and sympathetic-adrenomedullary responses in a model of quick habituation to repeated handling. Second hypothesis was that buspirone, a neuroendocrine probe into the serotonin-1A system, would result in larger pituitary-adrenocortical activation in EE rats. We kept a group of rats in large plexiglass ($1m^2$) cages, ten animals per cage, which contained variety of objects, toys, tunnels, swings and running wheels, exchanged three times a week. Control animals were reared in standard wire mesh cages, 4 rats per cage. Rats kept in EE had higher plasma corticosterone (CORT) levels and larger adrenals compared to controls. EE animals had lower CORT and adrenaline responses to initial handling. Exposure to repeated handling led to a more rapid extinction of CORT responses in EE rats. EE rats had higher CORT responses to buspirone challenge. Thus, environmental enrichment leads to pronounced changes in neuroendocrine regulation including larger adrenals and adrenocortical function, so far considered to be a negative indication of chronic stress. Moreover, EE modified adaptation processes and responses to pharmacological treatment. *The study was supported by the grant of VEGA 2/2007 and by European Commission support of Centre of Excellence ICA1-CT-2000-70008.*

Keywords: enriched environment, corticosterone, catecholamines, adaptation, buspirone

INHIBITORY EFFECT OF FORMALIN ADMINISTRATION ON EPINEPHRINE RELEASE

MRAVEC B.[1], BODNAR I.[1], KUBOVCAKOVA L.[1], NAGY G.M.[2], PALKOVITS M.[3], KVETNANSKY R.[1] [1]*Institute of Experimental Endocrinology, SAS, Bratislava, Slovakia,* [2]*Neuroendocrine Research Laboratory,* [3]*Neuromorphological Laboratory, Semmelweis University, Budapest, Hungary*

Subcutaneous (s.c.) injection of formalin is used as classical painful stressor, which produces a biphasic nociceptive response. We evaluated response of epinephrine (EPI) and norepinephrine (NE) plasma levels during s.c. administration of 4% formalin in comparison to saline administered animals. Application of formalin produced decrease of EPI in first 30 min. followed by increase started at one hour after administration; NE levels were increased from 30 min. To test capacity of formalin inhibitory effect on EPI release we exposed animals to two hours of immobilization (IMO) stress and formalin was administered before or during IMO. Formalin administration before the start of IMO decreased EPI levels during the first 30 min. without significant changes in plasma NE levels. Formalin administration after the start of IMO decreased EPI levels during the first 15 min. and increased after 60 min.; NE levels were increased after the 40th min. These data show that inhibitory process activated in the first phase by s.c. formalin administration is able significantly decrease EPI release not only during basal condition but also before or during exposure to stressors like immobilization. *Supported by the EU Center of Excellence Support ICA1-CT-2000-70008, VEGA 2/2090 and ETT 277/2001.*

Keywords: formalin, epinephrine, norepinephrine, immobilization stress

MOUSE MUTANTS AS TOOLS FOR HPA-AXIS RELATED DRUG DISCOVERY

MULLER M.B., KECK M.E., HOLSBOER F. *Max Planck-Institute of Psychiatry, Munich, Germany*

Genetically engineered mice with a specific deletion of targeted genes provide a novel and useful tool to study the endogenous mechanisms underlying aberrant behaviour. With respect to the stress hormone (hypothalamic-pituitary-adrenocortical; HPA) system refined molecular technologies have allowed to target individual genes involved in stress hormone regulation. Chronically elevated levels of circulating corticosteroid hormones are believed to enhance vulnerability to a variety of diseases, including human affective disorders. A more detailed knowledge of the signalling pathways, therefore, opens up the possibility to specifically modulate HPA system function. Corticosteroids are known to influence emotions and cognitive processes, such as learning and memory. In addition, corticosteroids play extremely important roles in modulating fear and anxiety-related behavior. The mechanisms by which corticosteroids exert their effects on behavior are often indirect, by modulating particular sets of neurons or neurotransmitter systems. In addition, the timing of corticosteroid increase (before, during or after exposure to a stressor) determines whether and how behavior is affected. Importantly, deletion of individual genes is not providing animal models for certain psychiatric disorders as these are caused by a manifold of minor changes in a series of so-called susceptibility genes. However, these gene targeting methods have become valuable tools to dissect the functions of individual components of complex biological systems in behavioural neuroscience: genetically engineered animals help to unravel the complex interactions and correlations between individual genes, hormonal regulation and behaviour, the most complex form of biological organization.

Keywords: stress, HPA system, mouse mutants, gene targeting

EFFECTS OF IMMOBILIZATION STRESS ON MUSCARINIC RECEPTORS, β-ADRENOCEPTORS AND ADENYLYL CYCLASE IN DIFFERENT HEART REGIONS

MYSLIVECEK J.[1,2], RICNY J.[2], PALKOVITS M.[3], KVETNANSKY R.[4] [1]*Institute of Physiology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic,* [2]*Institute of Physiology, Academy of Sciences, Prague, Czech Republic,* [3]*Department of Anatomy, Semmelweis University, Budapest, Hungary,* [4]*Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia*

Heart muscarinic receptors (MR) and β-adrenoceptors (BAR) belong to a large family of G protein coupled receptors. Although the role of catecholamines in the stress is under keen investigation for many years, the effects of immobilization on this pair of receptors with almost completely opposite action in the heart are not known yet. We have studied the effects of short-term immobilization (for 120 minutes) with different time of latention (0, 3 and 24 hours) on MR, BAR (β_1-AR and β_2-AR using radioligand binding studies) and adenylyl cyclase (AC; using HPLC detection of cAMP) in different rat heart regions (left and right atria with or without ganglionic cells, septum and left and right ventricles). The effects of one immobilization were first apparent after 24 hours. Stress brings about down-regulation of MR and BAR with decrease in AC activity. These effects were region specific and were predominantly expressed in right atrial tissue with ganglia and right ventricles. Our results indicate that the stress can influence not only BAR but also MR and that AC activity can be affected too. This is in good agreement with our previous hypothesis that this pair of receptors can change their number in membranes in parallel. *Supported by Grant 18/2003 from GAUK, VEGA 2/2090 and US Slovak Grant 002/2001.*

Keywords: muscarinic receptors, β-adrenoceptors, adenylyl cyclase, immobilization stress, heart

SALSOLINOL, AN ENDOGENOUS TETRAHYDROISOQUINOLINE, PLAYS A PIVOTAL ROLE IN STRESS-, AND SUCKLING-INDUCED RELEASE OF PROLACTIN

NAGY G.M.[1], FEKETE M.I.K[1]., BODNAR I[1,2]., TOTH B.E.[1], FULOP F.[3], MRAVEC B.[2], KVETNANSKY R[2]. [1]*Neuroendocrine Research Laboratory, Semmelweis University, Budapest, Hungary,* [2]*Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia,* [3]*Institute Pharm. Chemistry, Faculty of Pharmacy, Szent-Gyorgyi Univ., Szeged, Hungary*

Acute changes of prolactin (PRL) secretion usually occur following different challenges (like suckling stimulus or stress) of the homeostasis in mammals. The role of a prolactin-releasing factor (PRF) in these changes has been suspected for a long time. We have recently observed that 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, salsolinol (SAL), produced by the neuro-intermediate lobe (NIL) of the pituitary gland as well as by the hypothalamus, can selectively release PRL from the anterior lobe (AL). Moreover, binding sites for SAL have also been detected in areas like median eminence, NIL and AL. Therefore, it has been proposed that SAL is a putative endogenous PRF. We have also found that a structural analogue of SAL, 1-methyl-3,4-dihydroisoquinoline (1MeDIQ), is able to block dose-dependently SAL-, suckling-, immobilization (IMO)-, as well as formalin (FORM) stress induced release of PRL without having any influence on thyrotropin releasing hormone (TRH)-, or the, 5-hydroxytryptophan (5-HTP, serotonin precursor)-induced PRL responses. Neither SAL nor 1MeDIQ has any effect on corticosterone secretion. These results strongly suggest that SAL can play a key role in the regulation of PRL release induced by physiological-, and environmental stimuli, therefore, it can be considered as the strongest candidate for being the PRF in the hypothalamo-hypophysial system *Supported by OTKA 43370, ETT 277/2001; ICA1-CT-2000-70008 and VEGA 2-2090.*

Keywords: stress, suckling, PRL-release, salsolinol

EFFECTS OF HYPO- AND HYPERTHYROIDISM ON NORADRENERGIC ACTIVITY AND LIPOLYSIS IN THE HUMAN SUBCUTANEOUS ABDOMINAL ADIPOSE TISSUE

Nedvidkova J.[1], Bartak V.[1], Dostalova I.[1], Krsek M.[2], Svacina S.[2], Alesci S.[3,4], Pacak K[4]. [1]*Institute of Endocrinology, Prague,* [2]*3rd Dept of Med., 1st Fac. Med., Prague,* [3]*NICHD, NIH, Bethesda, USA* [4]*Clinical Neuroendocrinology Branch, NIMH, NIH, Bethesda, USA*

Thyroid function plays an important role in the regulation of overall metabolic rate and lipid metabolism. It is, however, uncertain whether thyroid hormones directly affect lipolysis-regulating catecholamines in adipose tissue. It was observed that chronic stress induces an alteration of the function of thyroid axis that alters the SNS activity and vice versa. Our study was designed to determine basal and isoprenaline-stimulated local lipolysis and local concentrations of lipolysis-regulating catecholamines in abdominal subcutaneous adipose tissue (AT) in hypothyroidism (HT), in hyperthyroidism (Tx) and healthy controls (C) using microdialysis technique. Plasma norepinephrine (NE) concentrations in HT were significantly higher than in both C and Tx group. In contrast to systemic, adipose NE levels in HT were decreased relative to C. HT, on the other hand, resulted in 4-fold higher adipose NE levels. Basal lipolysis measured by glycerol concentrations in the AT was significantly attenuated in HT and markedly increased in Tx in comparison with C. In addition to differences in basal lipolysis, HT resulted in attenuated and Tx in enhanced lipolytic response to local stimulation with β1,2-adrenergic agonist isoprenaline. These results demonstrate that lipolysis in the AT is strongly modulated by thyroid function. We suggest that thyroid hormones regulate lipolysis primarily by affecting local NE concentration and/or adrenergic postreceptor signaling.

Keywords: thyroid functions, catecholamines, adipose tissue, microdialysis

DIAGNOSTIC LOCALIZATION OF PHEOCHROMOCYTOMA BY 6-[^{18}F]FLUORODOPAMINE POSITRON EMISSION TOMOGRAPHY

Pacak K.[1], Carrasquillo J.A.[2], Chen C.A.[2], Eisenhofer G.[1], Goldstein D.S[3]. [1]*Pediatric and Reproductive Endocrinology Branch, NICHD,* [2]*Nuclear Medicine Department, Clinical Center, NIH,* [3]*NINDS, NIH, USA*

Current localization methods of pheochromocytoma include CT and MRI, which have good sensitivity but poor specificity. Functional imaging methods include radioiodinated meta-iodobenzylguanidine (MIBG) scanning and [18-F]-fluorodeoxyglucose, [11-C]-hydroxyephedrine or [11-C]-epinephrine PET scanning with either suboptimal sensitivity or specificity. Here we report results using 6-[^{18}F]fluorodopamine PET scanning in the localization of pheochromocytoma. Sixty three patients with known or clinically suspected pheochromocytoma underwent PET scanning with 6-[^{18}F]fluorodopamine. All patients but one enrolled underwent biochemical testing including the measurement of plasma catecholamine and metanephrine levels and imaging studies including CT, MRI, and 6-[18F]fluorodopamine PET imaging. A total of 63 patients were enrolled in this study (23 men, 40 women, mean ages 41.8 ± 3.2 and 45.8 ± 2.3 years). 29 of 63 patients were found to have pheochromocytoma, confirmed histologically in the 27 who underwent surgery. Overall, 6-[18F]fluorodopamine correctly identified pheochromocytoma in 27 of 29 patients subsequently shown to harbor the tumor (sensitivity 93%) and correctly yielded negative results in 33 of 34 patients who did not have the tumor (specificity 97%). 6-[18F]Fluorodopamine scans were negative in 33 of 34 patients where pheochromocytoma was suspected but excluded based on biochemical results. These results establish the sensitivity and specificity of 6-[18F]fluorodopamine scanning in the diagnostic localization of pheochromocytoma.

Keywords: pheochromocytoma, fluorodopamine, catecholamines

LOCALIZATION AND CHEMICAL CHARACTERIZATION OF THE AUDIOGENIC STRESS PATHWAY

PALKOVITS M., DOBOLYI A, HELFFERICH F., USDIN T.B. *Laboratory of Neuromorphology, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary and Laboratory of Genetics, National Institute of Mental Health, NIH, Bethesda, MD, USA*

Neuronal pathways involved in stress response to extreme somatosensory stimuli were investigated by immunostainings, viral tract-tracing and experimental brain surgery in rats. As a model, acute audiogenic stress was applied which elicits an immediate marked elevation in plasma ACTH and corticosterone concentrations. The neuronal pathway that interconnects the auditory system and the hypothalamus is constituted by axons of neurons located in the pontine *medial paralemniscal* (MPL) and the midbrain *parvicellular subparafascicular* (SPF) nuclei. Besides the components of the auditory pathway and stress-sensitive hypothalamic nuclei, loud noise (105 dB, 30 min) elicited *c-fos* activation in the MPL and SPF. Parvicellular neurons in the paraventricular nucleus (PVN) were double immunostained by corticotropin releasing hormone (CRH) and Fos protein. Injections of neurotropic viruses (pseudorabies, Bartha strain) into the PVN, MPL and SPF neurons were retrogradely infected. It has been shown by immunostaining that MPL and SPF neurons expressed a newly discovered neuropeptide, *tuberoinfundibular peptide of 39 residues* (TIP-39), which established a fine neuronal network in the PVN. Audiogenic stress elicited *c-fos* activation in TIP-39 positive neurons of the MPL and SPF. TIP-39 immunoreactivity disappeared from the PVN after transection of MPL and SPF projections to the nucleus. Both the MPL and SPF nuclei are in close topographical connections with brainstem catecholaminergic (A7, A8 and A11) neurons.

Keywords: audiogenic stress, c-fos, tract-tracing, double immunostaining

PRIOR ALCOHOL CONSUMPTION ALTERS THE STRESS RESPONSE IN MALE RATS

PATTERSON-BUCKENDAHL P., BLAKLEY G., KUBOVCAKOVA L.[1], KRIZANOVA O.[1], POHORECKY L.A., KVETNANSKY R.[1] *Center of Alcohol Studies, Rutgers University, Piscataway, NJ, USA and* [1]*Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic*

Humans and many wild animals voluntarily consume fermented food containing ethanol (EtOH), yet survival may depend on their response to stressful challenges. We hypothesized that EtOH might increase gene transcription for catecholamine synthesizing enzymes TH, DBH, and PNMT. 3 experiments: were conducted: (1) 4 wks, 10% w/v EtOH drinking water, lab chow *ad libitum* (AL) and pairfed (PF) controls n=10 per group; (2) 1 wk, 5% w/v EtOH in liquid diet (n=16, ELD, 35% of calories) with AL (n=12) or PF (n=12) receiving isocaloric liquid diet. Rats were cannulated on day 7 for blood collection on day 8 for norepinephrine (NE) and epinephrine (E) analysis. 7 AL, 7 PF, and 9 ELD rats underwent 2 hr foot restraint immobilization (FR), remainder served as unstressed controls; (3) 7 wks, 6% w/v EtOH drinking water, controls as for exp. 1, n=20 per group. Half of each group was restrained 2 hr daily in wire mesh cylinders (WMR). In all 3 expts, TH mRNA was elevated 80 to 90% by EtOH compared to AL. DBH and PNMT were unaffected by 10% EtOH, but increased by 6% EtOH drinking ($p<0.01$) and 5% ELD. mRNA for all three enzymes increased further after FR. Basal plasma NE and E were elevated in ELD and more so during FR. TH, DBH, and PNMT mRNA increased in 6% EtOH rats and PF controls compared to AL, less so in WMR EtOH and PF rats. These EtOH effects were similar to that of repeated cold or FR and may constitute a metabolic stressor. Elevated plasma NE and E may prime the animal for response to stress and may also account for increased aggression sometimes noted following provocation of EtOH consumers.

Keywords: alcohol consumption, stress, catecholamine, gene expression, mRNA levels

CATECHOLAMINE SYNTHESIZING CELLS IN THE EMBRYONIC HEART

PFEIFER K., BOE S., EBERT S. *National Institute of Child Health and Human Development, Bethesda, MD USA and Georgetown University Medical School, Washington DC, USA*

Catecholamines are essential for mouse heart development at e10.5 to e13.5. In these early embyros, the heart itself is the major and perhaps only source of catecholamines. Synthesis of catecholamine biosynthetic enzymes in the heart is transient and shows an intriguing dynamic pattern: the intrinsic cardiac adrenergic (ICA) cells are transiently and progressively associated with pacemaking and conduction tissue development (SA node, AV node, bundle of His, and ventricular septum). To study the function and fate of these cells in the developing mammalian heart, we have generated a novel mouse model whereby the *cre-recombinase* gene has been inserted into the endogenous *Pnmt* locus encoding the epinephrine biosynthetic enzyme, phenylethanolamine N-methyltransferase. We show that this knock-in mouse selectively produces cre recombinase in adrenergic cells. When crossed with an appropriate tester strain, cre recombinase activates β-galacotosidase expression in adrenergic cells and their descendents. Under these conditions, we show that β-galacotosidase is evident in the developing mouse heart as early as e8.5. By e10.5, extensive non-uniform labeling of ICA cells with β-galacotosidase is seen throughout the myocardium. Some of the strongest labeling was observed at the A-V and conoventricular junctions, but sporadic labeling was also observed in the ventricle and at the SA junction. By late fetal/early postnatal stages of development, b-galacotosidase positive cells were seen to contribute to significant portions of the heart, including proximal components of the pacemaking and conduction system tissue (SA and AV nodes, bundle of His) as well as much of the epicardium. These data suggest that ICA cells contribute much more extensively to cardiac development than previously thought.

Keywords: catecholamines, PNMT, mouse, heart

HYPERTONIC SALINE AND IMMOBILIZATION INDUCED FOS-EXPRESSION IN MOUSE BRAIN CATECHOLAMINERGIC CELL GROUPS: COLOCALIZATION WITH TYROSINE HYDROXYLASE (TH) AND NEUROPEPTIDE Y (NPY)

PIRNIK Z., MRAVEC B., KUBOVCAKOVA L., MIKKELSEN J.D.[1], KISS A. *Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia,* [1]*Department of Molecular Anatomy and Physiology, Neurosearch A/S, Ballerup, Denmark*

In the experiments neuronal activities in catecholaminergic (CA) areas (A1, A2, A5, A6, C1, C2, C3) and parabrachial nuclei (PBN), involved in the transduction of the ascending viscero- and somato-sensory signals, were studied in wild-type mouse exposed to 60 min of hypertonic (400 µl 1.5 M i.p.) saline (HS) or 120 min of immobilization (IMO) stress. Fos-signal, a general marker of cell activity, was identified by ABC-immuno procedure. To reveal the phenotypical nature of activated neurons colocalization of Fos with either TH or NPY was evaluated. Generally, HS elicited more extensive Fos-staining in the mouse brain than IMO. HS induced a massive Fos activation in the area postrema-NTS complex, PBN, and Fos-TH staining in the A1 group. In the other catecholaminergic areas from low to moderate Fos-TH colocalization was seen in both HS and IMO mice. NPY revealed dense innervation network in the area postrema-NTS complex, PBN, and locus coeruleus (A6). In A6 and A1 areas sparse Fos-NPY colocalization occurred in both HS and IMO mice. The data demonstrate that both viscerosensory (HS) and somatosensory (IMO) inputs in mice generally activate the same CA areas to transfer the nociceptive signals to higher centers. *The study was supported by EC ICA1-CT-2000-70008 and VEGA 2/2007and 2/2090 grants.*

Keywords: catecholaminergic areas, Fos-immunohistochemistry, stress, mouse

5-HT_{1A} RECEPTOR ACTIVATION PRIOR ACUTE STRESS COUNTERACTED THE INDUCED BEHAVIOURAL LONG-TERM EFFECTS

RIOJA J., SANTIN L.J., DONA A., DE PABLOS L., CUADRADO M.I., ALCALDE F.J., AGUIRRE J.A. *Dept. of Physiology, School of Medicine and Dept. of Psychobiology, School of Psychology, University of Malaga, Malaga, Spain*

The behavioural long-term consequences of acute immobilization (IMMO) in rats and the effects of 5-HT_{1A} receptor activation (8-OH-DPAT: 0.3 mgr/kg, s.c) was studied. Corticosterone levels and serum total antioxidant status (TAS) of IMMO with or without 8-OH-DPAT were also studied. The rats (3 h IMMO) were, 24 h later, performed in both conditioned (passive avoidance) and unconditioned (escape behaviour) anxiety tests in the elevated T maze. One animal per group was used for c-Fos immunohistochemistry. Pre-exposure to IMMO induces long-term behavioural changes in contrast with control rats. These behavioural alterations include an increase of anxiogenic responses such as exploratory behaviour and passive avoidance response. This effect was counteracted by 8-OH-DPAT pretreatment and reversed by WAY-100635 when administered prior 8-OH-DPAT. Serum corticosterone levels increased during the first hour of stress and after 8-OH-DPAT administration and TAS decreases only in IMMO group. Our results clearly support the hypothesis that involvement of acute stress is crucial in the formation of anxiety disorders and aversive memories and that 5-HT_{1A} receptors stimulation in capable to counteract this long-term effects induced by IMMO. *Supported by Spanish DGYCIT (PM-0159).*

Keywords: passive avoidance, escape behaviour, immobilization, 5-HT_{1A} receptors, corticosterone, c-Fos

ANGIOTENSIN II, AN IMPORTANT STRESS HORMONE: MULTIPLE REGULATORY SITES AND THERAPEUTIC OPPORTUNITIES

SAAVEDRA J.M., ANDO H., ARMANDO I., BAIARDI G., BREGONZIO C., JEZOVA M., ZHOU J. *Section on Pharmacology, Division of Intramural Research Programs, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA*

Angiotensin II (Ang II) is present in most organs, including the brain. The discovery that selective blockade of brain and peripheral Ang II AT_1 receptors profoundly decreases the response to stress established Ang II as one fundamental factor regulating, at multiple sites, the sensitivity and response to stress. In peripheral organs, Ang II promotes inflammatory and vasoconstrictor responses characteristic of the immediate pathological alterations of intense stressful conditions. In the adrenals, Ang II regulates catecholamine, glucocorticoid and mineralocorticoid secretion both under basal conditions and during stress. In turn, corticoids affect the brain through feedback mechanisms controlling the nature, extent, and duration of the response to stress at the hypothalamic-pituitary axis and higher limbic and cortical pathways. Brain Ang II influences the vulnerability and the response to stress controlling the cerebral circulation, catecholamine, CRH and vasopressin release, CRH_1 receptor expression and function and the cognitive response to stress. The recent finding that AT_1 receptor blockade is sufficient to completely prevent an acute stress disorder opens novel therapeutic avenues not only for the treatment but also for the prevention of these conditions.

Keywords: renin-angiotensin system, angiotensin II receptors, AT_1 receptor antagonists, stress therapy, stress prevention

MOLECULAR REGULATION OF GENE EXPRESSION OF CATECHOLAMINE BIOSYNTHETIC ENZYMES BY STRESS

SABBAN E.L.[1], SEROVA L.[1], NANKOVA B.[1], KVETNANSKY R.[2], LIU X.[1], [1]*New York Medical College, Valhalla, NY, USA,* [2]*Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic*

Stress triggers a large rise in gene expression of TH and DBH, in the sympathetic ganglia and in adrenal medulla (AM). However, distinct molecular mechanisms appear to regulate these genes in these locations. ACTH injection elevates TH and DBH expression in rat sympathetic ganglia but not AM. The superior cervical and stellate ganglia were found to express ACTH responsive MC2 receptor mRNA, which was elevated by immobilization (IMO) stress. In addition, augmented binding of CREB proteins to TH and DBH cAMP responsive promoter elements was observed in nuclear extracts of sympathetic ganglia upon injection of ACTH or exposure to stress. These results suggest that ACTH, via the MC2R, may be directly involved in the stress-elicited regulation of norepinephrine biosynthesis in sympathetic ganglia. In contrast the AM does not express MC2 receptor mRNA. Experiments were directed to determine the mechanism of elevation of TH and DBH gene expression in AM with cold and IMO for various times or repetition. IMO did not augment binding of CREB, but elevated its phosphorylation, induced Egr1 and several AP1 transcription factors depending on the duration of stress. Cold triggered different alterations in transcription factors, which could account for the adaptation of adrenal TH to prolonged cold, but not to IMO stress, and for the exaggerated response to novel stressors. The results have begun to elucidate stressor-specific signaling pathways responsible for changes in gene expression in AM and sympathetic systems.

Keywords: ACTH, adrenal medulla, DBH, sympathetic ganglia, tyrosine hydroxylase

DECIPHERING THE CHOLINERGIC COMPONENT OF STRESS-ASSOCIATED SYNDROMES

SOREQ H. *Institute of Life Sciences, The Hebrew University of Jerusalem, Israel*

The multileveled mechanisms that underlie mammalian stress responses have been addressed by molecular neurophysiology and behavior approaches. Physiological stress responses include alternative splicing, mRNA translocation into neuronal processes (Meshorer et al, Science, 2002, 295, 508-512), chronic induction of LTP impairments and intracellular mobilization and activation of neuronal protein kinase C (Birikh et al., 2003 PNAS 100, 283-288). These and related intracellular signaling processes maintain cholinergic neurotransmission, yet induce long-term hypersensitivity and exacerbate contextual fear responses following stress insults. When combined with human genotype analysis, this study points to polymorphisms in the cholinesterase genes as being associated with higher risk for stress-induced diseases. In elucidating post-transcriptional events in experimental animals, antisense oligonucleotides limited the accumulation of the stress-induced variant of acetylcholinesterase. Targeted at consensus RNA sequences, these limit the consequent stress-related pathophysiologies both in brain synapses (Cohen et al., 2002, Mol. Psych. 7, 874–885) and in neuromuscular junctions (Brenner et al., 2003, FASEB J., 17, 214-222; Argov et al., 2003, Neurology, in press).

Keywords: acetylcholinesterase variants, alternative splicing, antisense oligonucleotides, cholinergic neurotransmission, protein kinase C

OUR EXPERIENCES WITH PHEOCHROMOCYTOMA DIAGNOSIS

STEJSKAL D., ZUBER R., LACNAK B., HORALIK D., PROSKOVA J., ANDELOVA K., ADAMOVSKA S., JURAKOVA R., BARTEK J. *Hospital Sternberk, Department of Laboratory Medicine, Sternberk, Czech Republic*

Pheochromocytomas are tumours with low prevalence (about 0.1% of population), their diagnosis is time consuming and difficult. In our hospital the following diagnostic algorithm has been applied: 1) determination of free NMN and MN or in combination with determination of conjugated NMN+MN in urine. 2) In case of ambiguous factors we examine all catecholamines in the plasma and urine, metanephrines and VMA in urine, or the clonidine test is performed. Since 1998, the above-mentioned determinations (except free nephrines in the plasma) have been made using the HPLC-ECD method. Examinations have been made for outpatient centers and wards of Olomouc and Moravian-Silesian regions. From September 2002 to end-January 2003 (6 months) we made 54 examinations and pheochromocytoma was diagnosed in 5 patients (9%). Diagnostic sensitivity and negative predictive value for determination of free plasma MN and NMN achieved 100%. To compare the achieved result, we present data obtained in 2001 (123 patients) when determination of conjugated nephrines in the urine showed 100% sensitivity and 72% specificity, determination of urine catecholamines had 75% sensitivity and 74% specificity, determination of plasma catecholamines showed 88% sensitivity and 57% specificity. Referring to recent literary data we confirmed that examination of free metanephrines in the plasma is the method with highest diagnostic sensitivity and specificity, minimal pre-analytical requirements and a simple interpretation.

Keywords: pheochromocytoma, methoxyamines, sensitivity, specificity

STRESS-INDUCED MODULATION OF IL-1 AND CRF RECEPTORS IN THE HYPOTHALAMIC-PITUITARY-ADRENOCORTICAL AXIS

TAKAO T., HASHIMOTO K., DE SOUZA E.B.[1] *Kochi Medical School, Nankoku, Japan and* [1]*Archemix Corporation, Cambridge, MA, USA*

Stress and infection potently activate the hypothalamic-pituitary-adrenocortical (HPA) axis through actions on corticotropin releasing factor (CRF) and interleukin-1 (IL-1), respectively. We evaluated the modulation of IL-1 receptors and Type 1 IL-1 receptor mRNA (IL-1R1 mRNA) by stress, CRF and CRF receptor antagonists. Ether-laparotomy stress in mice reciprocally increased [125I]IL-1a binding and decreased [125I]oCRF binding in the pituitary with no significant alterations observed in the brain. Moreover, i.p. injection of r/h CRF dramatically increased [125I]IL-1a binding in the pituitary without affecting [125I]IL-1a binding in the hippocampus. The stress-induced upregulation of IL-1 receptors in the pituitary gland was attenuated by systemic administration of mixed CRF_1/CRF_2 receptor peptide antagonist D-Phe12-Nle21,38 human CRF_{12-41}NH2 or pretreatment with the non-peptide, selective CRF_1 receptor antagonist, CRA 1000. CRF-induced increases in IL-1 receptors in the pituitary appear to be regulated at the corticotroph level since treatment of AtT-20 mouse pituitary adenoma cells with CRF produced dose-dependent increases in cAMP and [125I]IL-1a binding. These data provide further support for a role for IL-1 in coordinating HPA responses to stress and infection and a role for CRF and CRF_1 receptors in mediating the effects of the cytokine.

Keywords: interleukin-1 receptor, cytokines, stress, corticotropin releasing factor$_1$ receptor, pituitary

MOLECULAR AND PHARMACOLOGICAL MECHANISMS MEDIATING THE STRESS-INDUCED REGULATION OF TYROSINE HYDROXYLASE GENE EXPRESSION

TANK A.W., SUN B., CHEN X., MICKELSEN D., STERLING C. *University of Rochester Medical Center, Rochester, New York, USA*

Tyrosine hydroxylase is induced in adrenal medulla, sympathetic ganglia and locus coeruleus by many types of stressors. In this report we investigate some of the molecular and pharmacological mechanisms that mediate this response. First, we have tested which receptors are essential for the response of the TH gene in the adrenal medulla to different stressors. Our results suggest that different receptors mediate the response to acute vs chronic treatments and that individual chromaffin cell receptors differentially regulate transcriptional and post-transcriptional processes that participate in the induction of TH enzyme protein. We have also tested whether the response of the TH gene in the rat locus coeruleus is mediated by transcriptional and/or post-transcriptional mechanisms, using a novel semi-quantitative RT-PCR assay to measure TH RNA primary transcripts. Finally, we have used PC12 cells to investigate the signaling pathways and transcription factors that regulate the TH gene in response to agonist occupation of adrenal chromaffin cell receptors. Our results suggest that the mechanisms mediating this induction are complex, involving multiple receptors, multiple intracellular signaling pathways and both transcriptional and post-transcriptional regulation.

Keywords: tyrosine hydroxylase, adrenal medulla, locus coeruleus, stress

A SPECIFIC ROLE FOR NOREPINEPHRINE IN MEMORY RETRIEVAL: IMPLICATIONS FOR POST-TRAUMATIC STRESS DISORDER

THOMAS S.A., MURCHISON C.F., ZHANG X.-Y., ZHANG W.-P., OUYANG M., LEE A. *Department of Pharmacology, University of Pennsylvania, Philadelphia, PA, USA*

Interfering with adrenergic signaling has been reported to diminish passive avoidance retention and cued fear conditioning, while enhancing contextual fear conditioning. To more specifically test the roles of adrenergic signaling in emotional learning and memory, we examined dopamine β-hydroxylase mutant (*Dbh*$^{-/-}$) mice, which cannot synthesize norepinephrine (NE), using a fear conditioning protocol. Prior studies by us demonstrated that passive avoidance retention is normal in the *Dbh*$^{-/-}$ mice. We now report that cued fear conditioning is also unchanged. In contrast, *Dbh*$^{-/-}$ mice exhibit impaired retention of contextual fear conditioning. The deficit is present between 2 hours and 4 days after training, but not at 1 hour or 1 week. Restoration of CNS NE before testing but not before training restored normal freezing to the shock context. Acute pharmacologic treatments mimicked these results in control mice and rats. Similar results were obtained for spatial reference memory in rats in the Morris water maze. These results indicate that emotional memory formation is not dependent on NE. Instead, they suggest that retrieval of an intermediate phase of memory that is typically dependent on the hippocampus requires intact adrenergic signaling. Such results may be relevant to the intrusive memories associated with post-traumatic stress disorder.

Keywords: norepinephrine, learning, memory, emotion, post-traumatic stress disorder

FUNCTIONAL ACTIVITY OF THYROID GLAND IN MEN EXPOSED TO COLD STRESS

TIGRANIAN R., KALITA N., ASADI MOBARHAN A.H. *Institute for Control of Drugs, Moscow, Russia*

Blood levels of TSH, total T4, free T4 (fT4), total T3, free T3 (fT3) and reverse (rT3) were studies in 13 men on a 91 day 1800 km transpolar skitrek from Russia to Canada via the North Pole. Samples were taken 18 and 11 days before the start; at days 12, 29, 57 and 74 of the trek; and 3 days after the finish. Standard techniques of RIA were used to determine the content of hormones. The blood concentrations of TSH were only increased significantly on day 29 of the expedition. Blood concentrations of total T3did not change, but fT3 decreased at all stages of the trek relative to initial values. Blood concentrations of rT3 did not change, but total T4 increased in the first half of the journey, returning to baseline values for the second half of the trek. FT4 decreased at stages 1-3 of the traverse. The thyrotropic function of adenohypophysis increased only at stage 2 of the expedition and increased TSH/T3 ratio suggest that the response threshold of the hypothalamus/hypophyseal/thyroid axis has been increased. Considering the ability of TSH to activate not only the synthesis and secretion of T4 in the thyroid gland, but also peripheral deiodination, the change in deiodination at stage 2 (the decrease T4/T3 ratio) may be due simply to the increased blood levels of TSH. Generally, there was some early inhibition of thyroid function, the most distinctive feature being an active inhibition of deiodination to T3. Changes that followed the trek suggested an intensification of thyroid secretory activity, linked to the increase in total T3 during the period of readaptation to normal climates.

Keywords: thyroid gland, cold, stress

A PATIENT WITH ANEMIA AND ORTHOSTATIC HYPOTENSION

TIMMERS H.J.L.M., DEINUM J., BOOMSMA F., WEVERS R.A., LENDERS J.W.M. *University Medical Centre St. Radboud and Erasmus Medical Center Rotterdam, The Netherlands*

A 34-year-old female was referred after she had extensive haematological investigations elsewhere for a normocytic anemia and orthostatic dizziness. Autonomic function testing revealed an abnormal response of blood pressure during Valsalva's maneuver with normal reflexive changes in heart rate, indicating sympathetic failure with intact parasympathetic function. Plasma noradrenaline and adrenaline levels were below detection level whereas dopamine was elevated (1.12 nmol/l). These findings are pathognomic for complete deficiency of the enzyme dopamine-ß-hydroxylase (DβH). Sympathetic failure in this patient is caused by failure of neurochemical transmission due to the complete absence of the predominant neurotransmitter noradrenaline. The absence of a pressor response and neuronal noradrenaline release after tyramine, the absence of DβH enzyme activity in plasma and the demonstration of a homozygous 764G>T mutation of the DβH gene at the 9q34 confirmed the congenital DβH deficiency. DβH deficiency is an extremely rare autosomal recessive disorder with severe orthostatic hypotension as the most important clinical hallmark. However, another less appreciated clinical feature of sympathetic failure is a moderate normocytic anemia, which is probably due to inappropriately low erythropoietine levels. Apparently a normally functioning sympathetic nervous system seems to be necessary for adequate erythropoietine production. We present a patient with normocytic anemia caused by sympathetic failure based on DβH deficiency, a rare but treatable genetic disorder of catecholamine metabolism. Although this patient underwent extensive and repeated investigations because of the anaemia, a straight-forward investigation of the severe orthostatic hypotension would have provided a timely diagnosis of sympathetic failure and in this case treatment of the DβH deficiency.

Keywords: orthostastic hypotension, dopamine-ß-hydroxylase

ADRENAL CATECHOLAMINE AND ANGIOTENSIN II AT_2 RECEPTOR RESPONSES TO RESTRAINT STRESS REQUIRE INTACT SEROTONIN TRANSPORTER FUNCTION.

TJURMINA O.A.[1*], ARMANDO I.[2*], SAAVEDRA J.M.[2], LI Q.[1], MURPHY D.L.[1] [1]*Laboratory of Clinical Science and* [2]*Section on Pharmacology, NIMH, NIH, Bethesda, MD, USA. *Contributed equally*

This study examined whether serotonin transporter (SERT) deficiency influences adrenal serotonin (5-HT), catecholamine and Angiotensin II systems responses, as well as hormonal responses to acute restraint stress. Control mice (+/+) expressed high numbers of SERT binding sites and a high 5-HT content in the adrenal medulla. 15 minutes restraint stress increased adrenal 5-HT in parallel with a strong increase in adrenomedullary tyrosine hydroxylase (TH) mRNA expression and plasma epinephrine (EPI) and norepinephrine levels without alterations in adrenal catecholamine content. The 5-HT and catecholamine responses to stress in control mice coincided with a significant increase in adrenomedullary Angiotensin II AT_2 receptor expression. Conversely, SERT deficient mice (-/-) did not express SERT binding sites in adrenal medulla, their basal 5-HT was significantly depleted and did not increase after stress. In SERT -/- mice, the stress-induced EPI release into plasma was higher than in SERT +/+, the increase in TH mRNA expression did not occur, adrenal catecholamine content was decreased, and instead of increased expression, stress induced a profound decrease in the number of adrenomedullary AT_2 receptors. SERT -/- also possessed decreased pituitary 5-HT, their pituitary ACTH was reduced after stress, but stress-induced increase in plasma ACTH and corticosterone was not different from those in +/+. Our results indicate that SERT function not only restrains stress-induced EPI release but also is required for the increase in adrenal catecholamine synthesis and AT_2 receptor expression.

Keywords: epinephrine, TH mRNA, angiotensin II receptors, serotonin, SERT deficiency

MOLECULAR MECHANISM OF EMOTIONAL STRESS- AND CATECHOLAMINE-INDUCED CARDIOMYOPATHY

UEYAMA T. *Department of Anatomy and Cell Biology, Wakayama Medical University, Wakayama, Japan*

A novel cardiac syndrome known as ”Tako-tsubo (Ampulla)“ cardiomyopathy mimicking acute myocardial infarction has recently been proposed by Japanese cardiologists. This is characterized by 1) acute onset, reversible left ventricular apical wall motion abnormality (”Tako-tsubo“ shape, or Ballooning) on LVG, 2) ST elevation on ECG, 3) minimal myocardial enzymatic release, 4) no significant stenosis or spasm on CAG, 5) high incidence in post-menopausal female, and 6) triggered by emotional or physical stress. Immobilization (IMO) of rats, an animal model of emotional stress can reproduce these LVG and ECG changes, both of which are normalized by pretreatment with αβ-adrenoceptor blockers. Increase of estradiol levels can also attenuate these changes. In response to IMO stress, a rapid activation of p44/p42 MAP kinase is observed in the heart, followed by a transient up-regulation of immediate early genes (IEG) and HSP in the heart. Natriuretic peptide genes are also up-regulated in the myocardium. Sequential gene expressions can be considered as an adaptive response to emotional stress. Blocking of both a-and b- adrenoceptors eliminates the up-regulation of IEG and MAP kinase induced by stress, while α- or β-agonist up-regulates IEG and MAP kinase in the perfused heart. Taken together, activation of α- or β-adrenoceptors is the primary trigger of emotional stress-induced unique cardiomyopathy.

Key words: animal model, cardiomyopathy, gene expression, adrenoceptor, estrogen

THE EXCRETION RATES OF STRESS HORMONES UNDER RADIOFREQUENCY ELECTROMAGNETIC RADIATION AND 24-HOUR SHIFTS

VANGELOVA K.K., ISRAEL M.S., VELKOVA D.P. *National Center of Hygiene, Medical Ecology and Nutrition, Sofia, Bulgaria*

The aim of the study was to follow the time-of-day variations of adrenaline, noradrenaline and cortisol in operators under radiofrequency electromagnetic radiation (EMR) and 24-hour shifts. The excretion rates of stress hormones were followed in 36 male operators, divided in three groups as follows: I group - 12 operators from a broadcasting station, II group - 12 operators from a TV station and III group - 12 operators from a satellite station. The catecholamines were assessed using spectrofluorimetric method and cortisol with radioimmunological kits on four-hour intervals during the 24-hour shifts. The EMR exposure and the confounding work stressors were assessed. The dosimetric data show high level radiofrequency EMR exposure with I group, low level one with II group and very low radiofrequency EMR with III group. The latter was used as control group. The tests of between subject effects revealed significant effect of high level radiofrequency EMR exposure on adrenaline ($F_{(1,143)}$=4.941, p=0.028), noradrenaline ($F_{(1,143)}$=20.980, p<0.001) and cortisol ($F_{(1,143)}$=12.724, p<0.001) excretion rates. The effect of low level exposure did not reach significance, but it was in the frame of trend for cortisol. No significant differences in working conditions, work content, work control and work related social support were found. The significant and dose-dependant effect of radiofrequency EMR on catecholamines and cortisol levels rises health concerns and further studies are needed to clarify the effect EMR with different frequency and intensity parameters on stress system and their significance.

Keywords: catecholamines, cortisol, electromagnetic radiation, radiofrequency band, shiftwork

BASAL AND STRESS-INDUCED DIFFERENCES IN HPA AXIS, 5-HT RESPONSIVENESS AND HIPPOCAMPAL CELL PROLIFERATION IN TWO MOUSE LINES

VEENEMA A.H.[1], KOOLHAAS J.M.[1], DE KLOET E.R.[2] [1]*Dept. of Animal Physiology, Univ. of Groningen,* [2]*Div. of Medical Pharmacology, LACDR/Leiden University Medical Center, The Netherlands*

To characterize neuroendocrine and neurobiological correlates of stress coping, two lines of wild house-mice were studied. These mice are genetically selected for high and low aggression and show distinctly different behavioural coping styles. SAL (Short Attack Latency) mice display an 'active' coping style, whereas LAL (Long Attack Latency) mice show a 'passive' coping style. We studied two systems involved in stress adaptation, i.e. the hypothalamus-pituitary-adrenal (HPA) axis and the serotonergic (5-HT) system, and also hippocampal cell proliferation. Under basal conditions, the corticosterone output in LAL mice was more sensitive to adrenocorticotropic hormone (ACTH), but showed less day-night variation than in SAL mice. LAL mice showed lower hippocampal 5-HT_{1A} receptor gene expression and lower 5-HT turnover in striatum and amygdaloid region. Basal hippocampal cell proliferation rate (using the endogenous marker Ki-67) was slightly lower in LAL than in SAL mice. Exposure to acute stress (forced swimming for 5 min.) resulted in a hyperreactive HPA axis and lower 5-HT turnover in several brain regions in LAL compared to SAL mice. The same stressor induced an almost two-fold lower hippocampal cell proliferation rate (using the exogenous marker BrdU) in LAL compared to SAL mice. In conclusion, these studies show that a genetic trait in behavioural coping style in wild house-mice is associated with differences in HPA regulation, 5-HT responsiveness and hippocampal cell proliferation rate. This might have implications for a differential susceptibility to chronic stress and stress-related mood disorders.

Keywords: cell proliferation, coping style, HPA axis, 5-HT, stress

GENETICS MECHANISMS FOR ADRENERGIC CONTROL DURING STRESS

WONG D.L., TAI T.C., WONG-FAULL D.C., KVETNANSKY R.[1] ,CLAYCOMB R. *Harvard Medical School and McLean Hospital, Belmont, MA USA,* [1]*Institute of Experimental Endocrinology, SAS, Bratislava, Slovakia*

Cortisol and epinephrine released in response to stress are replenished via activation of the hypothalamic-pituitary-adrenal (stress or HPA) axis. Immobilization (IMMO) stress in rats stimulates epinephrine production in part via the gene encoding the epinephrine-synthesizing enzyme, phenylethanolamine N-methyltransferase (PNMT). PNMT mRNA rises up to 3.5-fold with acute or chronic stress (30 or 120 min IMMO once or daily for 7 days). Two transcription factors mediating stress induction of the PNMT gene are the glucocorticoid receptor (GR) and Egr-1, which interact with -533, -759 and -773 bp and -165 bp binding sites in the PNMT promoter respectively. To identify molecular mechanisms involved, effects of hypoxia on PNMT promoter activity were examined in PC12 cells transfected with the PNMT promoter-luciferase reporter gene construct pGL3RP893. Oxygen reduction to 5% increased PNMT promoter-driven luciferase expression, with maximum activity at 6 hr. Pretreatment of the cells with PKA and PKC inhibitors H-89 and GF109203X respectively attenuated the rise in luciferase. Similarly, PKA-deficient PC12 cells transfected with pGL3RP893 and exposed to hypoxia also showed attenuated PNMT promoter driven-luciferase expression. Mutation of the Egr-1 binding site completely prevented PNMT promoter activation, indicating that Egr-1 is essential to the stress response. Consistent with this result, hypoxia increased Egr-1 protein. Hypoxia also increased endogenous PNMT mRNA. However, a shift to intron-retaining mRNA from which truncated, non-functional protein is produced, occurred, suggesting that post-transcriptional regulation may be an important genetic mechanism controlling adrenergic expression and hence, epinephrine, during stress.

Keywords: PNMT, stress, genetic mechanisms

COLD STRESS INDUCES THE ALTERNATIVE SPLICING OF DBH AND NET mRNAS

YATSENKO O.P., SENNIKOV S.V. *Institute of Clinical Immunology, Novosibirsk, Russia*

Influence of cold stress (1 h at 4°C) on dopamine β-hydroxylase (DBH) and norepinephrine transporter (NET) in mouse adrenal medulla was studied. RT-PCR analysis with DBH cDNA specific primers revealed two PCR fragments: major correspondent to normal length and short-cut minor. The amplification products were characterized after isolation by subsequent bidirectional sequencing. Deletion of 147 bp region corresponding to exon 2 was detected in minor PCR fragment. The deletion of exon 2 does not result in a frameshift. Thus, this alternatively spliced DBH mRNA form (DBH-d2) may code for a shorter, truncated DBH protein, missing 49 amino acids (118-166) overlapping with the DoH domain (60-142). DoH domains were identified in four mammalian proteins (dopamine β-hydroxylase, its paralogue, mono-oxygenase X, SDR2 and CG-6). Probably it can posses a catecholamine-binding function provided negative feedback mechanism with norepinephrine binding to the dopamine β-hydroxylase DoH domain thereby allosterically inhibiting the protein's enzymatic activity. Slight, but significant increase of DBH mRNA level was shown in the cold stress group in comparison with the control animals. Same time the DBH-d2 mRNA was detected in adrenal glands of control mice at very low levels and was considerably induced by cold stress (at least 10 times). Using same cDNA samples we demonstrated that cold stress can differentially induce norepinephrine transporter mRNA isoforms lucking 13-14 exons. Further studies are needed to elucidate whether this is an aberrant splicing or regulatory mechanism on the base of the physiological functions for this putative truncated proteins.

Keywords: alternative splicing, cold stress, DBH, NET

MATERNAL GENOTYPE INFLUENCES STRESS REACTIVITY OF BRATTLEBORO RATS GENETICALLY LACKING VASOPRESSIN

ZELENA D., MERGL ZS., MAKARA G.B. *Hungarian Academy of Sciences, Institute of Experimental Medicine, Budapest, Hungary*

The role of vasopressin (AVP) in stress is debated, since normal as well as reduced adrenocorticotrop hormone (ACTH) rise to an acute challenge have been reported in Brattleboro rats genetically lacking AVP (AVP-). Since AVP- pups could be born either from AVP+ (heterozygous) or from AVP- mothers, and maternal influence is known to modify adult responsiveness, we asked whether the influence of maternal genotype could explain the variability. Adult rats from mothers with different genotypes were stressed with 60 min restraint and at the end of stress trunk blood was collected for measuring hormone content by radioimmunoassay. All offspring of AVP+ mothers had similar ACTH responses to restraint, while the AVP- rats born to, and raised by AVP- mothers showed reduced ACTH reactivity to restraint. The AVP- rats show elevated water turnover and require a clean cage every day, which means frequent handling. To offset the role of handling in the next experiments all animals were given clean cages every day, but the results were the same as in the first series. To find out if the behavior of the mother or some other factors during the pregnancy is responsible for the differences, pups from AVP+ mothers were raised by AVP- mother and conversely. We found that the genotype of parental mother is more important than that of the nursing mother. Our results suggest that AVP is not an indispensable factor for ACTH release and the AVP- genotype of the parental mother can decrease the stress reactivity of the AVP- Brattleboro rats. In studies with mutant animals also the experimenter should control the rearing conditions.

Keywords: ACTH, handling, nursing, Brattleboro rats

OXYTOCIN- AND CORTICOTROPIN-RELEASING FACTOR NEURONS MEDIATE THE CARDIOVASCULAR RESPONSE INDUCED BY SUBSTANCE P IN THE FOREBRAIN

ZHAO Y., JIRIKOWSKI G.[1], UNGER T., CULMAN J. *Institute of Pharmacology, Christian-Albrechts University of Kiel and* [1]*Institute of Anatomy II, Friedrich-Schiller University of Jena, Germany*

Substance P (SP), a natural ligand for NK_1 receptors, has been implicated in the initiation and regulation of cardiovascular, behavioural and endocrine responses to stress, which are generated in the hypothalamus. To address the role of oxytocin (OT), vasopressin (AVP) and corticotropin-releasing factor (CRF) neurons in the paraventricular nucleus (PVN) we investigated the effects of central pretreatment of rats with OT, AVP and CRF antisense (AS) and mixed base (MB) oligodeoxy-nucleotides (ODNs) on mean arterial pressure (MAP), heart rate (HR) and behavioural responses (BR) induced by stimulation of periventricular NK_1 receptors. Male Wistar received two intracerebroventricular (i.c.v.) injections of vehicle (controls), AS ODN directed against the initiation codon of the respective mRNA or MB ODN. The time interval between the two i.c.v. injections was 4 h. Four h after the second i.c.v. injection, SP (50 pmol) was injected i.c.v. and MAP, HR and BR were recorded. I.c.v. pretreatment with OT AS ODN attenuated both, the MAP and HR responses to i.c.v. SP while CRF AS ODN reduced only the MAP response. AVP AS ODN or MB ODNs did not affect the cardiovascular response to SP. Our results demonstrate that OT neurons in the PVN innervating the cardiovascular brain stem areas transmit signals generated by SP in the forebrain to induce increases in blood pressure and HR. CRF neurons participate in the generation of hypertensive reactions in response to SP. OT and CRF neurons may also transmit signals which are generated by SP in the hypothalamus and responsible for the sympathoadrenal activation upon stress.

Keywords: substance P, oxytocin, vasopressin, CRF, brain

AT_1 RECEPTOR BLOCKADE DOWN-REGULATES STRESS-LINKED HEAT SHOCK PROTEINS IN BRAIN MICROVESSELS FROM SPONTANEOUSLY HYPERTENSIVE RATS

ZHOU J., JEZOVA M., ANDO H., SAAVEDRA J.M. *Section on Pharmacology, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA*

We purified rat brain microvessels and used microarrays to determine gene transcription. We studied the stress-vulnerable strain of Spontaneously Hypertensive Rats (SHR) and their normotensive controls Wistar Kyoto Rats. Each strain was divided in two groups, treated with vehicle or an antagonist of Angiotensin II (Ang II) AT_1 receptors, administered peripherally. The objective was to determine the effects of hypertension and of blockade of the Ang II system on gene transcription in cerebral microvessels, to clarify mechanisms of protection against ischemia by this class of compounds. We found changes in gene transcription of heat shock proteins (Hsps), highly expressed during the heat shock response, characterized by exposure of cells and tissues to extreme stress conditions. We found that Hsps genes, including hsp 70, hsp 27 and hsp 90, were up-regulated in SHR when compared with WKY rats. This up-regulation was prevented by the AT_1 receptor antagonist candesartan (sc., 0.3 mg/kg/day, 28 days). We hypothesized that Hsps are involved in the mechanisms of increased vulnerability to stress in SHR rats, and that inhibition of AT_1 receptors in brain microvessels participates in the mechanism of protection from stress, that is characteristic of this class of compounds.

Keywords: microvessels, ischemic stress, heat shock proteins, AT_1 receptors, angiotensin II

GENOMIC CONTROL OF CARDIAC ADRENALINE SYNTHESIZING ENZYMES

ZIEGLER M.G., BAO X., MAHATA S., KENNEDY B.P., JOINER A. *UCSD, San Diego CA, USA*

In the fetal rat immunostaining tyrosine hydroxylase (TH) was visible in paraspinal primordial ganglia and gut tubes by embryonic day 11 (e 11) and by e 16 axons staining for TH were growing toward the heart. In contrast, dopa decarboxylase activity peaked in the developing heart by day e 16 and phenylethanolamine N methyltransferase (PNMT) activity peaked by e 12. PNMT activity in the heart fell rapidly toward day e 16, when adrenal A and cardiac NA were increasing. PNMT in the adrenal was absent in glucocorticoid receptor deficient mice, but they still had cardiac PNMT. PNMT mRNA in the adrenal was present in a single form, but PNMT mRNA in the heart was present in intron retained and intronless forms. The intron retained PNMT mRNA contained a stop codon in the first intron and translated a truncated PNMT protein. Glucocorticoid treatment enhanced removal of the intron from PNMT mRNA and was associated with greater production of the whole PNMT molecule and greater levels of PNMT enzymatic activity. Thus, glucocorticoids enhanced PNMT production in the heart by a different mechanism than in the adrenal. PNMT activity in the fetal heart was able to produce A when pregnant rats were fed L-DOPA. A was not otherwise detectable in fetal heart, either because it was present in levels too low to measure or because of the absence of TH activity. In the adult rat heart, measurable levels of A remained, even after adrenalectomy, suggesting local PNMT activity. The burst of PNMT in early fetal cardiac development suggests a possible role in embryogenesis. It will be possible to study this in a PNMT knockout mouse currently breeding. It may also be possible to study low PNMT activity in man, since a naturally occurring human PNMT promoter containing 161A/367A rather than the wild type 161G/367G has only 20% normal activity when linked to a luciferase reporter gene.

Keywords: PNMT, heart, glucocorticoid, embryo

AUTHOR INDEX FOR ABSTRACTS