

**EIGHTH SYMPOSIUM  
ON CATECHOLAMINES  
AND OTHER NEUROTRANSMITTERS  
IN STRESS**

**SMOLENICE CASTLE, SLOVAKIA**

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

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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 Brain septum was immediately withdrawn and incubated *in vitro* to measure basal, ethanol and hyposmotic stimulated TRH release.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**

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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**

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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**

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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

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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<sub>1</sub> and AT<sub>2</sub> receptors regulate basal catecholamine synthesis controlling the TH transcription factor Fra-2. AT<sub>1</sub> receptor stimulation additionally regulates TH transcription through participation of pCREB and ERK2. During stress, AT<sub>1</sub> 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<sub>1</sub> receptors control CRH formation and release. AT<sub>1</sub> receptor blockade resets the HPA axis tone and makes animals less vulnerable and responsive to stress. When stress occurs under AT<sub>1</sub> receptor blockade, the release of ACTH, corticosterone and vasopressin is substantially decreased. The prevention of stress-induced cortical CRH<sub>1</sub> receptor decrease, and the anti-anxiety effects of AT<sub>1</sub> antagonists suggest a modulation of the cognitive response to stress. This explains how selective AT<sub>1</sub> 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<sub>1</sub> receptors, AT<sub>2</sub> receptors, CRH receptors

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

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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**

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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<sub>1</sub> RECEPTOR BLOCKADE REDUCES STRESS-INDUCED RELEASE OF CATECHOLAMINES, GLUCOCORTICOIDS AND VASOPRESSIN**

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We have recently reported that a two-week pretreatment with an angiotensin II (Ang II) AT<sub>1</sub> antagonist prevented the adrenomedullary and hormonal response to acute isolation stress (Armando et al., *Endocrinology*, 2001). We now report that AT<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> receptor antagonist is long lasting, differs between stress-prone SHR and WKY, and that specific components of the stress response react differently to AT<sub>1</sub> receptor blockade.

**Keywords:** isolation stress, angiotensin AT<sub>1</sub> receptors, stress, catecholamines, vasopressin

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

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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**

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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<sup>-1</sup> 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**

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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 (ICAI-CT-2000-70008), VEGA 2/2090 and ETT 277/2001.*

**Keywords:** salsolinol, immobilization stress, catecholamines

**ANGIOTENSIN II AT<sub>1</sub> RECEPTOR BLOCKADE PREVENTS GASTRIC ULCERS DURING COLD RESTRAINT STRESS**

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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<sub>1</sub> receptor antagonist candesartan before cold-restraint stress. AT<sub>1</sub> 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  $\alpha$  (TNF- $\alpha$ ), adhesion protein ICAM-1 in arterial endothelium and neutrophil infiltration in the gastric mucosa, and decreased PGE<sub>2</sub> content. AT<sub>1</sub> receptor blockers prevent stress-induced ulcerations by a combination of gastric blood flow protection, decreased sympathoadrenal activation, anti-inflammatory effects with reduction in TNF- $\alpha$ , and ICAM-1 expression leading to reduced neutrophil infiltration, while maintaining the protective glucocorticoid effects and PGE<sub>2</sub> release. Ang II has a crucial role, through stimulation of AT<sub>1</sub> receptors, in the production and progression of stress-induced gastric injury, and AT<sub>1</sub> receptor antagonists could be of therapeutic benefit.

**Keywords:** stress ulcers, inflammation, angiotensin II receptors, AT<sub>1</sub> receptor antagonists



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

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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 °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**

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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?

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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  $24 \pm 0.8$ ) 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 (ICAI-CT-2000-70008).*

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

### THE NEUROENDOCRINOLOGY OF THE HUMAN STRESS RESPONSE

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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  $\beta$ -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**

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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<sub>1</sub> (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 NK<sub>1</sub> receptor knock-out mice or after pharmacological blockade of central NK<sub>1</sub> receptors by the selective, non-peptide NK<sub>1</sub> 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<sub>1</sub> 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 serotonergic system in the brain participates in the SP-induced inhibition of the HPA, and 2) genetic disruption or pharmacological blockade of the NK<sub>1</sub> 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**

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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 thermogenic capacity. As the indicator of the SNS function intensity the activity of serum dopamine-β-hydroxylase (DBH) and IBAT monoamine oxidase (MAO) were examined in male Wistar rats chemically thyroidectomized by methimazole (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**

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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- $\beta$ -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**

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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 (1<sup>st</sup> wounds to the right ventricle of heart, 2<sup>nd</sup> wounds to the left ventricle of heart, and 3<sup>rd</sup> 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**

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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**

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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

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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 (CORT) 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

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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 hypokinesia. 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**

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Anxiety is an emotional state believed to be related to stress response. However, only limited information is available on the links between anxiety 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 anxiety 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 anxiety 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 anxiety 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 anxiety 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**

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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  $\beta 1$ -adrenergic receptors. On the other hand,  $\alpha 1$ -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?**

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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**

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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- $\alpha$  and interferon (IFN)- $\gamma$ , whereas they stimulate the production of IL-10 and transforming growth factor (TGF)- $\beta$ . 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- $\alpha$  production, while ATP via the P2X7 receptor may activate the post-translational processing of IL-1 $\beta$  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**

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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 ( $\beta$ -ARS) IN EPIDIDYMAL ADIPOSE TISSUE FROM STRESSED RATS**

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We have demonstrated that adipocytes isolated from epididymal 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  $\beta$ -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  $\beta$ -ARs. Results: Foot shock stress decreased GR,  $\beta_1$ -AR and  $\beta_3$ -AR protein levels, but increased  $\beta_2$ -AR. Conclusions: Our results confirm that the supersensitivity to isoprenaline and subsensitive to norepinephrine previously reported are associated with an increase of  $\beta_2$ -AR and a decrease of  $\beta_1$ -AR and  $\beta_3$ -AR. These alterations on proteins expression are modulated by high corticosterone levels, which did also down-regulate its own receptor. *Financial Support: FAPESP.*

**Keywords:**  $\beta$ -adrenoceptors, foot shock stress, glucocorticoid receptor, adipose tissue, rats

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

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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**

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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-associated 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**

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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**

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*Objective:* Neuroprotective agents to dopaminergic neurons exposed to oxidative stress. *Methods:* Primary dopaminergic cultures were treated with MPP<sup>+</sup>, L-DOPA or rotenone together with dopamine agonists and coenzyme Q<sub>10</sub>. Cell viability, dopamine uptake, and lactate were determined. Striatal slice cultures of adult mice were subjected to MPP<sup>+</sup> treatment and coenzyme Q<sub>10</sub>. *Results:* The number of dopaminergic neurons in primary culture is markedly reduced by treatment with MPP<sup>+</sup>, 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<sub>10</sub> protected tyrosine hydroxylase, respiratory chain complexes and hexokinase from loss in activity after MPP<sup>+</sup>-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**

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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**

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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 reinnervation. 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 reinnervation.

**Keywords:** human heart, transplantation, PNMT

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

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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, neurotrophin, 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, serotonergic, 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**

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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 increasingly 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**

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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**

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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

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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 (1X10<sup>6</sup> 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

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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 ( $46.8 \pm 7.9$  ng/ml and  $32.1 \pm 13.1$  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

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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  $\text{VO}_{2\text{MAX}}$  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

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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 [<sup>18</sup>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 [<sup>76</sup>Br]MJL 1-109-2 was proven by blocking with 10<sup>-6</sup> M  $\alpha$ -CRH or sauvagine and by 10<sup>-8</sup> 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**

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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**

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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

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Acute hypercapnia (>15% CO<sub>2</sub>), in humans, increases sympatho-adrenomedullary (SAM) and hypothalamo-pituitary-adrenal (HPA) axis output, and elicits panic-like symptoms. Since 1) evidence suggests that elevated CO<sub>2</sub> is detected centrally within brainstem noradrenergic (NA) and adrenergic (A) nuclei, and 2) glucocorticoids may alter the excitability of CO<sub>2</sub>-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% CO<sub>2</sub>) 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 CO<sub>2</sub> 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

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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**

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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 INTER-ACTIONS**

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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- $\kappa$ B DNA-binding activity and downregulation of I $\kappa$ B 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*<sup>-/-</sup>) and *Crh*<sup>+/+</sup> mice. Our findings revealed delayed corticosterone rise in the *Crh*<sup>-/-</sup> mice, together with very elevated plasma TNF $\alpha$  and chemokines, such as MIP-1 and MIP-2, at the very early stages of endotoxemia. Interestingly, the susceptibility of the *Crh*<sup>-/-</sup> mice to a moderate dose of LPS (120 $\mu$ g/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*<sup>+/+</sup> mice. We have previously shown altered epinephrine secretion during inflammation in *Crh*<sup>-/-</sup> 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, TNF $\alpha$ , epinephrine

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

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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**

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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<sub>3</sub> RECEPTORS IN RAT HEART**

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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<sub>3</sub> 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<sub>3</sub> receptors. Contrary to single immobilization, repeated immobilization decreased a gene expression of the type 1 and 2 IP<sub>3</sub> receptors, and also the protein levels of IP<sub>3</sub> receptors. Although the physiological relevance of our observations remains to be elucidated, we propose that decrease in IP<sub>3</sub> 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**

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Inositol 1,4,5-trisphosphate receptors (IP<sub>3</sub> receptors) are intracellular calcium channels, releasing calcium from the sarcoplasmic reticulum. In the heart, IP<sub>3</sub> 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<sub>3</sub> receptor. However, in stellate ganglia, which innervate the heart, no changes in the mRNA of the type 1 IP<sub>3</sub> receptors were observed after single immobilization stress. In renal medulla, a moderate decrease in both, mRNA and protein levels of IP<sub>3</sub> receptors was observed after single immobilization exposure. After repeated immobilization, mRNA and protein levels of the type 1 and 2 IP<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> receptors, immobilization stress, heart, gene expression



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

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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 5<sup>th</sup> day of SF, on the 2<sup>nd</sup> and 16<sup>th</sup> days after the landing in the first Slovak astronaut, and before and on the 5<sup>th</sup> 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**

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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 hypothalamic-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 ADRENO-CORTICAL RESPONSES TO PSYCHOSOCIAL STRESS**

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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 (*BclI* RFLP, N363S, ER22/23EK) on cortisol and ACTH responses to psychosocial stress and pharmacological stimulation. Compared to subjects with the rather common allele pattern '*BclI* 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 *BclI* 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 *BclI* 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<sub>1-24</sub> 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**

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Stress-induced mRNA levels of tyrosine hydroxylase (TH), dopamine-β-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**

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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**

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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**

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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?**

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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 recanalizes 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 TOPOGRAPHICAL- LY ORGANISED SUBSETS OF SEROTONERGIC NEURONES

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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

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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**

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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**

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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**

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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**

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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 <sup>125</sup>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**

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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-vasopressin, 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**

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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- $\beta$ -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 002/2001 and 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**

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The HPA axis can be stimulated by serotonin via both 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors. This study was undertaken to localize the neurons affected by either a 5-HT<sub>1A</sub> receptor (8-OH-DPAT) or 5-HT<sub>2A</sub> 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<sub>1A</sub> and 5-HT<sub>2A</sub> 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**

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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 delivered 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**

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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**

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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<sup>2</sup>) 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**

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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 40<sup>th</sup> 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**

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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,  $\beta$ -ADRENOCEPTORS AND ADENYLYL CYCLASE IN DIFFERENT HEART REGIONS**

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Heart muscarinic receptors (MR) and  $\beta$ -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 ( $\beta_1$ -AR and  $\beta_2$ -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,  $\beta$ -adrenoceptors, adenylyl cyclase, immobilization stress, heart

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

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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

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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  $\beta$ 1,2-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-[<sup>18</sup>F]FLUORODOPAMINE POSITRON EMISSION TOMOGRAPHY

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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 [<sup>18</sup>F]-fluorodeoxyglucose, [<sup>11</sup>C]-hydroxyephedrine or [<sup>11</sup>C]-epinephrine PET scanning with either suboptimal sensitivity or specificity. Here we report results using 6-[<sup>18</sup>F]fluorodopamine PET scanning in the localization of pheochromocytoma. Sixty three patients with known or clinically suspected pheochromocytoma underwent PET scanning with 6-[<sup>18</sup>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-[<sup>18</sup>F]fluorodopamine PET imaging. A total of 63 patients were enrolled in this study (23 men, 40 women, mean ages  $41.8 \pm 3.2$  and  $45.8 \pm 2.3$  years). 29 of 63 patients were found to have pheochromocytoma, confirmed histologically in the 27 who underwent surgery. Overall, 6-[<sup>18</sup>F]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-[<sup>18</sup>F]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-[<sup>18</sup>F]fluorodopamine scanning in the diagnostic localization of pheochromocytoma.

**Keywords:** pheochromocytoma, fluorodopamine, catecholamines



**LOCALIZATION AND CHEMICAL CHARACTERIZATION OF THE AUDIOGENIC STRESS PATHWAY**

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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**

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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**

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Catecholamines are essential for mouse heart development at e10.5 to e13.5. In these early embryos, 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  $\beta$ -galactosidase expression in adrenergic cells and their descendants. Under these conditions, we show that  $\beta$ -galactosidase is evident in the developing mouse heart as early as e8.5. By e10.5, extensive non-uniform labeling of ICA cells with  $\beta$ -galactosidase 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,  $\beta$ -galactosidase 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)**

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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 visceros- and somato-sensory signals, were studied in wild-type mouse exposed to 60 min of hypertonic (400  $\mu$ l 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 2/2007 and 2/2090 grants.*

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

**5-HT<sub>1A</sub> RECEPTOR ACTIVATION PRIOR ACUTE STRESS COUNTERACTED THE INDUCED BEHAVIOURAL LONG-TERM EFFECTS**

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The behavioural long-term consequences of acute immobilization (IMMO) in rats and the effects of 5-HT<sub>1A</sub> 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 pre-treatment 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<sub>1A</sub> receptors stimulation is capable to counteract this long-term effects induced by IMMO. *Supported by Spanish DGCYT (PM-0159).*

**Keywords:** passive avoidance, escape behaviour, immobilization, 5-HT<sub>1A</sub> receptors, corticosterone, c-Fos

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

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Angiotensin II (Ang II) is present in most organs, including the brain. The discovery that selective blockade of brain and peripheral Ang II AT<sub>1</sub> 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<sub>1</sub> receptor expression and function and the cognitive response to stress. The recent finding that AT<sub>1</sub> 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<sub>1</sub> receptor antagonists, stress therapy, stress prevention

**MOLECULAR REGULATION OF GENE EXPRESSION OF CATECHOLAMINE BIOSYNTHETIC ENZYMES BY STRESS**

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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**

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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 pathophysiologicals 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

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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 nephines 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 nephines 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

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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<sub>1</sub>/CRF<sub>2</sub> receptor peptide antagonist D-Phe12-Nle21,38 human CRF<sub>12-41</sub>NH<sub>2</sub> or pretreatment with the non-peptide, selective CRF<sub>1</sub> 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<sub>1</sub> receptors in mediating the effects of the cytokine.

**Keywords:** interleukin-1 receptor, cytokines, stress, corticotropin releasing factor<sub>1</sub> receptor, pituitary

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

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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**

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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  $\beta$ -hydroxylase mutant (*Dbh*<sup>-/-</sup>) 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*<sup>-/-</sup> mice. We now report that cued fear conditioning is also unchanged. In contrast, *Dbh*<sup>-/-</sup> 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**

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Blood levels of TSH, total T4, free T4 (fT4), total T3, free T3 (fT3) and reverse (rT3) were studied 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 T3 did 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 suggests 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**

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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- $\beta$ -hydroxylase (D $\beta$ H). 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 D $\beta$ H enzyme activity in plasma and the demonstration of a homozygous 764G>T mutation of the D $\beta$ H gene at the 9q34 confirmed the congenital D $\beta$ H deficiency. D $\beta$ H 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 D $\beta$ H 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 D $\beta$ H deficiency.

**Keywords:** orthostatic hypotension, dopamine- $\beta$ -hydroxylase

### ADRENAL CATECHOLAMINE AND ANGIOTENSIN II AT<sub>2</sub> RECEPTOR RESPONSES TO RESTRAINT STRESS REQUIRE INTACT SEROTONIN TRANSPORTER FUNCTION.

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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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> receptor expression.

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

### MOLECULAR MECHANISM OF EMOTIONAL STRESS- AND CATECHOLAMINE-INDUCED CARDIOMYOPATHY

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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  $\alpha\beta$ -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  $\alpha$ - and  $\beta$ -adrenoceptors eliminates the up-regulation of IEG and MAP kinase induced by stress, while  $\alpha$ - or  $\beta$ -agonist up-regulates IEG and MAP kinase in the perfused heart. Taken together, activation of  $\alpha$ - or  $\beta$ -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

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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

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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 adrenocorticotrophic hormone (ACTH), but showed less day-night variation than in SAL mice. LAL mice showed lower hippocampal 5-HT<sub>1A</sub> 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

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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

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Influence of cold stress (1 h at 4°C) on dopamine  $\beta$ -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  $\beta$ -hydroxylase, its paralogue, mono-oxygenase X, SDR2 and CG-6). Probably it can possess a catecholamine-binding function provided negative feedback mechanism with norepinephrine binding to the dopamine  $\beta$ -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 lacking 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**

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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<sup>-</sup>). Since AVP-pups could be born either from AVP<sup>+</sup> (heterozygous) or from AVP<sup>-</sup> 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<sup>+</sup> mothers had similar ACTH responses to restraint, while the AVP<sup>-</sup> rats born to, and raised by AVP<sup>-</sup> mothers showed reduced ACTH reactivity to restraint. The AVP<sup>-</sup> 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<sup>+</sup> mothers were raised by AVP<sup>-</sup> 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<sup>-</sup> genotype of the parental mother can decrease the stress reactivity of the AVP<sup>-</sup> 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**

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Substance P (SP), a natural ligand for NK<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> RECEPTOR BLOCKADE DOWN-REGULATES STRESS-LINKED HEAT SHOCK PROTEINS IN BRAIN MICROVESSELS FROM SPONTANEOUSLY HYPERTENSIVE RATS**

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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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> receptors, angiotensin II

**GENOMIC CONTROL OF CARDIAC ADRENALINE SYNTHESIZING ENZYMES**

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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

- Abe K. 113  
 Adameova A. 83  
 Adamovska S. 127  
 Adrian J. 106  
 Aguilera G. 83  
 Aguirre J.A. 125  
 Akana S.F. 92  
 Alcalde F.J. 125  
 Alesci S. 84, 122  
 Amaral M.E. 97  
 Andelova K. 127  
 Ando H. 84, 88, 125, 134  
 Armando I. 85, 86, 88, 125, 130  
 Armario A. 85  
 Arun C.P. 86  
 Asadi Mobarhan A.H. 129  
 Bacova Z. 83  
 Bagaeva T.R. 98  
 Baiardi G. 85, 86, 88, 125  
 Bakalov V.K. 96  
 Bakos J. 87  
 Bali B. 108  
 Bao X. 134  
 Barna I. 115  
 Bartak V. 87, 122  
 Bartek J. 127  
 Blakley G. 123  
 Blazicek P. 104, 114  
 Bodnar I. 88, 120, 121  
 Boe S. 124  
 Booltink E. 116  
 Boomsma F. 129  
 Bregonzio C. 85, 86, 88, 125  
 Brtko J. 89, 109  
 Buske-Kirschbaum A. 102  
 Bussolino D. 89  
 Cancela L. 89  
 Carrasquillo J. A. 122  
 Cervenakova Z. 90  
 Chandras C. 107  
 Chen C. A. 122  
 Chen X. 128  
 Choi D.C. 102  
 Chrousos G.P. 84, 90, 96  
 Claycomb R. 132  
 Contoreggi C. 104  
 Cuadrado M.I. 125  
 Culman J. 91, 133  
 Cuttitta F. 117  
 Cvijic G. 91  
 Dallman M.F. 92  
 Davidovic V. 91  
 Davydova N.A. 92  
 De Jong I.E.M. 93  
 de Kloet E.R. 93, 93, 131  
 De Pablos L. 125  
 De Souza E.B. 127  
 Deinum J. 129  
 Dixon R. 99  
 Djordjevic J. 91  
 Dobolyi A. 123  
 Dona A. 125  
 Dorfman A.G. 92  
 Dostalova I. 87, 122  
 Dronjak S. 94  
 Dubovicky M.A. 94  
 Duncko R. 87, 95, 105, 116, 119  
 Dunn A.J. 95  
 Ebert S. 124  
 Eckelman W.C. 104  
 Eisenhofer G. 96, 122  
 Elenkov I.J. 96  
 Esler M. D. 97  
 Fabian J. 100  
 Farias-Silva E. 97  
 Federenko I. S. 111  
 Fekete M.I.K. 88, 121  
 Fickova M. 95  
 Figueiredo H.F. 102  
 Filaretova L.P. 98  
 Filipenko M.L. 98, 111  
 Fischer-Brügge U. 116  
 Fisk N. 118  
 Fleisher T. 96  
 Fries E. 102  
 Fulop F. 121  
 Gasperikova D. 99  
 Gay J. 107  
 Gille G. 99  
 Gioio A.E. 107  
 Glover V. 103, 118  
 Gold P.W. 96  
 Goldstein D. 96, 96, 100, 122  
 Gomez F. 92  
 Goncalvesova E. 100  
 Goosen K.D. 103  
 Gorbunova A.V. 101  
 Grassi-Kassisse D.M. 97  
 Grigoriev A.I. 114  
 Gulyaeva L.F. 98  
 Hashimoto K. 127  
 Hashimoto R. 101  
 Hashiramoto A. 96  
 Hay-Schmidt A. 118  
 Helfferich F. 123  
 Hellhammer D.H. 102, 111  
 Herman J.P. 102  
 Hernandez-Pando R. 103  
 Hollis J.H. 103, 106  
 Holsboer F. 120  
 Horalik D. 127  
 Houshyar H. 92  
 Huckova M. 90  
 Hudecova S. 109  
 Hung S.-T. 99  
 Igosheva N. 103  
 Imrich R. 104  
 Israel M.S. 131  
 Jagoda E.M. 104  
 Jakubek M. 95  
 Jamal B. 83  
 Jaroscakova I. 105  
 Jeong K.H. 115  
 Jezova D. 87, 90, 94, 94, 95, 96, 105, 116, 119, 119  
 Jezova M. 84, 85, 86, 88, 125, 134  
 Jirikowski G. 133  
 Johansson B.B. 119  
 Johnson P.L. 106  
 Joiner A. 134  
 Jurakova R. 127  
 Kaciuba-Uscilko H. 106  
 Kalita N. 129  
 Kaplan B.B. 107  
 Karalis K. 107, 115  
 Katina S. 116  
 Keck M.E. 120  
 Keilhoff G. 105  
 Kennedy G. 134  
 Kieseewetter D.O. 104  
 Kiss A. 83, 87, 118, 124  
 Kitraki E. 108  
 Kittas C. 108  
 Klimes I. 99  
 Knopp J. 89  
 Koolhaas J.M. 131  
 Koper J. W. 111  
 Kopin I.J. 96  
 Koska J. 90, 104, 110, 114  
 Kovacs K.J. 108  
 Kovacs P. 99  
 Kremmyda O. 108  
 Krepsova K. 109, 109, 117  
 Krizanova O. 100, 109, 109, 110, 111, 112, 117, 123  
 Krsek M. 122  
 Ksinantova L. 100, 104, 110, 114  
 Kubovcakova L. 83, 88, 109, 110, 111, 120, 123, 124  
 Kumsta R. 111  
 Kuper M. 116  
 Kvetnansky R. 83, 88, 89, 90, 94, 96, 99, 100, 104, 109, 109, 110, 110, 111, 112, 114, 117, 120, 121, 121, 123, 126, 132

- La Fleur S.E. 92  
Lacnak B. 127  
Landgraf R. **112**  
Lang L. 104  
Lee A. 128  
Lee E.W. 113  
Lenders J.W.M. **113**, 129  
Li L. **113**  
Li Q. 130  
Lightman S.L. 103, 106  
Liu X. 126  
Loder I. 95, 116  
Lowry C.A. 103, 106, **114**  
Macejova D. 89  
Macho L. 90, 110, **114**  
Mahata S. 134  
Majzoub J. 110, **115**  
Makara G.B. **115**, 133  
Makatsori A. 87, 95, 105, **116**  
Manalo S. 92  
Manz B. **116**  
Martinez A. **117**  
Matta S. 103  
Mergl Zs. 115, 133  
Mickelsen D. 128  
Micutkova L. 100, 109, 109, 111, 112, **117**  
Mikhailova O.N. 98  
Mikkelsen J.D. **118**, 124  
Mikulski T. 106  
Miller N. **118**  
Mizutani M. 101  
Mlynarik M. **119**  
Modi N. 118  
Moncek F. 95, 105, 116, **119**  
Moratalla R. 106  
Morinaga Y. 105  
Mravec B. 88, 100, 111, **120**, 121, 124  
Mueller N.K. 102  
Muller M.B. **120**  
Murchison C.F. 128  
Murphy D.L. 130  
Myslivecek J. **121**  
Nagatsu T. **101**  
Nagy G.M. 88, 120, **121**  
Nankova B. 126  
Nazar K. 106  
Nedvidkova J. 87, 122  
Nikitina O.V. 92  
Noskov V.B. 114  
Novotova M. 109  
Ohta T. 101  
Oitzl M.S. 93  
Omura I. 1, 01  
Orozco H. 103  
Ouyang M. 128  
Pacak K. 84, 87, 107, 114, **122**, **122**  
Pacchioni A. 89  
Palamarchouk V. 95  
Palkovits M. 109, 112, 120, 121, 122, **123**  
Patterson-Buckendahl P. **123**  
Pecoraro N. 92  
Penesova A. 90  
Perez M. 89  
Petrovic N. 91  
Petrovic V.M. 91  
Pfeifer K. **124**  
Pirnik Z. 87, **124**  
Podvigina T.T. 98  
Pohorecky L.A. 123  
Proskova J. 127  
Rabadan-Diehl C. 83  
Rausch W.-D. 99  
Reichmann H. 99  
Rice K.C. 104  
Rigny J. 121  
Rioja J. **125**  
Rook G.A.W. 103  
Rovensky J. 96  
Rupniak N.M.J. 91  
Ryckova N. 111  
Saavedra J.M. 84, 85, 86, 88, **125**, 130, 134  
Sabban E.L. 110, 111, 112, 117, **126**  
Samani N.J. 99  
Santin L.J. 125  
Santos I.N. 97  
Schild L. 105  
Schommer N. 102  
Sebokova E. 99  
Sennikov S.V. 132  
Serova L. 126  
Smorawinski J. 106  
Smriga M. 105  
Soreq H. **126**  
Spadari-Bratfisch R.C. 97  
Stejskal D. **127**  
Sterling C. 128  
Strbak V. 83  
Sun B. 128  
Svacina S. 122  
Swiergiel A.H. 95  
Tai T.C. 132  
Takao T. **127**  
Takeuchi K. 98  
Tanaka A. 98  
Tank A.W. **128**  
Tauer U. 91  
Thomas S.A. **128**  
Thomson J.R. 99  
Tibenska E. 104  
Tigranian R. **129**  
Timmers H.J.L.M. 113, **129**  
Tjurmina O.A. **130**  
Toth B.E. 121  
Ueyama T. **130**  
Unger T. 133  
Usdin T.B. 123  
van Rossum E.F. 111  
Vangelova K.K. **131**  
VanVlerken L. 107  
Veenema A.H. **131**  
Velkova D.P. 131  
Venihaki M. 107, 115  
Vietor I. 110  
Vigas M. 90, 104, 110, 114  
Volpi S. 83  
Vybiral S. 87  
Wagner E. 110  
Weninger S.C. 115  
Weston K. 99  
Wevers R.A. 129  
Wilder R.L. 96  
Wolf G. 105  
Wong D.L. **132**  
Wong-Faull D.C. 132  
Wuest S. 102  
Wust S. 111  
Yatsenko O.P. **132**  
Youlatos D. 108  
Zelena D. 115, **133**  
Zhang W.-P. 128  
Zhang X.-Y. 128  
Zhao Y. 91, **133**  
Zhou J. 84, 125, **134**  
Ziegler M.G. **134**  
Zuber R. **127**  
Zukowska Z. **113**