DIFFERENTIAL RESPONSES TO STRESS STIMULI OF LEWIS AND FISCHER RATS AT THE PITUITARY AND ADRENOCORTICAL LEVEL

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Objective. Histocompatible rat strains Lewis (LEW) and Fischer 344 (F344) are often used to study hypothalamo-pituitary-adrenocortical axis function in relation to immune system activity. It has been suggested that LEW rats have a defect in the hypothalamic production of corticotropin-releasing hormone. The aim of this study was to clarify differential responsiveness of LEW and F344 rat strains to acute stress by measuring adrenocorticotropic hormone (ACTH) and corticosterone concentrations in plasma, corticosterone in adrenal cortex and proopiomelanocortin (POMC) gene expression in the pituitary and spleen.

Methods. Two separate experiments were performed. In the first, indwelling catethers were used for blood sampling from conscious animals during immobilization stress. In the second experiment, rats were immobilized for two hours and decapitated after another 3 hrs for organ collection.

Results. Our results show that LEW strain hyporeactivity was evident from significantly lower ACTH and corticosterone levels compared to those in F344 at all time intervals during stress studied. Measurement of POMC gene expression in the pituitary revealed that the difference in hormone secretion was consistent with POMC mRNA concentrations in these strains of rats. On the other hand, corticosterone concentrations in the adrenal cortex after stress were significantly higher in LEW rats compared to F344. No differences in spleen POMC mRNA concentrations between LEW and F344 rats were found.

Conclusion. The results show that differential reactivity of LEW and F344 rats is associated with different POMC gene expression in the pituitary and probably other factors on the adrenocortical level.

Key words: Lewis – Fischer – Stress – ACTH – corticosterone – POMC mRNA – pituitary – adrenals

The hypothalamic-pituitary-adrenal (HPA) axis is activated to adjust the metabolic response of the body to stressful stimuli. The cascade begins with neural impulses converging on parvocellular neurosecretory neurones in hypothalamic paraventricular nucleus (PVN) which deliver corticotopin-releasing hormone (CRH) to the hypophysial portal vasculature in median eminence. When CRH reaches pituitary, it stimulates corticotrophs to secrete adrenocorticotropic hormone (ACTH). The final effectors of the HPA axis cascade are glucocorticoid hormones (VALE et al. 1981; SAWCHENKO et al. 1993).

The responsivity of the HPA axis to stress stimuli is not uniform but exhibits great differences depending on the stimulus and many other factors (VIGAS et al. 1984; AGUILERA, 1994; JEZOVA AND SKULTETYOVA, 1997), including genetic predisposition. Genetically related histocompatible rat strains Lewis (LEW) and Fischer 344 (F344) differ in the activation of their HPA axis to various acute and chronic stressors. LEW rats have low-

er plasma corticosterone and ACTH levels and CRH gene expression in the PVN in response to physical-psychological stress stimuli or immunologic challenge (Sternberg et al. 1989a; Dhabhar et al. 1993).

Rats of LEW and F344 strains have been repeatedly used as a valuable tool to understand different immune parameters and mechanisms of immunologic disease development. LEW rats readily develop exogenously induced arthritis, while F344 rats are relatively immune (Wilder et al. 1987). LEW animals are also being utilized to study neural correlates of addictive behavior, as they are vulnerable to self-administration of drugs of abuse (George and Goldberg, 1989). In addition, F344 strain has been employed as a model of aging and cancer (Miller and Nadon, 2000).

It is suggested that many of pathophysiological differences between these two strains may be attributed to their altered HPA axis function. However, there is some inconsistency as to the localization of the defect. Most authors showed major differences in hypothalamic CRH neuron function (Sternberg et al. 1989a; Calogero et al. 1992), while others observed changes in the pituitary (Bernardini et al. 1996), or adrenal cortex (Gomez et al. 1996). The aim of this study was to contribute to the understanding of differential responsiveness of LEW and F344 rat strains at the pituitary and adrenocortical levels. POMC mRNA gene expression, adrenal content of corticosterone, as well as plasma ACTH and corticosterone concentrations in response to an acute stress stimulus were evaluated. Moreover, POMC mRNA levels were analyzed also in the spleen, an organ involved in immune responses.

Materials and Methods

Animals. Male Lewis (Lew/Crl/CrlBR) and Fischer (CDF(F-344)/CrlBR) from Charles River Breeding Laboratories Deutschland, Germany, weighing between 260-310 g. They were housed 3-4 per cage under controlled conditions (23 ± 2 °C, lights on between 6.00 and 18.00 h), with free access to rat chow pellets and tap water.

Cannulations. Under sodium pentobarbital anesthesia (50 mg/kg, i.p.) indwelling cannulas were inserted into the tail artery for blood collection. Treatments and blood sampling were performed in conscious animals 24 h later.

Blood sampling. Blood samples were collected into cooled polyethylene tubes with EDTA and centrifuged immediately at 4°C to separate plasma, which was stored at -20°C until analyzed. Blood samples were taken just before and 5, 20, 60, 120 minutes after the beginning of immobilization.

Stress stimuli. The animals were immobilized by taping their four limbs to metal mounts attached to a board. Rats bearing indwelling cannulas were immobilized for 2 h. In a second experiment, rats were immobilized for 2 h and sacrificed after another 3 h for organ collection.

Organ collection. Pituitaries and spleens for mRNA measurement by in situ hybridization were immediately frozen on dry ice and isopentane at -30°C, respectively. Adrenal gland cortices were frozen in liquid nitrogen for the determination of corticosterone. All organs were stored at -70°C until analyzed.

Corticosterone extraction. Adrenal cortices were mechanically homogenized in 1 ml of physiological solution with a motor-driven Teflon pestle. The homogenate was delipidated by the addition of 750 µl of heptane and centrifugated 10 min at 2500 rpm. The heptane was removed by suction. Aqueous homogenate (50 µl) or plasma (10 µl) were diluted with 500 µl of water and corticosterone was extracted with 1 ml of dichloromethane. After centrifugation for 10 min at 2500 rpm the water was removed by suction.

Plasma hormone estimation. ACTH in plasma was analyzed by a specific radioimmunoassay (RIA) described previously (Jezova et al. 1987). Plasma corticosterone was measured by RIA of dichloromethane extracts of 10 µl of plasma or 50 µl of adrenal cortex homogenate, using ^{3}H-corticosterone (Amersham, Buckinghamshire, UK) and a corticosterone antiserum raised in the laboratory of Experimental Neuroendocrinology (INSERM U297, Marseille, France), as described previously (Jezova et al. 1994). The sensitivity of corticosterone assay was 0.5 µg/100ml plasma (5 ng/ml). The intra- and interassay coefficients of variations were 6 and 8 %, respectively.

Protein estimation. Adrenal cortex homogenate (20 µl) was diluted by 180 µl of physiological solution and 1100 µl of 2% Na_2CO_3 in 0.1N NaOH was added. After 10 min, 110 µl 50 % Folin solution was added and mixed thoroughly. Protein content was measured after 30 min photometrically against bovine albumin as standard.

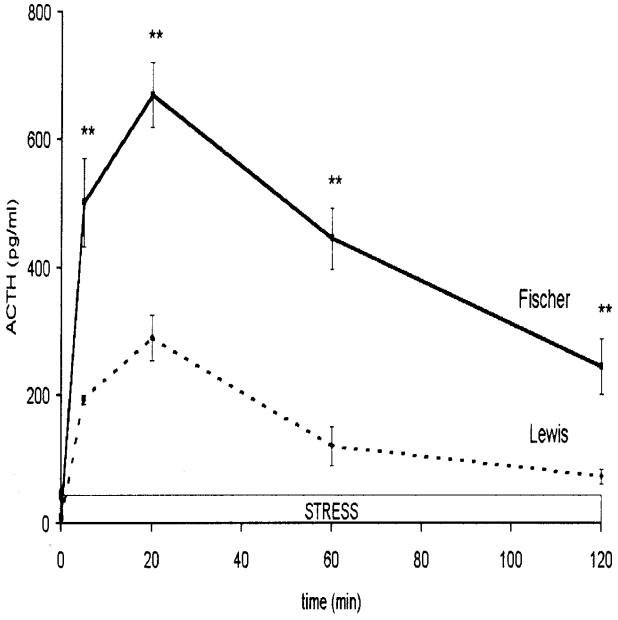

Figure 1
Effect of IMO stress on plasma ACTH concentrations in LEW (n=5) and F344 (n=7) rats. Values represent means ± SEM. *P<0.05, **P<0.01 between strains.

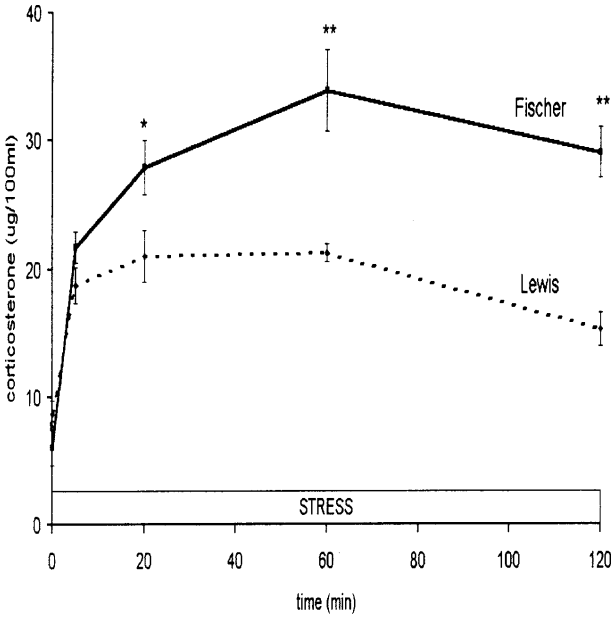

Figure 2
Effect of IMO on plasmatic corticosterone concentrations in LEW (n=5) and F344 (n=7) rats. Values represent means ± SEM. *P<0.05, **P<0.01 between strains.

In situ hybridization. Coronal 12 μm sections were hybridized according to the protocol described previously (SKULTETYOVA et al. 1997; SKULTETYOVA AND JEZOVA, 1999). The probes were 48-mer oligonucleotides complementary to the bases corresponding to amino acids 102-117 of rat POMC (a gift from G Aguilera, USA). The autoradiographic hybridization signal was quantified using computerized image analysis system (Scion Image for Windows, Beta 4.0.2, NIH, USA).

Statistical evaluation. In all experiments two-way ANOVA was used to evaluate differences among treatments, except adrenal weight where students t-test was used. When the results of the ANOVA were significant, Tukey's post hoc test was used to find intervals of significance within groups. Calculations were performed on Jandel SigmaStat for Windows 2.0 statistical software.

Results

Experimental series 1.

Plasma ACTH: Under basal conditions, no strain differences in plasma ACTH concentrations were observed (Figure 1). ACTH levels rose in response to IMO in both groups of rats. Peak values of 290±36 in LEW and 671±50.9 pg/ml in F344 animals were reached at 20 min. Two-way ANOVA revealed statistically significant differences in the mean values of ACTH concentrations among different levels of strain ($P<0.001$, $F=77.2$), time ($P<0.001$, $F=35.5$) and the interaction between the two ($P<0.001$, $F=6.3$). Post hoc Tukey test showed that there was a statistically significant difference between the strains ($P<0.01$), with higher levels in F344 rats. The interaction between the two levels showed more exactly that IMO caused an elevation in ACTH levels in LEW strain at 5 ($P<0.05$) and 20 min ($P<0.01$). In F344 rats, the rise was significant at all time intervals studied ($P<0.01$). Moreover, strain differences in ACTH release during stress were significant ($P<0.01$) at all time intervals investigated (Figure 1).

Plasma corticosterone. Plasma corticosterone concentration also rose in both strains of rats in response to IMO (Figure 2). Peak levels of 21.3±0.7 in LEW and 33.9±3.2 μg/100 ml in F344 groups were reached at 60 min. Two-way ANOVA revealed sta-

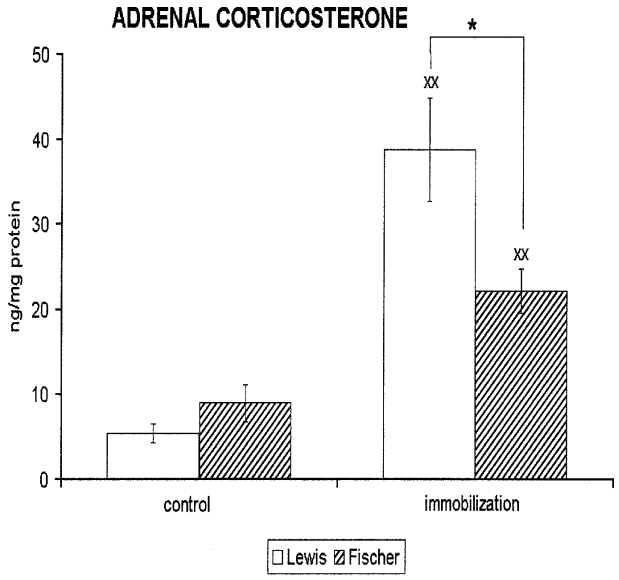

Figure 3
Effect of IMO on adrenal corticosterone levels in LEW and F344 rats. Values represent means ± SEM. *P<0.05 between strains, xx P<0.05 control vs. IMO stress.

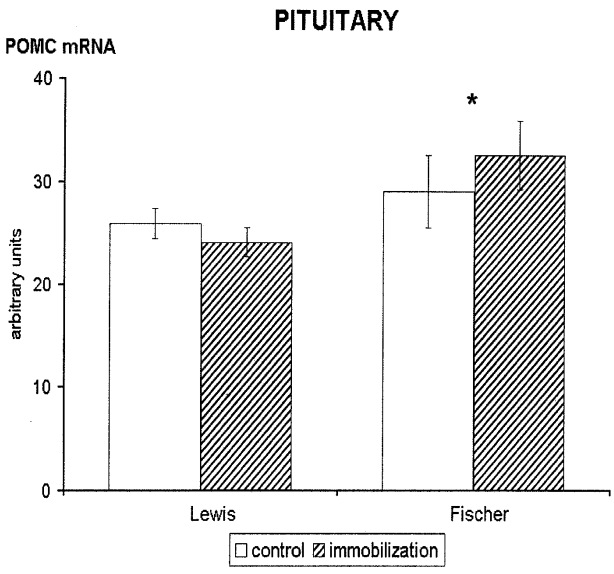

Figure 4
The effect of IMO on POMC mRNA concentrations in the pituitary of LEW (n=5) and F344 (n=7) rats. Values represent means ± SEM. *P<0.05 between strains.

tistically significant differences in the mean values of corticosterone concentration among different levels of strain (P<0.001, F=29.5), time (P<0.001, F=31.8) and the interaction between the two (P<0.001, F=6.2). Post hoc Tukey test showed significant difference of both strain (P<0.01) and time (P<0.01). The levels were higher in F344 rats. Closer inspection of the data showed that corticosterone concentrations during stress were elevated in LEW at 5 (P<0.05) 20, and 60 min (P<0.01). In F344 animals, the rise was significant at all time intervals studied (P<0.01). Differences between strains reached significance at 20 (P<0.05) 60, and 120 min (P<0.01).

Experimental series 2.

Adrenal weight: The weight of adrenal glands was significantly higher (t-test, P<0.05) in F344 (22.2±0.5 mg) compared to LEW rats (20.1±0.7 mg).

Adrenal corticosterone. Adrenal corticosterone concentration increased from 5.5±3 to 40.6±20.9 in LEW rats and 9.0±6.3 to 22.2±8.3 (ng/mg protein) in F344 rats in response to IMO stress for 2 h (Figure 3). Two-way ANOVA showed that there was no clear cut effect of strain (p>0.1, F=2.8), although there was an effect of treatment (P<0.001, F=35.5) with significant interaction between the two (P<0.05, F=6.65). While no differences were found in basal levels of corticosterone concentration between these two strains of rats, there was a significant difference in the increase after IMO. Interestingly, the concentration was lower in F344 rats (Tukey test, P<0.05).

Pituitary POMC mRNA. The results showed that there was a significant difference in pituitary POMC concentrations between the rat strains studied (two-way ANOVA: P<0.05, F=4.6; Tukey test: P<0.05). However, no significant increase after IMO was noticed (Figure 4).

Spleen POMC mRNA: No differences in spleen POMC mRNA concentrations between LEW and F344 rats were found (Figure 5).

Discussion

In this study, we found that the expression of POMC in the anterior pituitary, which is directly influenced by hypothalamic CRH release, was higher in F344 compared to LEW rats in both control and IMO groups. These results are in good agreement with changes in CRH mRNA levels in hypothalamic

PVN during stress in these strains of rats (Sternberg et al. 1989a). It has been suggested that LEW rats have a central nervous system defect in biosynthesis of corticotropin-releasing hormone in response to a number of stress stimuli (Sternberg et al. 1989a; Dhabhar et al. 1993). Our data show the same difference at the level of POMC gene expression. Accordingly, F344 animals have larger number of corticotrophs in anterior pituitary compared to LEW rats (Zelazowski et al. 1992). However, we did not observe a significant increase in POMC mRNA levels in the pituitary after IMO stress in either group of rats. As it is known that the levels of mRNA are the result of its synthesis and degradation, it may be due to the time interval selected.

In agreement with previous data (Dhabhar et al. 1997) the rise of plasmatic ACTH during stress was significantly higher in F344 than in LEW animals. However, the kinetics of this reaction has not yet been studied in detail. As found in the present experiments, the kinetics was similar in both LEW and F344 rats, with a sharp initial rise and a peak in the 20th minute.

Differences in plasmatic corticosterone levels between these two strains reached lower statistical significance than the corresponding ACTH concentrations. Again, the rise was significantly greater in F344 rats which was also observed by others (Sternberg et al. 1989b). Corticosterone concentrations peaked in the 20th minute in F344, but LEW rats reached the peak already in the 5th minute, after which the levels rose no further. As was published previously (Jezova et al. 1987), both small and large increases in plasma ACTH levels were accompanied by increases in plasma corticosterone of comparable magnitudes.

Local production of POMC derived peptides in the spleen was suggested (Jessop et al. 1994; De Bold et al. 1988). Our data show no differences between these strains of rats in POMC mRNA concentrations. Neither did the POMC gene expression change after stress exposure.

Finally, we also measured corticosterone concentrations in adrenal cortex and their changes in response to IMO. Surprisingly, the levels were significantly lower in F344 than in LEW rats. This is unexpected because both ACTH and corticosterone levels in plasma were higher in F344 animals. We may speculate that possible differences in concentrations of corticosteroid-binding globulin (CBG) between

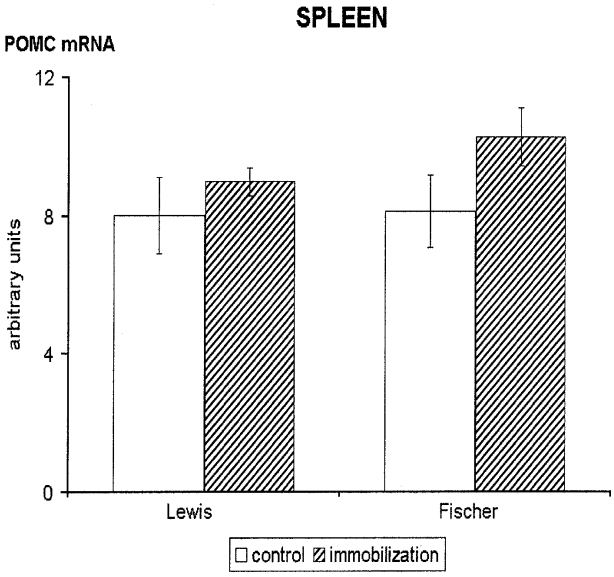

Figure 5
The effect of IMO on POMC mRNA concentrations in the spleen of LEW (n=5) and F344 (n=5) rats. Values represent means ± SEM.

these strains of rats could be involved in this phenomenon. It has been found that F344 rats have higher levels of CBG in plasma, spleen and thymus than LEW rats (Dhabhar et al. 1993). These differences may be further exaggerated by stress exposure after which the CBG levels could be downregulated (Tinnikov, 1993). Thus, low levels of CBG in LEW rats could cause an accumulation of corticosterone in the adrenal cortex observed in the present study. Another contributing factor could be related to vasopressin release. LEW rats hypersecrete vasopressin to compensate for the defect in CRH secretion from the PVN (Patchev et al. 1993; Patchev et al. 1992). Vasopressin is also produced locally in the adrenal cortex (Perraudin et al. 1993). It may be possible that LEW rats have an increased AVP production by the adrenal cortex during stress, which could also contribute to the larger adrenal corticosterone concentrations in this strain compared to F344. ACTH causes vasodilatation of afferent arterioles and increases blood flow through the adrenal gland (Vinson et al. 1994). It is possible that lower levels of ACTH in LEW rats during stress result in lower blood flow and cumulation of steroids in adrenals.

In conclusion, increased ACTH and corticosterone release in F344 rats is associated with more intensive POMC gene expression, but decreased corticosterone content in the adrenal cortex after stress, compared to LEW rats.

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