Glucocorticoid negative feedback on the HPA axis in five inbred rat strains

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The activation of the hypothalamic-pituitary-adrenal (HPA) axis represents one primary manifestation of exposure of animals to both physical and psychological stressors. The importance of the HPA axis in stress derives mainly from two well-known facts. 1) Glucocorticoids have a major role in physiopathology, particularly by inhibiting the immune system, and 2) the degree of activation of the HPA axis is related to the intensity of stress experienced by animals and is also sensitive to the process of habituation to a repeated stressor. The HPA axis is not only one of the main components of the biological response to stress, but it is related to psychopathology as well. Thus abnormalities of the HPA axis have been described in patients with depression, anorexia nervosa, and post-traumatic stress disorders (PTSD). The inhibition of the HPA axis caused by administration of the synthetic glucocorticoid dexamethasone (Dex), the so-called Dex suppression test (DST), is one of the most frequently used tests in these studies to characterize the integrity of negative feedback mechanisms of glucocorticoids on the axis. Depressed patients are characterized by a resistance to suppression by Dex, and an enhanced suppression appears to exist in PTSD patients.

Therefore, the present study was to study the influence of altering glucocorticoid negative feedback on both basal activity of the hypothalamic-pituitary-adrenal (HPA) axis and its response to acute stress (tail shock) in five inbred rat strains known to differ in some depression-like behaviors: Brown Norway (BN), Fischer 344 (F344), Lewis (Lew), spontaneously hypertensive (SHR), and Wistar-Kyoto (WKY) rats. Two complementary approaches were used: 1) enhancement of negative feedback by administration of 0.05 and 0.2 mg/kg dexamethasone (Dex) and 2) attenuation of negative feedback by pharmacological adrenalectomy (PhADX). The results indicate that 1) Lew rats consistently show adrenocorticotropic hormone (ACTH) and corticosterone hyporesponsiveness to stress, 2) interstrain differences in the effect of Dex on the HPA axis were very weak and not related apparently to differences in the metabolism of the steroid, 3) the suppressive effect of the highest dose of Dex on basal corticosterone levels was lower in BN rats than in the other strains, and 4) after PhADX, an increase in ACTH levels was observed in response to acute stress in BN, F344, and WKY but not in Lew and SHR rats, suggesting possible interstrain differences in pituitary sensitivity to neural stimuli induced by stress. In summary, our results indicate that there are differences among the strains with regard to both 1) the suppressive effect of Dex on the HPA axis, BN rats showing a certain degree of resistance, and 2) the capability of PhADX rats to respond to acute stress, which suggests a defective release of ACTH in Lew and SHR rats. The biological meaning of these alterations of corticosteryl negative feedback among the five inbred strains studied remains to be established.

hypothalamic-pituitary-adrenal axis; Brown Norway rats; Fischer 344 rats; Lewis rats; spontaneously hypertensive rats; Wistar-Kyoto rats
MATERIAL AND METHODS

Animals

Male BN, F344, Lew, SHR, and WKY rats 65–70 days old at the beginning of the experiments were used. They were obtained from Charles River. Animals were kept two per cage under standard conditions of light (photoperiod from 0730 to 1930) and temperature (22 ± 1°C) for at least 2 wk before and throughout the experiments. Animals had free access to food and water. In experiment 3, saline (0.9%) was given to animals after adrenalectomy (ADX).

Experimental Procedures

The experimental procedures used in this work were previously approved by the ethical committee for animal experimentation of the Universitat Autònoma de Barcelona. Stress procedure and blood sampling. The experiments were performed between 0900 and 1300 to minimize any circadian influence. In experiments 1 and 2, basal and stress levels of HPA hormones were determined. Just before exposure to stress (time 0), a blood sample was taken from each rat by the tail cut procedure within 2 min of the time they had been taken from the animal room. These samples were considered as basal levels. Immediately, rats were introduced into opaque plastic tubes (22 × 6 cm) provided with small holes to facilitate breathing and heat dissipation, and electric tail shocks were delivered. Rats received shocks of 1-mA intensity, 5-s duration, and 25-s intershock interval for 30 min, and blood samples were taken at 15 and 30 min. Blood samples of 300 µl were collected in EDTA-coated capillary system tubes and centrifuged. The plasma was stored at −20°C until adrenocorticotropic hormone (ACTH) and corticosterone levels were assayed.

Experiment 1: DST. In a trial experiment, 5 mg/kg Dex was given to animals (8 animals per strain), and 2 h later a blood sample was taken under resting conditions and plasma Dex was assayed by radioimmunoassay (RIA) to test possible interstrain differences in Dex bioavailability. In experiment 1, eight animals per strain and dose of Dex were used. Animals were injected intraperitoneally at 0800 with either vehicle (saline 0.9%) or 0.05 or 0.2 mg/kg Dex. Two hours later, samples from basal and stress conditions (see Stress procedure and blood sampling) were obtained.

Experiment 2: PhADX. Eight animals per strain and treatment were used. In pharmacologically adrenalectomized (PhADX) groups, synthesis of endogenous corticosteroids was blocked following a protocol previously reported (23). In brief, the 11β-hydroxylase inhibitor metyrapone (20 mg/100 g body wt sc) was administered 8 and 2 h before the first blood sampling (the injection given at lights off was made under red lights). The 20α-hydroxylase inhibitor aminoglutethimide (20 mg/100 g body wt sc) was administered 45 min before the start of blood sampling. Drugs (kindly provided by Ciba-Geigy, Barcelona, Spain) were dissolved in dimethylsulfoxide (Sigma, St. Louis, MO). We injected control groups with vehicle, maintaining the same time schedule of injections.

Experiment 3: In vitro cytosol binding assay of mineralocorticoid receptor and glucocorticoid receptor. Eight stress-naive rats per strain were adrenalectomized by dorsal approach under ether anesthesia to eliminate endogenous corticosterone. Rats were killed under resting conditions by decapitation ~20–24 h after ADX, and the trunk blood was collected in EDTA-coated tubes for further plasma corticosterone analysis. Brains were quickly removed, and the hippocampus and the hypothalamus were dissected on a chilled ice plate, frozen on dry ice, and stored at −80°C until assay.

Hormone Measurements

Total plasma corticosterone was measured by RIA according to the method described previously (8), but protein interference was eliminated by treating the samples at 70°C for 30 min; rabbit anticorticosterone-3-OCMO serum (Biodin, Cardiff, UK) was used. Total plasma Dex was measured by RIA according to the method described for corticosterone, except that heat treatment of the samples was not done because Dex does not bind to the corticosteroid-binding globulin (CBG). [3H]Dexamethasone (specific activity 41 Ci/mmol; Du Pont-NEN, Madrid, Spain) and sheep antidexamethasone-21-hemisuccinil-bovine serum albumin (BSA) (Biodin) were used. Plasma ACTH was assayed immunoradiometrically with the use of a commercial kit (Nichols Institute, San Juan Capistrano, CA). The analysis of basal ACTH (time 0) was omitted in experiment 1 because there are no differences among strains in basal plasma ACTH levels (8) and because of the extremely low levels of ACTH expected after Dex in nonstressed rats.

Corticosteroid Receptors

In experiment 3, two to three pooled hippocampuses or three to four pooled hypothalamuses per strain were homogenized in ice-cold 5 mM tris(hydroxymethyl)aminomethane containing 5% (vol/vol) glycerol, 10.0 mM sodium molybdate, 1.0 mM β-mercaptoethanol, and 1.0 mM EDTA (Tris-EDTA-molybdate-glycerol buffer, pH 7.4). The homogenate (1–2 mg protein/ml cytosol) was centrifuged at 100,000 × for 1 h at 2°C. To determine the mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) binding capacity (Bmax) and affinity (Kd), 100 µl of cytosol were incubated with [3H]corticosterone concentrations varying from 0.1 to 30 nM, and cold RU-28362 at a 50-fold excess was used to prevent [3H]corticosterone from binding to GR. The specific binding to GR was calculated by subtracting specific binding to MR from total binding. Nonspecific binding was obtained in a parallel set of tubes containing a 500-fold excess of cold corticosterone. Tubes were incubated overnight at 4°C. Thirty microliters of the incubates were taken and counted to determine the total concentration of ligand. Separation of bound and free fraction was done as previously described (33), using polyethyleneimine-pretreated glass fiber filters in a cell harvester system. Binding parameters were estimated by nonlinear regression fit, using Inplot 4.0 (GraphPad-Software, San Diego, CA). Protein content was measured using the Lowry method, with BSA as the standard.

Statistical Analysis

One-way analysis of variance (ANOVA) followed by post hoc individual comparisons with the Student-Newman-Keuls (SNK) test (P < 0.05) were used to assess statistical significance of interstrain differences in Dex levels in the trial experiment and in ACTH and corticosterone levels within each treatment and time in experiments 1 and 2. Paired r-tests were used within each strain to study the statistical significance of HPA axis response to stress. When necessary, data were log transformed to achieve homogeneity of variances. If this was not achieved, nonparametric one-way Kruskal-Wallis (KW) test followed by the Mann-Whitney U test (U test) was used.

RESULTS

Trial Experiment

The one-way ANOVA revealed no significant effect of strain on plasma Dex levels (ng/dl) [(means ± SE; n =
Experiment 1

As Fig. 1 shows, the one-way ANOVA of saline-treated groups subjected to tail shock revealed no significant effect of strain on ACTH levels after 15-min tail shock but did reveal a significant effect after 30-min tail shock ($P < 0.005$), in that Lew rats showed lower ACTH levels than BN, F344, and WKY rats (SNK test). No differences among strains were found in rats treated with 0.05 mg/kg Dex at any time. After 0.2 mg/kg Dex, inhibition of ACTH levels was so important that some ACTH values (especially those observed in F344 and Lew rats) were nondetectable for the technique used. Therefore, nonparametric tests were used. After 0.2 mg/kg Dex, a significant effect of strain was found only after 15-min exposure to shock (KW test, $P < 0.03$), in that F344 and Lew rats showed lower ACTH levels than WKY rats (U test).

Figure 2 depicts plasma corticosterone levels, including basal (time 0) values. In saline-treated groups, the one-way ANOVAs revealed no differences among strains at time 0, but there were differences after 15 ($P < 0.05$) and 30-min ($P < 0.02$) tail shock. At both times, Lew rats showed lower corticosterone levels than SHR (SNK test), the other strains showing intermediate levels. After 0.05 mg/kg Dex, interstrain differences ($P < 0.02$) were only observed at 30 min after starting tail shock; Lew rats showed lower corticosterone levels than F344 and SHR rats. After 0.2 mg/kg Dex, interstrain differences were also found after times 0 ($P < 0.03$), 15 ($P < 0.001$), and 30 min ($P < 0.04$) of exposure to tail shock. At time 0, BN showed higher corticosterone levels than the other strains; after 15-min shock, Lew rats showed lower corticosterone levels than the other strains; and after 30-min shock, the differences among Lew rats and the other strains were restricted to SHR rats. The paired $t$-tests revealed that plasma corticosterone levels were increased by 15- and 30-min stress in all strains after vehicle and 0.05 mg/kg Dex administration.
tion, but after 0.2 mg/kg Dex, Lew rats failed to respond to stress (Fig. 2).

**Experiment 2**

Pharmacological blockade of steroid synthesis was clearly associated with increases in plasma ACTH levels in basal and stress conditions (Fig. 3). In vehicle-treated groups, interstrain differences were observed at time 0 (one-way ANOVA, P = 0.01) and after 15 (KW test, P = 0.005)- and 30-min (one-way ANOVA, P = 0.005) tail shock. At time 0, BN rats showed higher ACTH levels than Lew, SHR, and WKY rats (SNK test); after 15-min tail shock, Lew rats showed lower ACTH levels than BN and F344 rats (U test); and after 30-min stress, F344 showed higher corticosterone levels than Lew, SHR, and WKY (SNK test); and after 30 min, Lew rats showed the lowest corticosterone level compared with the other strains (SNK test). After blockade of corticosterone synthesis by PhADX, differences among strains were only found at time 0 (P < 0.004); BN showed higher corticosterone levels than Lew and SHR rats, and F344 rats also showed higher corticosterone levels than SHR (SNK test). The paired t-tests revealed that, in vehicle-treated groups, all the strains showed a corticosterone increase in response to 15- and 30-min stress compared with time 0. However, such increases were suppressed by PhADX.

When corticosterone levels were studied in vehicle-treated groups (Fig. 4), differences were observed at time 0 (KW test, P < 0.01) and after 15 (one-way ANOVA, P < 0.01)- and 30-min stress (one-way ANOVA, P < 0.01). At time 0, BN showed higher corticosterone levels than Lew, SHR, and WKY (U test); after 15-min stress, F344 showed higher corticosterone levels than Lew, SHR, and WKY (SNK test); and after 30 min, Lew rats showed the lowest corticosterone level compared with the other strains (SNK test). After blockade of corticosterone synthesis by PhADX, differences among strains were only found at time 0 (P < 0.004); BN showed higher corticosterone levels than Lew and SHR rats, and F344 rats also showed higher corticosterone levels than SHR (SNK test). The paired t-tests revealed that, in vehicle-treated groups, all the strains showed a corticosterone increase in response to 15- and 30-min stress compared with time 0. However, such increases were suppressed by PhADX.

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**Fig. 3.** Plasma ACTH levels after vehicle (A) or pharmacological adrenalectomy (PhADX, B) treatment in BN, F344, Lew, SHR, and WKY rats at times 0, 15, and 30 min after exposure to tail shock. Results are expressed as means ± SE (6–8 rats/strain). Within the same treatment and time, bars labeled with different letters are statistically different (SNK or Mann-Whitney U tests, P < 0.05). *Statistically significant effects of stress compared with respective time 0 values (P < 0.05, paired t-test).

**Fig. 4.** Plasma corticosterone levels after vehicle (A) or PhADX (B) treatment in BN, F344, Lew, SHR, and WKY rats at times 0, 15, and 30 min after exposure to tail shock. Results are expressed as means ± SE (6–8 rats/strain). Within the same treatment and time, bars labeled with different letters are statistically different (SNK or Mann-Whitney U tests, P < 0.05). *Statistically significant effects of stress compared with respective time 0 values (P < 0.05, paired t-test).
Experiment 3

Because the data were obtained from the analysis of three pools per strain and area, except for the hypothalamus of WKY in which just one pool was analyzed, no statistical analysis could be done. Nevertheless, F344 rats appear to have higher levels of both MR and GR in the hippocampus than do the other strains (20–30%). Differences were not apparent in the hypothalamus. No differences among the strains appear to exist in the Kd of MR and GR for [3H]corticosterone in either the hippocampus or the hypothalamus (Table 1).

DISCUSSION

In the present study, we have characterized the HPA axis response to experimental “manipulation” of endogenous negative glucocorticoid feedback in five inbred rat strains known to have clear differences in their behavior in the FST, which is considered here as a procedure to evaluate depression-like behavior in rodents. Dex caused an overall inhibition of plasma ACTH and corticosterone levels in all strains, the most important difference among the strains being a partial resistance in BN rats. After pharmacological blockade of corticosterone synthesis, circulating ACTH increased to the same extent in all strains in nonstressful conditions. In contrast, after superimposition of an acute stressor (tail shock), a further significant increase in ACTH was observed in BN, F344, and WKY but not in Lew and SHR rats, suggesting a defect in HPA activation in the two latter strains.

According to most previous results from our own and other laboratories (2, 4, 7–10, 16, 28, 29), no differences in basal morning corticosterone levels were observed among the five inbred strains studied. Although some differences were observed in plasma ACTH and corticosterone levels obtained at time 0 in experiment 2, this condition cannot be strictly considered as basal due to handling associated with PhADX (see Experimental Procedures). These data indicate that during such handling conditions, some strain differences might appear that could be erroneously interpreted as differences in resting levels.

Whereas basal corticosterone levels were not strain dependent, the magnitude of the ACTH and corticosterone responses to the acute stress caused by tail shock and blood sampling were consistently lower in Lew rats than in the other strains; therefore, these data agree with most of the previously published reports (2, 7, 9, 15, 29). In one report (28), no differences in the ACTH response to various stressors were observed in male adult rats of F344 and Lew strains. Differences were only observed in female rats’ response to some particular stressors. Similarly, Grota et al. (9) found in adult rats a lower corticosterone response to stressors in Lew than in F344 rats, although the differences were lower in magnitude than those usually observed in young animals. Therefore, it appears that both sex and age can influence HPA response to stress in Lew and F344 rats despite the fact that, in our laboratory, males showed a consistently low HPA response to stressors (2, 15). These gender differences cast some doubts about the adequacy of the data obtained in females to explain what occurs in males. Thus the defective corticotropin-releasing factor (CRF) response to stressors and the reduced ACTH response to CRF observed in female Lew rats compared with F344 rats (29–31) might not be relevant in males (28). On the basis of the response to foot shock stress and immune challenge of immediately genes and mRNA for CRF, it appears that functional activation within parvocellular CRF neurons is normal in adult male Lew rats (26). In addition, the reduced HPA activation is not due, at least in males, to low CRF gene expression, either in basal conditions or after chronic stress (8), or to a lower pituitary sensitivity to CRF and arginine vasopressin (AVP) (28). In adult male Lew rats, the primary hypothalamic defect appears to reside in the parvocellular CRF neurosecretory system coexpressing AVP, which would limit the amount of secreted AVP into portal blood (35). Nevertheless, a defect at the pituitary and adrenal levels should not be disregarded (19), particularly when we consider the low corticosterone response to ACTH administration observed in Lew rats compared with F344 rats in both sexes (9).

The present results failed to find differences in the HPA response to stress between SHR and WKY. Inconsistent results exist in the literature regarding the HPA response to stress in these strains, because a similar ACTH response to stress (2, 4) or an enhanced response in SHR (10) has been reported. Also a reduced ACTH response to CRF administration in SHR has been observed compared with WKY rats (4, 10, 11) that could be age dependent (11). Furthermore, the response to AVP was strongly dependent on the use of freely moving (10) or anesthetized (11) rats. In addition, a higher corticosterone response to exogenous ACTH administration has been observed in SHR compared

### Table 1. MR and GR Bmax and Kd in hippocampus and hypothalamus of BN, F344, Lew, SHR, and WKY rats

<table>
<thead>
<tr>
<th>Strain</th>
<th>MR Hippocampus</th>
<th>MR Hypothalamus</th>
<th>GR Hippocampus</th>
<th>GR Hypothalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td>BN</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>11.0 ± 1.1</td>
<td>9.6 ± 1.9</td>
</tr>
<tr>
<td>F344</td>
<td>0.5 ± 0.1</td>
<td>0.3 ± 0.0</td>
<td>10.6 ± 0.4</td>
<td>7.3 ± 0.2</td>
</tr>
<tr>
<td>Lew</td>
<td>0.4 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td>7.9 ± 1.6</td>
<td>9.1 ± 0.2</td>
</tr>
<tr>
<td>SHR</td>
<td>1.5 ± 1.3</td>
<td>0.2 ± 0.1</td>
<td>6.3 ± 3.7</td>
<td>9.2 ± 1.2</td>
</tr>
<tr>
<td>WKY</td>
<td>0.6 ± 0.2</td>
<td>0.3</td>
<td>13.8 ± 2.7</td>
<td>9.9</td>
</tr>
</tbody>
</table>

Kd, nM; Bmax, fmol/mg protein; BN, Brown Norway; F344, Fischer 344; Lew, Lewis; SHR, spontaneously hypertensive rats; MR, mineralocorticoid receptor; GR, glucocorticoid receptor; Bmax, maximal binding capacity; Kd, apparent affinity.

Results are expressed as means ± SE obtained from 3 different pools (2–3 hippocampuses or hypothalami each) except for hypothalamus of Wistar-Kyoto (WKY) rats, in which only a single value from 3 pooled hypothalamuses is shown. BN, Brown Norway; F344, Fischer 344; Lew, Lewis; SHR, spontaneously hypertensive rats; MR, mineralocorticoid receptor; GR, glucocorticoid receptor; Bmax, maximal binding capacity; Kd, apparent affinity.
with WKY (27) rats, although neither our results (2, and present data) nor those by Castanon et al. (4) showed evidence for an altered ACTH/corticosterone ratio in WKY rats. Because the contribution of each secretagogue to ACTH release depends on intensity, duration, and type of stressor (22), the different response of these strains to CRF and AVP could, at least under certain conditions, explain why no consistent differences in their HPA response to stress have been found.

Both doses of Dex caused an overall inhibition of stress levels of ACTH in all strains, and the percent inhibition compared with respective vehicle-treated rats was similar in all strains. Regarding Lew and F344 rats, these data agreed with the absence of differences between the two strains in the peripheral target tissues response to glucocorticoids (13). The interpretation of the finding that F344 and Lew rats showed the lowest ACTH levels after 0.2 mg/kg Dex is confounded by the limitations of the assay when it is performed at such low ACTH levels. However, it seems that, in basal conditions, the effect of 0.2 mg/kg Dex was less effective at reducing corticosterone levels in BN rats than in the other strains. After tail shock, percent inhibition of corticosterone levels was always in the lowest range in BN rats and in the highest range in Lew rats, perhaps due to dissociation of the effect on ACTH and corticosterone. The minor differences observed cannot be apparently explained by differential Dex bioavailability (in the trial experiment, no differences among strains were found in circulating Dex levels 2 h after administration of 5 mg/kg Dex). Redei et al. (25) have suggested that WKY rats might be resistant to negative glucocorticoid feedback, but the great differences in circulating corticosterone observed in WKY (compared with F344 or outbred Wistar rats) in ADX rats receiving corticosterone pellets made their results difficult to interpret.

To know the possible relationship between the effects of Dex and brain corticosteroid receptors, we analyzed the $B_{max}$ and $K_d$ of MR and GR in the hippocampus and in the hypothalamus. Although the low number of data generated after pooling brain areas for various rats strictly precluded statistical analysis, it appears that hippocampal MR and GR $B_{max}$ are higher (20–30%) in F344 rats than in the other strains, whereas no differences were apparent in the hypothalamus. Also, no differences in $K_d$ of MR and GR were observed in any area. It has been suggested that Dex suppression might be more important in the pituitary than in other areas (5, 6). Therefore, data of GR binding capacity in the pituitary would have been of interest. Unfortunately, the high CBG levels in this area made it necessary to use $[^3H]$dexamethasone as a labeled steroid for the receptor assay, and the method used to separate free and bound fractions (see MATERIAL AND METHODS) was not good enough for this steroid. The increase in hippocampal GR is compatible with the greater effect of Dex on ACTH levels in F344 rats. However, Dabhar et al. (7) reported no differences between F344 and Lew rats in corticosteroid receptors. Discrepancies might arise from methodological differences in the binding assay.

The present data regarding BN rats might support previous results suggesting that glucocorticoid feedback could become deficient during aging in BN rats (32). The functional relevance of resistance to Dex in BN beyond that related to aging in BN is unclear at present. BN and WKY rats are more passive than F344, Lew, and SHR rats in the FST (2), a test initially developed for the screening of antidepressant drugs (24) that some authors consider to be a reflection of depression-like behavior (14, 21, 34). Apart from the controversies regarding the interpretation of the rat behavior in the FST (18), the partial resistance of BN to Dex fits with the passive behavior of this strain in the FST. However, the weak differences in resistance to Dex of BN rats and the normal response of WKY rats compared with the other strains do not correlate with the major differences in the FST behavior.

To further characterize negative corticosteroid feedback in these strains, the contribution of basal corticosterone levels to tonic regulation of HPA axis was studied by subjecting the animals to PhADX. As expected, PhADX suppressed stress corticosterone levels and increased basal circulating ACTH levels in all strains. When the response to tail shock was studied, it was found that in intact rats, all strains showed a significant ACTH response to stress, although that of Lew rats was lower than that of the other strains. In PhADX animals, exposure to tail shock stress resulted in a significant ACTH response in BN, F344, and WKY rats, but Lew and SHR failed to respond. Interestingly, F344 and Lew rats showed the highest and the lowest ACTH levels after stress, confirming that these two strains are markedly different in HPA activity. These data agree well with the well-known defect of the HPA axis observed in Lew rats reflected in blunted ACTH and corticosterone responses to stressors.

In addition to Lew rats, SHR also failed to increase ACTH levels in response to tail shock stress. In contrast to that observed in Lew rats, the defect in ACTH response to stress was observed in SHR rats after PhADX only, when the prestress ACTH levels were already very high. It is therefore possible that a defect exists in SHR rats that is only unmasked when a strong release of ACTH is required. This hypothesis could be compatible with the lower ACTH response to CRF in SHR compared with WKY rats (4, 10). However, the defect might be also located at a suprapituitary level, similar to the reduced in vitro hypothalamic CRF release after incubation with a low dose of $K^+$ found in SHR (10).

The contribution of MR to the altered resting and stress levels of ACTH in PhADX animals should be considered, because the amount of corticosterone was enough to occupy MR. In this regard, the blunted ACTH response to tail shock in Lew and SHR rats might be due to a greater efficacy of hippocampal MR to inhibit the HPA axis in these two strains. Although no differences in MR were apparent in the present study after surgical ADX, a greater MR binding capacity has been reported in Lew compared with outbred Wistar rats (19), suggesting that at least under more appropriate
experimental conditions, differences might have appeared in MR in Lew rats. In addition, PhADX resulted in low but significantly higher levels of corticosterone than surgical ADX so that we do not know exactly the status of MR after PhADX.

In summary, our results indicate that there are differences among the strains studied with regard to the suppressive effect of Dex on the HPA axis, BN rats showing a certain degree of resistance and Lew rats showing the greater inhibition. These changes were not apparently related either to differential metabolism of Dex or differences in MR and GR in the hippocampus and the hypothalamus. After attenuation of corticosterone negative feedback by PhADX, BN, F344, and WKY rats show an increase in plasma ACTH after acute stress, but Lew and SHR rats failed to do so, suggesting a defective release of ACTH in these two strains, perhaps related to a greater efficacy of MR in restricting the activity of the HPA axis. The physiological meaning of these alterations of corticosteroid negative feedback among the five inbred strains studied remains to be established.

Perspectives

The most reliable biological abnormalities in depression are related to the HPA axis. On the assumption that at least some particular syndromes of depression might be modeled in the laboratory, it was then hypothesized that those animals showing differences in some tests presumably measuring depression-like behavior (for instance the FST) might also show differences in the regulation of the HPA axis. To this end, the characteristics of the negative feedback of glucocorticoids on the HPA axis were studied in five inbred rat strains known previously to differ in their behavior in the FST. Although some minor interstrain differences in the negative feedback of Dex on the HPA axis were found, the present data did not support the hypothesis that passive behavior in the FST is strongly related to resistance to Dex in the rat. In fact, minimal differences in the efficacy of Dex to suppress the HPA axis were found among the strains studied. In contrast, important differences in the negative feedback of glucocorticoids have been described after manipulation of the animals during some critical developmental periods. We can conclude that 1) FST behavior is, if at all, weakly related to Dex suppression and 2) negative glucocorticoid feedback might be more strongly modulated by environmental than genetic factors.

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