Strain Differences in Corticosteroid Receptor Efficiencies and Regulation in Brown Norway and Fischer 344 Rats

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Key words: rat strains, adrenal steroid receptor regulation, plasma transcortin, corticosterone efficiency, body weight, thymus.

Abstract

During the dark phase of the diurnal cycle, and during recovery from restraint stress, Brown Norway (BN) rats secrete less corticosterone than Fischer 344 (F344) rats. These strains also display different levels of corticosteroid receptors in the hippocampus, and of plasma transcortin. Because corticosteroid receptors, plasma transcortin and corticosterone secretion are mutually regulated, we examined brain and pituitary mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) expression and some of the parameters modulated by these receptors (i.e. body and thymus weight, fluid intake, plasma transcortin) in BN and F344 rat strains, by comparing the effects of either hormone deprivation by long-term (21 days) adrenalectomy (ADX), or chronic elevation of corticosterone given in drinking fluid to ADX rats. In BN rats, body weight gain and fluid intake were insensitive to corticosterone deprivation, suggesting that MR-related mechanisms are constitutively active in this strain. Body weight (b.w.) gain, plasma transcortin and thymus weight were reduced to a greater extent by chronic corticosterone in BN rats than in F344 rats, possibly as a consequence of higher free, active fraction of plasma corticosterone due to lower plasma transcortin concentrations and/or a greater efficiency of GR-related mechanisms in BN rats. F344 rats displayed twofold higher brain and pituitary MR levels than BN rats, whereas tissue-and strain-specific regulations were observed for GR levels. The differences in MR levels observed between BN and F344 strains cannot completely explain the differences in corticosterone actions, suggesting that strain differences in response to ADX or corticosterone treatment result from variable receptor efficiencies.

Receptors which mediate the biological actions of corticosteroids were initially detected in the central nervous system by uptake and retention of radioactive [^{3}H]-corticosterone (1). [^{3}H]-corticosterone, the pure synthetic glucocorticoids [^{3}H]-dexamethasone and [^{3}H]-RU 28362 enabled researchers to differentiate two receptor subtypes in the rat brain (2, 3). The *mineralocorticoid receptor* (type I or MR) is preferentially located in the limbic system, and exhibits a high affinity for corticosterone and aldosterone ($Kd = 0.5–1.0$ nM) and a lower affinity for dexamethasone ($Kd = 2.5–5.0$ nM). The *glucocorticoid receptor* (type II, or GR), has a more widespread distribution in the brain (4, 5), and binds dexamethasone and RU 28362 with high affinity ($Kd = 0.5–1.0$ nM), corticosterone with a lower affinity ($Kd = 2.5–5.0$ nM) and aldosterone with a very low affinity ($Kd > 15$ nM). Thus both receptor subtypes bind corticosterone, but whereas low corticosterone concentrations are sufficient to saturate MRs, higher concentrations, achieved during stress or at circadian peak, are necessary to occupy GRs (6, 7). Both receptor types are present in the hypothalamus and hippocampus, which are closely involved in the control of pituitary-adrenal activity (5, 8). Both are also present in the pituitary (4) where they regulate adrenocorticotropic hormone (ACTH) synthesis and release (9, 10). Mineralocorticoid receptors regulate basal secretion of corticosterone and the threshold of the stress response, whereas GRs are involved in the feedback action of corticosterone on the hypothalamo-pituitary axis (6, 11). Corticosteroid receptors are regulated by many factors, including glucocorticoids themselves (12–16). In the brain, long-term adrenalectomy (ADX)

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increases and corticosterone treatment decreases GR binding via both auto- and hetero-regulation (5, 17). Contradictory results, however, have been published for MR regulation by corticosterone (5, 15, 16, 18).

Individual rats differ in their neuroendocrine and behavioural responses to stress, and these differences reflect both epigenetic (19) and genetic factors (20). Inbred rat strains have been extensively used to study these mechanisms. For instance, Brown Norway (BN) and Fischer 344 (F344) rat strains differ in their hypothalamic-pituitary adrenal (HPA) axis activity and reactivity to stress (21, 22). We have previously shown that, compared to F344 rats, BN rats secrete less corticosterone during the dark phase of the diurnal cycle and during recovery from restraint stress (22). These differences may be associated with lower MR levels in the hippocampus of BN rats. Plasma transcortin concentrations are also markedly lower in BN rats than in F344 rats. Since these structural proteins and corticosterone are involved in retroactive loops, it is difficult to identify the origin of the difference(s) in protein expression and in HPA axis activity/reactivity between BN and F344 rat strains. This study was designed to compare the effects of hormone deprivation by long-term ADX, and of controlled chronic elevation of corticosterone, on the content of MRs or GRs in brain and pituitary, and on some of the parameters modulated by these receptors: body weight gain (23–25), thymus weight (26), fluid intake (27, 28) and plasma transcortin (29, 30).

Results

Rats were adrenalectomized on day 0 and watered with saline with (ADX + CORT group) or without (ADX group) corticosterone for 21 days. Body weight (b.w.) and fluid intake were periodically estimated. Thymus weight, plasma CBG, and MR and GR levels in the hippocampus, hypothalamus and pituitary were determined and compared to 1 day ADX rats (control group).

Body weight gain and fluid intake

The weight gain was calculated in relation to the initial weight on day 0 (Fig. 1). ANOVA showed significant strain ($P<0.05$) and treatment effects ($P<0.001$), and a strain–treatment interaction ($P<0.001$). This interaction probably resulted from the large differences observed between the strains in their response to treatments. Whereas BN and F344 control groups displayed normal growth patterns, significant differences ($P<0.001$) were shown between the two strains after ADX. There was no significant difference in weight gain between the control and the ADX groups in BN rats, while ADX caused a large decrease ($P<0.001$) of b.w. in F344 rats in the first week of the experimental period, although the body growth of the two strains became identical afterwards. Both strains experienced weight loss under corticosterone treatment ($P<0.001$), but to a larger extent in BN rats than in F344 rats ($P<0.05$).

Since fluid intake measurements could not be made individually but only by cage, no statistical analysis could be applied to these data. Nevertheless, ADX appeared to induce a marked progressive increase in fluid intake in F344 rats,

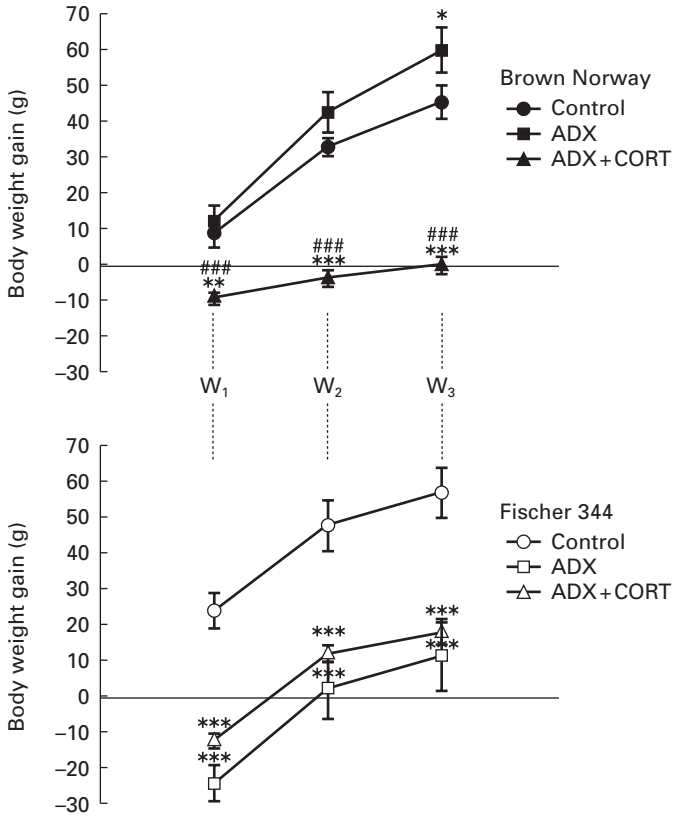

FIG. 1. B.w. gain according to time. W_n corresponds to the weight measured the week *n*. B.w. gain was insensitive to long-term ADX in BN rats, but reduced to a greater extent by corticosterone treatment in BN rats than in F344 rats. Different from the control group designed as $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Different from the ADX group designed as $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$.

whereas no significant change was observed in BN rats (Table 1). Chronic corticosterone restored the fluid intake of F344 rats to control group levels.

Thymus weight

ANOVA revealed strong strain and treatment effects ($P<0.001$) and a strain–treatment interaction ($P<0.001$) for thymus weight. As shown in Fig. 2, the thymus gland was heavier in BN rats than in F344 control rats ($P<0.001$) and ADX induced a significant increase in thymus weight ($P<0.001$), similar in both strains. Chronic corticosterone administration induced a strong involution of the thymus, larger (−64.6% *vs* ADX) in BN rats than in F344 rats (−48.0%, $P<0.05$).

Plasma transcortin

ANOVA showed strain ($P<0.01$) and treatment effects ($P<0.001$) and a strain–treatment interaction ($P<0.05$). As shown in Fig. 2, transcortin concentrations were higher in F344 than in BN control rats (+65.2%, $P<0.05$), but this strain difference disappeared after ADX. ADX increased transcortin concentrations, and corticosterone restored them

TABLE 1. Fluid intake according to time.

	Brown Norway			Fischer 344		
	Control	ADX	ADX +CORT	Control	ADX	ADX +CORT
1st week	11.0	10.3	10.7	10.9	10.2	9.7
2nd week	9.0	10.5	9.7	9.3	15.6	10.2
3rd week	8.2	10.2	9.4	8.6	22.0	10.9

Results are expressed as ml/100g of b.w./day. Contrary to F344, BN rats did not show the expected ADX-induced increase of fluid intake. Since BN and F344 rats had the same drink intake, they received the same dose of corticosterone in μg/g/b.w.

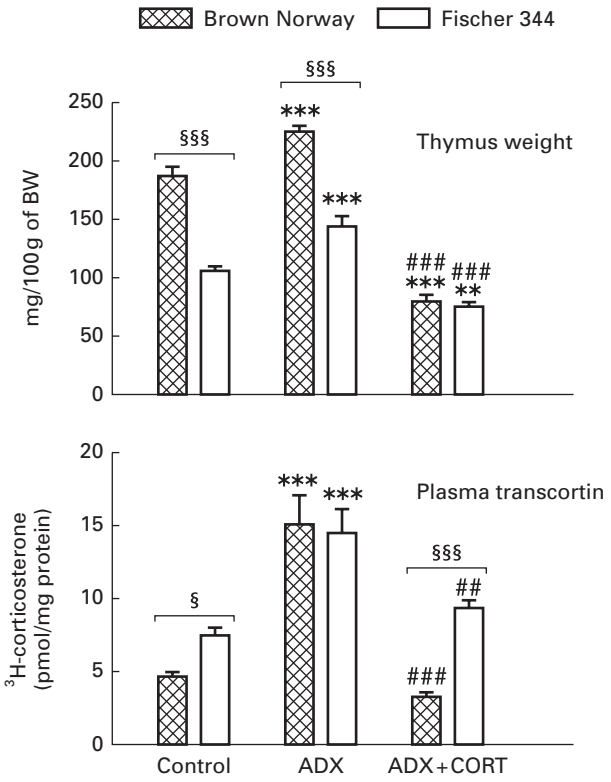

FIG. 2. Thymus weight and plasma transcortin concentrations. Thymus weight and plasma transcortin were reduced *vs* ADX to a greater extent by chronic corticosterone in BN rats than in F344 rats. Different from the control group designed as $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Different from the ADX group designed as $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$. BN different from F344 designed as $^{\S}P<0.05$, $^{\S\S}P<0.01$, $^{\S\S\S}P<0.001$.

to control concentrations. When compared to the ADX group values, the percentage of inhibition induced by corticosterone treatment was twice larger in BN (−78.1%) than in F344 rats (−35.6%).

MR binding assays

Pituitary

Strain and treatment effects ($P<0.001$) were revealed by ANOVA (Fig. 3). MR levels were higher in F344 rats than in BN rats. In both strains, ADX induced a threefold increase ($P<0.001$), and corticosterone treatment restored MR pituitary levels to those observed in control groups.

Hypothalamus

A significant treatment effect was shown by anova ($P<0.01$). In both strains, ADX increased MR hypothalamic levels ($P=0.01$) and corticosterone treatment restored them to those observed in control groups (Fig. 3).

Hippocampus

Significant effects of treatment ($P<0.05$) and strain ($P<0.001$) were shown by ANOVA (Fig. 3). MR levels were twofold greater in F344 rats than in BN rats. In both strains, ADX increased hippocampal MR levels ($P<0.05$), and corticosterone treatment restored them to those observed in control groups.

Kd was slightly higher in BN rats than in F344 rats (0.19 ± 0.03 *vs* 0.13 ± 0.01 nM, $P<0.05$) and the treatment effect was marginally significant (control 0.22 ± 0.04 nM, ADX 0.14 ± 0.02 nM, ADX+CORT 0.12 ± 0.01 nM, $P<0.05$).

GR binding assays

Pituitary

ANOVA did not reveal any significant effects (Fig. 4).

Hypothalamus

A significant treatment effect ($P<0.001$) was shown by anova (Fig. 4). Although the BN control group exhibited higher GR levels in hypothalamus than F344 control group ($P<0.01$), no significant strain difference was shown in ADX or ADX+CORT groups. No effect of ADX was observed in BN rats, whereas in F344 rats GR levels increased by 73.7% after long-term ADX ($P<0.05$). Chronic corticosterone reduced GR levels by a similar extent in the two strains (−50% *vs* ADX).

Hippocampus

Significant strain (F344>BN, $P<0.05$) and treatment ($P<0.01$) effects were shown by ANOVA (Fig. 4). ADX

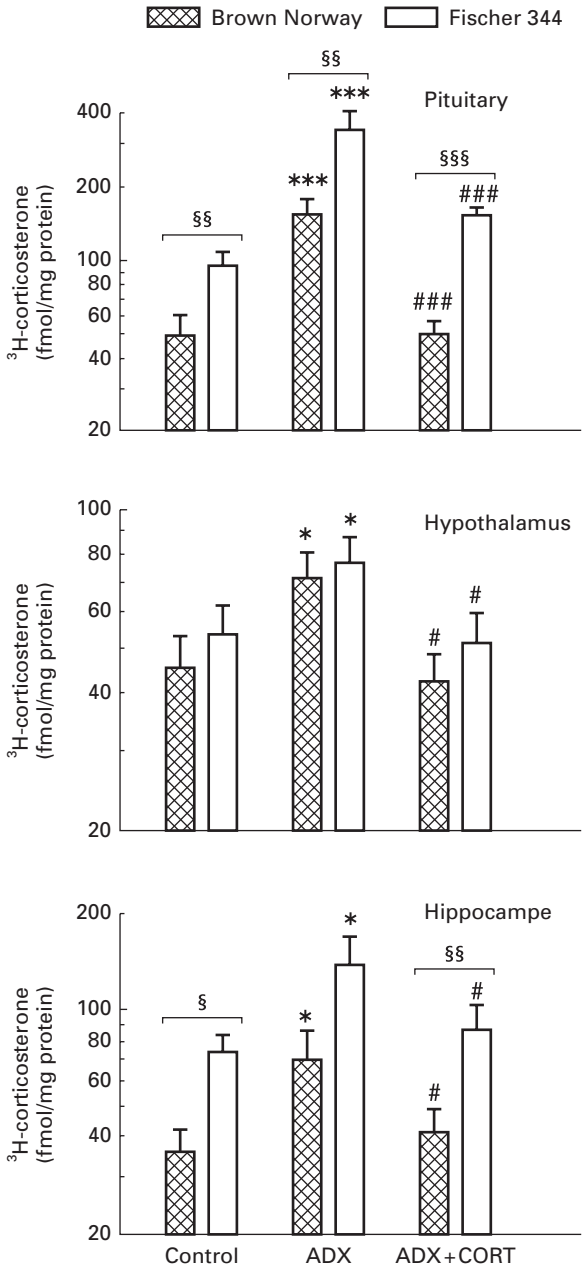

FIG. 3. MR binding in the pituitary, hypothalamus and hippocampus. F344 rats displayed higher brain and pituitary MR levels than BN rats. Long-term ADX increased MR levels whereas chronic corticosterone restored them to the control values. Different from the control group designed as $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Different from the ADX group designed as $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$. BN different from F344 designed as $^{\S}P<0.05$, $^{\S\S}P<0.01$, $^{\S\S\S}P<0.001$.

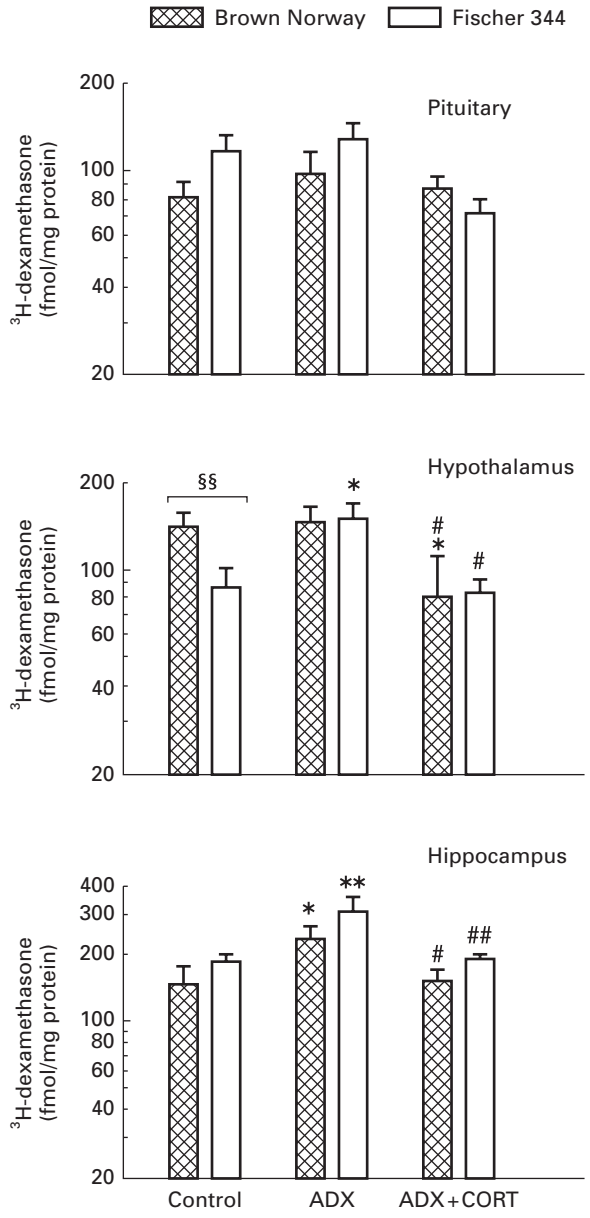

FIG. 4. GR binding in the pituitary, hypothalamus and hippocampus. Tissue- and strain-specific regulations were observed for GR levels. Different from the control group designed as $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Different from the ADX group designed as $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$. BN different from F344 designed as $^{\S}P<0.05$, $^{\S\S}P<0.01$, $^{\S\S\S}P<0.001$.

increased hippocampal GR level both in BN rats (+58%, $P<0.01$) and in F344 rats (+68%, $P<0.01$). This effect was abolished by corticosterone treatment.

Kd was not different between strains (BN 0.72 ± 0.07 nM, F344 0.64 ± 0.06 nM) and experimental groups (control 0.73 ± 0.10 nM, ADX 0.70 ± 0.10 nM, ADX+CORT 0.62 ± 0.04 nM).

Discussion

This study was designed to determine the effects of corticosterone on constitutive proteins of the HPA axis and on some corticosterone-sensitive physiological end points in BN and F344 rats, strains that differ in their corticosterone secretory activity both in basal conditions and in response to stress (22). The examination of long-term ADX rats allows us to characterize differences between strains in the absence of regulation by corticosterone. By examining changes induced by fixed corticosterone concentrations in ADX rats, we are

able to determine the sensitivity of the mechanisms involved in regulation by corticosterone, whereas levels in control rats result from variable initial expression, as well as different free corticosterone concentrations and receptor sensitivity to corticosterone.

The effects of corticosterone on b.w. follow a bell-shape curve with increasing concentrations of hormone (23) as a result of different components of corticosterone actions on consumatory behaviours and metabolism (24, 25, 31). Optimal growth is reached at around physiological concentrations of corticosterone. Growth is reduced in ADX rats, restored to normal by low doses of corticosterone acting via MRs, and decreased by high doses as a result of the catabolic effect of corticosterone on fat and protein stores via GRs (24, 25, 31). Our study demonstrated that F344 rats followed the expected curve whereas BN rats differed in two respects: their b.w. appeared to be insensitive to corticosterone deprivation, but more sensitive to a supraphysiological excess of corticosterone. These data suggest that MR mechanisms involved in the metabolic and/or behavioural actions of corticosterone are active independently of the presence of their ligands, i.e. constitutively active in BN rats. The greater sensitivity of GR actions to corticosterone may be due to a higher concentration of the free, active fraction of corticosterone in blood and/or a greater efficiency of GR mechanisms (i.e. stronger effects for the same dose of corticosterone) in the BN strain.

BN rats did not show the expected ADX-induced increase of fluid intake, possibly reflecting constitutive activation of MR mechanisms regulating salt and water balance at the levels of the kidneys and/or the brain (27, 32, 33). The aldosterone-like effect of corticosterone on hydric balance through GRs may allow F344 rats to regulate their fluid intake (28). Since BN and F344 rats had the same drink intake, they received the same dose of corticosterone in μg/g of b.w.

To test the hypotheses about differential GR effects of corticosterone, we determined plasma transcortin concentrations. No difference was observed between strains in 21 days-ADX rats. As expected, transcortin concentrations were negatively regulated by chronic corticosterone via GRs (29, 30) and again, to a greater extent in BN rats. BN control and corticosterone-treated rats exhibited lower transcortin concentrations than F344 rats. As a result, the BN strain had a greater fraction of free corticosterone in plasma. This difference may partly explain the apparently higher sensitivity of GR mechanisms described above. Consequently, the differences in control rats resulted from the combination of higher total corticosterone concentrations in F344 rats, but a larger free fraction, and possibly a higher efficiency, of corticosterone action via GRs in BN rats (22).

Thymus weight was higher in BN ADX rats (+77.1% *vs* F344). This difference is therefore structural, that is to say, a difference which persists without any regulation by corticosterone. As expected, thymus weight was negatively regulated by corticosterone via GRs (26). Chronic corticosterone decreased thymus weight of the two strains, but to a greater extent in BN rats. This result agrees with the previous suggestion that GRs are more stimulated and/or more active in BN than in F344 rats.

In all treatment conditions, pituitary and hippocampal MR levels were twice as high in F344 as in BN rats, showing that this strain difference is structural and independent of regulation by corticosterone. No strain difference was detected in the hypothalamus. As previously described (16, 18), long-term ADX increased MR levels in all the structures studied, and chronic corticosterone administration returned MRs to the levels observed in control rats.

Recent data suggest that GR and GR mRNA are regulated by both auto- and heteroregulation (17, 34, 35), and that MR regulation of GRs is tissue-specific (17). In our case, for GR levels, both strain and treatment effects were tissue-specific. Neither strain nor treatment effects were observed in the pituitary. Treatment effects in the hippocampus were similar to those described for MRs, with higher levels in F344 rats and a negative regulation by corticosterone, compatible with an autoregulation via GRs. Conversely, no structural difference between strains was detected in the hypothalamus of long-term ADX rats, and the regulation by corticosterone was more complex. Although ADX increased GR levels in F344 rats, no change was observed in BN rats, suggesting a MR-mediated mechanism (constitutively active in BN rats) and/or different systems of regulation of GR in BN rats and F344 rats. Under chronic corticosterone treatment, GRs were similarly down-regulated in both strains, suggesting a GR-mediated mechanism. This succession of MR- and GR-regulations with increasing doses of corticosterone is reminiscent of the observations on regulation of b.w. Most publications report similar results concerning GR regulation by corticosterone in pituitary (16, 36), hypothalamus and hippocampus (16, 37–39).

The present results demonstrate the diversity of mechanisms involved in HPA axis activity/reactivity and corticosterone action in BN and F344 rats. Ultimately, the level of regulation by corticosterone results from: (1) the plasma concentration of free corticosterone; (2) variable receptor efficiencies; and (3) modulation of the two corticosteroid receptor types through auto- and hetero-regulations. MR mechanisms appear to be constitutively active in BN rats, since they appear insensitive to removal of their endogenous ligands by ADX. On the other hand, GR actions are larger in BN rats even when corticosterone concentrations are stabilized by fixed concentrations in drinking fluid. The differences observed between BN rats and F344 rats in corticosteroid receptor levels cannot completely explain the strain differences in the biological actions of corticosterone. Thus, we assume that the efficiency of transduction mechanisms (intracellular hormone availability, dissociation of the heat shock protein complex, receptor phophorylation, nuclear translocation, DNA binding, etc.) also differ between BN rats and F344 rats (40). On the other hand, the higher sensitivity of transcortin down-regulation by corticosterone in BN rats may also contribute to the greater efficiency of corticosterone on GR mechanisms in this strain.

The strain differences in corticosteroid receptor activation can explain the functional differences in corticosterone secretion. Indeed, the faster return of corticosterone concentrations to baseline after the evening secretory peak and after restraint stress that we have previously documented in BN rats, as compared to F344 rats (22), may be associated with a larger

GR activation, as a result of higher levels of GRs in hypothalamus, lower circulating transcortin concentrations (and therefore a larger free fraction of corticosterone), and possibly with a better efficiency of GR mechanisms in BN rats.

Materials and methods

Animals

Male inbred BN and F344 rats, 7 weeks old, weighing 200–250 g, were purchased from IFFA Credo (L'Arbresle, France). Rats were housed four per cage in a temperature-controlled room (23 ± 1 °C) with a light/dark cycle of 12/12 h (lights on at 07.00 h). Food and water were provided *ad libitum*. Rats were allowed to adapt to the animal room for 10 days before being randomly assigned to one of the three experimental groups ($n = 6$–8 per group).

(1) The first group consisted of rats left intact during the whole experimental period and adrenalectomized 24 h before they were killed, to remove endogenous circulating corticosteroids, and thus to release all available binding sites without modifying receptor levels (5). This group (designated as control) was watered with 0.9% saline and 1% ethanol for 21 days.

(2) The second group consisted of rats adrenalectomized on the first day (d_0) of the experimental period and watered with 0.9% saline and 1% of ethanol for 21 days (designated as ADX).

(3) The third group consisted of rats adrenalectomized on the first day of the experiment and watered with saline and 1% of an alcoholic solution of corticosterone (100 µg of corticosterone per ml of drinking fluid, provided by Sigma (St Louis, MO, USA) for 20 days. The corticosterone treatment was ended the day before they were killed (12). This third group was designated as ADX + CORT. ADX was performed through small bilateral incisions (1–1.5 cm) under pentobarbital anaesthesia. Incisions were closed with surgical gut and wound clips.

Fluid intake was measured every morning by weighing the bottles. Rats were weighed at the time of surgery and every 7 days during the experimental period.

Rats were killed by rapid decapitation in the morning. The brain was quickly removed and the pituitary, hypothalamus and hippocampus were rapidly dissected on ice, frozen on dry ice and stored at −80 °C until use. Trunk blood was collected into chilled tubes coated with a 10% EDTA solution and centrifuged (4500 g, 15 min, 4 °C) in order to extract plasma, which was stored at −80 °C for subsequent measurement of corticosterone and transcortin. Thymus glands were removed, blotted and weighed.

Plasma transcortin

Plasma transcortin measurement was performed as described by Sarrieau and Mormède (22). Plasma was diluted 50-fold with TEGMD buffer (30 mM Tris-HCl (pH 7.4), 1 mM disodium EDTA, 10% (v/v) glycerol, 10 mM Na_2MoO_4, and 1 mM dithiothreitol). aliquots of diluted plasma (225 µl) were incubated with 150 µl of 50 nM [^{3}H]-corticosterone (88.4 Ci/mmol, purchased from NEN products, Boston, MA, USA). Non-specific binding was measured with a 500-fold excess of unlabelled corticosterone. The incubation lasted 18–20 h at 4 °C. Bound was separated from unbound steroid using the method described below for receptors. Plasma proteins were measured with the method of Bradford (41) with bovine serum albumin as the standard to express results as pmol of [^{3}H]-corticosterone bound per mg/protein.

Receptor assays

All procedures for the receptor assays were performed at 4 °C as described by Sarrieau *et al.* (42). Frozen tissues were homogenized in cold TEGMD buffer (one hypothalamus or one pituitary in 1.2 ml or one hippocampus in 3.3 ml). The homogenate was centrifuged at 105,000 g for 60 min at 4 °C. The resulting supernatant was referred to as cytosol.

For the determination of pituitary and hypothalamic MRs, 150 µl of cytosol were added to 100 µl of 5 nM [^{3}H]-corticosterone in the presence of a 250-fold excess of the specific GR receptor agonist RU 28362 (3, 43) to prevent binding of [^{3}H]-corticosterone to GR. A 500-fold excess concentration of unlabelled aldosterone was used to define non-specific binding in parallel incubations. Transcortin has very little affinity for aldosterone (44, 45) and thus [^{3}H]-corticosterone binding to any transcortin present in traces of contaminating blood or in pituitary tissue were included in the nonspecific binding. GR measurements were performed with a 5 nM concentration of [^{3}H]-dexamethasone (45.0 Ci/mmol, obtained from NEN products, Boston, MA, USA). Non-specific binding was determined with a 500-fold excess concentration of unlabelled RU 28362. For both receptors in the hippocampi, maximal binding (B_{max}) and dissociation constant (Kd) were measured with seven concentrations (0.1–10 nM) of [^{3}H]-ligand. A parallel incubation with a 500-fold excess of unlabelled ligand was used to assess nonspecific binding.

After 20–24 h of incubation at 4 °C, 100 µl aliquots were applied in duplicate to Sephadex LH 20 columns to separate bound label-receptor complexes from the free ligand (42). The columns were made up in 1 ml disposable plastic pipette tips equilibrated with TEGMD buffer. Thirty min later, 500 µl of TEGMD was added and the eluate collected into scintillation vials. Scintillation fluid (3.5 ml) was added, and samples were counted at ≈60% efficiency by a liquid scintillation counter (LS 6500, Beckman Instruments, Gagny, France). Protein content in the cytosol was determined by the method of Bradford (41). The binding data were expressed as femtomoles [^{3}H]-steroid bound per mg protein. B_{max} and Kd values were derived from Scatchard analysis (46) for hippocampi.

Plasma corticosterone

Plasma corticosterone concentrations were determined following extraction with absolute ethanol and using [^{3}H]-corticosterone as radioligand and transcortin from rhesus monkey plasma as binder as previously described (22). Long-term ADX rats, except three which were excluded from the analysis, showed plasma corticosterone concentrations below assay detection limits. The final number of rats was six for BN control and ADX groups, eight for BN ADX + CORT group, eight for F344 control, seven for F344 ADX and eight for F344 ADX + CORT groups.

Data analysis

Results are expressed as means ± SEM. Data were analysed by a two-way ANOVA with strain (BN, F344) and treatment (control, ADX, ADX + CORT) as main factors. *Post-hoc* Newman-Keuls tests were performed when the anova was significant. A 'two between' (strain, treatment) and 'one within' (time) factors anova was applied to analysis of body weights. A logarithmic transformation was carried out to normalize the variances of receptor binding results. $P < 0.05$ was considered as significant.

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