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A SINGLE CORTICOSTERONE PRETREATMENT INHIBITS
THE HYPOTHALAMIC-PITUITARY-ADRENAL RESPONSES TO
ADRENERGIC AND CHOLINERGIC STIMULATION

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The purpose of the present study was to determine whether an increased plasma corticosterone or dexamethasone levels induced by a single corticosterone or dexamethasone injection to conscious rats affects the hypothalamic-pituitary-adrenocortical (HPA) activity induced by adrenergic and cholinergic agonists. Male Wistar rats were pretreated subcutaneously (s.c.) with a single dose of dexamethasone (5 mg/kg) or corticosterone (25 mg/kg) 24 or 48 h before intraperitoneal (i.p.) administration of adrenergic agonists: phenylephrine, an α₁-adrenergic receptor agonist, clonidine, a β₂-adrenergic agonist and noradrenaline acting predominantly on α₁-adrenoceptors, and cholinergic agonists: carbamaze, a predominant muscarinic receptor agonist and nicotine, a nicotinic receptor agonist. Dexamethasone profoundly decreased the resting ACTH levels in control rats and given 24 h before each of the stimulatory agonist abolished the adrenergic and cholinergic agonists-induced ACTH and corticosterone responses. Pretreatment with corticosterone of control rats did not substantially alter the resting plasma ACTH and serum corticosterone levels measured 24 and 48 h later. A single pretreatment with corticosterone abolished or powerfully inhibited, perhaps by a feedback mechanism, the ACTH and corticosterone responses induced 24 and 48 h later by all adrenergic and cholinergic agonists used in this study. These results indicate that prolonged administration of corticosterone is not necessary to induce almost complete suppression of the HPA responsiveness to adrenergic or cholinergic stimulation. Chronic treatment with corticosteroids to achieve glucocorticoid receptors desensitization does not seem to be required.

Key words: single corticosterone pretreatment, dexamethasone, ACTH, corticosterone, HPA axis, depression.

INTRODUCTION

It is generally accepted that increased central secretion of corticotropin releasing hormone (CRH) under prolonged stressful stimulation and consequent hyperactivity of the HPA axis is connected with development of depression (1—3). Hyperactivity of CRH neuronal systems during depression is
thought to result from desensitization of glucocorticoid receptors in structures involved with feedback inhibition of the HPA axis (4—6). Therefore, central glucocorticoid receptor (GR) signaling is impaired in depression, resulting in increased production and secretion of CRH (7, 8). The hypothesis that reducing HPA hyperactivity and remission of clinical symptoms of depression are related is supported by the fact that prolonged antidepressant treatment normalizes the HPA axis. These drugs act at neurotransmitter and signaling pathways that regulate HPA activity (9, 10). However, until now, the question whether hypersecretion of CRH or GR dysfunction plays a primary role or any role in the physiological and behavioral anomalies associated with stress-related disorders is not resolved (6).

Although CRH is one of the major physiologic stimulus for the pituitary-adrenocortical axis leading to secretion of ACTH and cortisol in humans or corticosterone in rodents, this neuropeptide acts in concert with other neuropeptides and neurotransmitters during almost any stimulation of the HPA axis in both physiological and pathological conditions. Experimental data clearly show that some neurotransmitters are far more potent stimuli of the HPA axis than CRH itself. Coregulatory role of different neurotransmitter systems in the HPA axis activity during depression has not been elucidated. The aminergic systems, e.g. catecholaminergic, serotonergic or cholinergic projecting to the hypothalamus also modulate glucocorticoid secretion. On the other hand glucocorticoids, the major adrenal corticosteroids, are known to regulate the actions of several classical neurotransmitters and peptides (11). Corticosterone alters the responses of neurotransmitter receptors which are linked to G proteins (12).

In some experimental models of depression prolonged HPA hypersecretion is imitated by daily injections of corticosterone lasting for weeks in order to examine alterations in particular glucocorticoid receptors and/or neurotransmitter system involved in the HPA stimulation. Glucocorticoids are known to be major regulators of a fast negative feedback inhibition of the HPA axis via repression of CRH and ACTH release.

The aim of the present study was to determine whether chronic hypersecretion of glucocorticoids is necessary to induce desensitization of GR and diminished HPA responsiveness to neurotransmitter stimulation and to what extent, a single pretreatment with corticosterone affects the HPA responses to adrenergic and cholinergic stimulation.

MATERIALS AND METHODS

Male Wistar rats weighing 170—200 g were used in these studies. The animals were housed in solid bottom cages with sawdust litter, 6 per cage and were fed commercial food and water ad libitum. The animal room was maintained on a 12 h light, 12 h dark cycle. All animals were given
a one week acclimation period before the onset of experimentation. The experiments were approved by the local Ethical Committee.

General procedures

The experiments were performed in five groups. The rats of control group were pretreated i.p. with saline or s.c. with Tween 1% solution. Experimental groups were pretreated s. c with single dose of dexamethasone (5 mg/kg) or corticosterone (25 mg/kg). In these groups of rats the effect of subsequent administration of adrenergic agonists and cholinergic agonists, 24 h and 48 h later, on ACTH and corticosterone secretion was investigated.

Induction of ACTH and corticosterone secretion

The rats pretreated with solvent, dexamethasone or corticosterone were injected i.p. with adrenergic agonists, phenylephrine (0.2 mg/kg), an α₁-adrenergic receptor (AR) agonist, clenbuterol (0.1 and 0.025 mg/kg), a β₂-AR agonist and noradrenaline (0.1 mg/kg) a predominant α₁-AR agonist, or with cholinergic receptor agonists, carbachol (0.25 mg/kg), a predominant muscarinic receptor agonist and nicotine (2.5 mg/kg), a selective nicotinic receptor agonist.

Preparation of drugs

Drugs used in this study were corticosterone acetate, dexamethasone acetate, phenylephrine hydrochloride, (-) artenol, noradrenaline bitartrate, clenbuterol, carbamylcholine hydrochloride (Carbachol) and nicotine, all from Sigma. The doses used are expressed in terms of salts. Corticosterone and dexamethasone were prepared for injection by sonication in 1% Tween 80 solution, while adrenergic and cholinergic agonists were dissolved in saline. Solutions were prepared immediately before use. The drugs or solvent were administered s.c. or i.p. in a volume of 0.2 ml per 100 g body weight. Corticosterone in a dose of 25 mg/kg was injected 24 or 48 h before each of adrenergic or cholinergic receptor agonists and dexamethasone, 5 mg/kg, was given 24 h before each of agonist.

One hour after the last injection the rats were decapitated immediately after their removal from the cage and their trunk blood samples were collected on ice in plastic conical tubes containing 200 µl of a solution of 5 mg/ml EDTA and 500 TIU of aprotinin (Sigma). Control rats were decapitated concurrently with the experimental group. Plasma was separated by centrifugation in a refrigerated centrifuge within 30 min and frozen at -20°C until the time of assay. Plasma ACTH concentrations were measured using the double antibody ¹²³I radioimmunoassay obtained from CIS Bio International and calculated as µg/ml of plasma. The concentration of serum corticosterone was measured fluorometrically and expressed as µg per 100 ml. To avoid circadian variability, all experiments were performed between 10—11 a.m. and all decapitations between 11—12 a.m., when plasma hormones are at a relatively low levels.

Statistics

The results were calculated as a group mean±standard error of the mean. Statistical evaluation was performed by an analysis of variance, followed by individual comparisons with Duncan's test. The results were considered to be significantly different when p<0.05.
RESULTS

Effect of dexamethasone on ACTH and corticosterone responses to adrenergic stimulation

Dexamethasone injected s.c. in a single dose of 5 mg/kg markedly decreased 24 h later the resting plasma ACTH level to 12 pg/ml from 73.6 pg/ml in saline-injected controls and did not substantially alter the resting corticosterone level.

Dexamethasone injected 24 h before i.p. administration of adrenergic agonists used in this study was able to totally prevent the HPA axis stimulation by these agonists observed in control rats. A significant and dose-related stimulation of ACTH and corticosterone secretion by phenylephrine, an α1-adrenergic receptor agonist, was totally prevented in rats pretreated with dexamethasone. The phenylephrine (0.2 and 0.5 mg/kg) — induced increases in plasma ACTH levels (189.6 and 727.8 pg/ml) were totally reduced (12.8 and 36.7 pg/ml) below the resting ACTH level (Table 1). Likewise, the

Table 1. Effect of dexamethasone on plasma ACTH and serum corticosterone levels induced by adrenergic and cholinergic agonists.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg i.p.</th>
<th>ACTH pg/ml</th>
<th>Corticosterone μg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>0.2 ml/kg</td>
<td>73.6 ± 9.0</td>
<td>7.8 ± 1.2</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>5.0</td>
<td>12.0 ± 1.5</td>
<td>6.3 ± 0.2</td>
</tr>
<tr>
<td>Adrenergic agonists</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>0.2</td>
<td>189.6 ± 25.0*&lt;sup&gt;+&lt;/sup&gt;</td>
<td>20.7 ± 4.6*&lt;sup&gt;++&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dex + Phenylephrine</td>
<td>5.0 + 0.2</td>
<td>12.8 ± 3.0**</td>
<td>2.8 ± 0.3**</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>0.5</td>
<td>727.8 ± 120**</td>
<td>60.5 ± 10**</td>
</tr>
<tr>
<td>Dex + Phenylephrine</td>
<td>5.0 + 0.5</td>
<td>36.7 ± 3.2**</td>
<td>3.6 ± 0.4**</td>
</tr>
<tr>
<td>Clenbuterol</td>
<td>0.1</td>
<td>521.3 ± 95**</td>
<td>46.3 ± 5.3**</td>
</tr>
<tr>
<td>Dex + Clenbuterol</td>
<td>5.0 + 0.1</td>
<td>50.0 ± 8.2**</td>
<td>3.1 ± 1.0**</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>0.1</td>
<td>248.± 41**</td>
<td>36.1 ± 1.5**</td>
</tr>
<tr>
<td>Dex + Noradrenaline</td>
<td>5.0 + 0.1</td>
<td>18.3 ± 1.5**</td>
<td>3.7 ± 1.0**</td>
</tr>
<tr>
<td>Cholinergic agonists</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbachol</td>
<td>0.25</td>
<td>549.0 ± 98**&lt;sup&gt;+&lt;/sup&gt;</td>
<td>38.5 ± 9.2**</td>
</tr>
<tr>
<td>Dex + Carbachol</td>
<td>5.0 + 0.25</td>
<td>33.5 ± 3.5**</td>
<td>2.9 ± 0.5**</td>
</tr>
<tr>
<td>Carbachol</td>
<td>0.5</td>
<td>742.0 ± 154**&lt;sup&gt;+&lt;/sup&gt;</td>
<td>60.7 ± 7.3**</td>
</tr>
<tr>
<td>Dex + Carbachol</td>
<td>5.0 + 0.5</td>
<td>125.0 ± 27**&lt;sup&gt;+&lt;/sup&gt;</td>
<td>13.3 ± 5.6**</td>
</tr>
<tr>
<td>Nicotine</td>
<td>2.5</td>
<td>376.3 ± 63**&lt;sup&gt;+&lt;/sup&gt;</td>
<td>31.4 ± 2.9**</td>
</tr>
<tr>
<td>Dex + Nicotine</td>
<td>5.0 + 2.5</td>
<td>40.9 ± 7.3**&lt;sup&gt;+&lt;/sup&gt;</td>
<td>6.1 ± 0.2**</td>
</tr>
</tbody>
</table>

Dexamethasone (Dex) was dissolved in 1% Tween solution and injected s.c. 24 h before i.p. adrenergic or cholinergic agonists. One hour after the last injection the rats were decapitated and trunk blood was collected for hormone determinations. Values represent the mean ± SEM of 4—6 rats. *<sup>+</sup>p < 0.01 vs. solvent control group, **p < 0.001 vs. respective agonist treated group.
corresponding phenylephrine-induced corticosterone levels, (20.7 and 60.5 µg/dl) were suppressed (2.8 and 3.6 µg/dl) below the resting control level. A potent stimulatory action of clenbuterol (0.1 mg/kg) a β₂-adrenergic receptor agonist, was also abolished in dexamethasone pretreated rats. Dexamethasone lowered the clenbuterol-induced ACTH and corticosterone levels, from 521.3 pg/ml and 46.3 µg/dl to 50.0 pg/ml and 3.1 µg/dl, respectively. Likewise, dexamethasone abolished the noradrenaline (0.1 mg/kg)-evoked increase in ACTH and corticosterone secretion, from 248.5 pg/ml and 36.1 µg/dl to 18.3 pg/ml and 3.7 µg/dl, respectively (Table 1).

Effect of dexamethasone on ACTH and corticosterone responses to cholinergic stimulation

Dexamethasone (5 mg/kg s.c.) given 24 h before carbachol, a predominantly muscarinic cholinergic receptor agonist, abolished its potent stimulatory effect on ACTH and corticosterone secretion observed in control, solvent-injected rats. The carbachol (0.25 and 0.5 mg/kg i.p.) — increased ACTH levels, (549 and 742 pg/ml), were reduced by dexamethasone (33.5 and 125 pg/ml), and respective corticosterone levels, (38.5 and 60.7 µg/dl), were diminished (2.9 and 13.3 µg/dl) to the resting levels. The nicotine (2.5 mg/kg) — induced significant increase in ACTH and corticosterone levels (376.3 pg/ml and 31.4 µg/dl) were suppressed by dexamethasone injected 24 h earlier, (40.9 pg/ml and 6.1 µg/dl) to the resting levels in vehicle-pretreated rats (Table 1).

Effect of corticosterone pretreatment on adrenergic agonists-induced ACTH and corticosterone responses

The significantly increased plasma ACTH levels (190 and 192 pg/ml) induced by phenylephrine (0.2 mg/kg i.p.) were abolished (19 and 104 pg/ml) by preteratment of rats 24 or 48 h earlier with corticosterone (25 mg/kg s.c.). Likewise, phenylephrine administered 24 or 48 h after pretreatment with corticosterone did not augment serum corticosterone levels above the control levels in corticosterone pretreated rats (Fig. 1).

In control saline-pretreated rats clenbuterol (0.025 mg/kg s.c.), considerably increased ACTH secretion. Pretreatment with corticosterone 24 or 48 h earlier profoundly inhibited this stimulatory action on ACTH, by 80 and 73%, respectively. Corticosterone pretreatment also considerably impaired, by 71 and 67%, the clenbuterol-induced corticosterone response 24 and 48 h later, respectively (Fig. 2).
Fig. 1. Effect of pretreatment with corticosterone (25 mg/kg i.p.) on ACTH and corticosterone response to phenylephrine (PHEN) (0.2 mg/kg i.p.) given 24 or 48 h later. In Fig. 1—5 adrenergic or cholinergic agonist was injected i.p. and 1 h later the rats were decapitated. Values represent the mean ± SEM of 5—6 rats. **p < 0.01 vs. solvent control group. *p < 0.05 and **p < 0.01 vs. corresponding agonist treated group.

Fig. 2. Effect of pretreatment with corticosterone on ACTH and corticosterone response to clenbuterol (CLEN) (0.025 mg/kg i.p.) given 24 or 48 h later. See text to Fig. 1.

Corticosterone administered 24 or 48 h before noradrenaline (0.1 mg/kg s.c.) totally abolished the noradrenaline-induced increase in ACTH secretion. The corticosterone response to noradrenaline was abolished in rats pretreated with corticosterone 48 h earlier and was significantly diminished in rats pretreated 24 h before noradrenaline (Fig. 3).
Effect of corticosterone pretreatment on cholinergic-induced ACTH and corticosterone responses

Carbachol potently increased ACTH secretion in control, solvent-pretreated rats. Corticosterone (25 mg/kg s.c.) given 24 h earlier abolished the rise in carbachol-induced ACTH secretion and injected 48 h earlier considerably (by 70%) diminished this secretion. Also carbachol-elicited increase in corticosterone secretion was significantly impaired (by 43 and 67%) in rats pretreated 24 and 48 h earlier (Fig. 4).

**Fig. 3.** Effect of pretreatment with corticosterone on ACTH and corticosterone response to noradrenaline (NA) (0.1 mg/kg i.p). See text to Fig. 1.

**Fig. 4.** Effect of pretreatment with corticosterone on ACTH and corticosterone response to carbachol (CARB) (0.25 mg/kg i.p.). See text to Fig. 1.
The stimulatory action of nicotine (2.5 mg/kg i.p.) on ACTH secretion was also considerably reduced (74 and 73%) by single pretreatment 24 and 48 h earlier with corticosterone (25 mg/kg s.c.). Likewise, the nicotine-evoked significant rise in corticosterone secretion was profoundly impaired, by 70% and 89%, respectively, by pretreatment with corticosterone, 24 and 48 h earlier (Fig. 5).

**Fig. 5.** Effect of pretreatment with corticosterone on ACTH and corticosterone response to nicotine (NIC) (2.5 mg/kg i.p.). See text to Fig. 1.

**DISCUSSION**

In the present experiment the stimulation of the HPA axis was induced by activation of adrenergic and cholinergic systems which are indispensable regulators of the HPA activity, particularly during stress conditions and development of neuroendocrine disorders associated with CRH hyperactivity (13). We investigated the influence of endogenous and synthetic glucocorticoids, corticosterone and dexamethasone, on the HPA response to adrenergic and cholinergic stimulation. Corticosterone has a very high affinity for mineralocorticoid receptor (MR) predominantly found in the hippocampus and lower affinity for glucocorticoid receptors (GR) widely expressed in the brain. On the other hand the synthetic dexamethasone has a high affinity for GR but a very low affinity for MR (14, 15).

In the present experiment 24 h after administration, dexamethasone itself in relatively large dose (5 mg/kg s.c.) strongly decreased the resting plasma ACTH levels (from 73.6 to 12 pg/ml). This indicates preferential and major inhibitory action of dexamethasone on anterior pituitary corticotrophs. A single pretreatment of rats with dexamethasone totally abolished significant increase
in ACTH and corticosterone secretion induced by subsequent administration 24 h later of adrenergic receptor agonists: phenylephrine, an $\alpha_1$-adrenergic receptor agonist, clenbuterol, a $\beta_2$-adrenergic agonist and noradrenaline a preferential $\alpha_1$-adrenergic agonist, or cholinergic agonists: carbachol, a muscarinic receptor agonist, and nicotine, a nicotinic receptor agonist.

Since this synthetic glucocorticoid poorly penetrates the blood-brain barrier therfore, after systemic administration dexamethasone may act on median eminence of the hypothalamus (16) and anterior pituitary, structures not protected by the blood-brain-barrier. Although dexamethasone given systemically may inhibit the release of CRH from hypothalamic paraventricular neurons (4) stimulated by adrenergic and cholinergic agonists, its effect on pituitary ACTH content is of greater magnitude than its effect on hypothalamic CRH (17, 18).

Corticosterone administered s.c. readily penetrates central nervous system and can directly affect neuroendocrine regulation. Pretreatment with a single dose of corticosterone (25 mg/kg s.c.) totally abolished the phenylephrine-induced rise in plasma ACTH and serum corticosterone levels observed in control vehicle-pretreated rats. This inhibitory effect was equally potent both 24 and 48 h after corticosterone administration. Corticosterone given s.c. only slightly increased serum corticosterone levels 24 h but not 48 h later in comparison with the levels in vehicle-treated rats. Pretreatment with corticosterone considerably decreased, but did not abolish, the clenbuterol-induced ACTH and corticosterone responses 24 h and 48 h later. The inhibition of the clenbuterol-induced ACTH and corticosterone responses by pretreatment with corticosterone was somewhat weaker in comparison with a total reduction of the phenylephrine-induced responses. It is known that at physiological concentrations endogenous adrenal glucocorticoids facilitate $\beta$-receptor function in vivo and enhance $\beta$-receptor mediated responses by regulating the coupling of $\beta$ receptors to G proteins and adeny cyclase activation (19) or upregulating $\beta_2$-adrenoceptors (20). $\beta_2$-adrenergic receptor agonists salbutamol or salmeterol translocated GR into the nucleus beginning 30 min after treatment and corticosteroids-induced activation of GR occured at the same time in human lung fibroblast and vascular smooth muscle cells (21). Therefore, corticosterone pretreatment may augment the clenbuterol-induced ACTH and corticosterone responses. However, in the present experiment exogenous corticosterone considerably impaired the $\beta_2$-receptor-induced ACTH and corticosterone secretion 24 and 48 h after administration. This observation indicates that corticosterone-induced feedback inhibition, via glucocorticoid receptors, of the HPA axis was much stronger than a potential enhancement by corticosterone of the clenbuterol-stimulated hormone responses. Pretreatment of rats with relatively high dose of corticosterone (25 mg/kg s.c.) was also sufficient to abolish 24 and
48 h later the noradrenaline-induced ACTH and corticosterone secretion. This effect may be interpreted as a result of strong feedback inhibition of pituitary corticotrophs by previously injected corticosterone.

Systemically administered cholinergic agonists stimulate the HPA axis through a centrally-mediated CRH-dependent mechanism (22). Intracerebroventricular administration of carbachol (23) or a cholinesterase inhibitor neostigmine (24) significantly increase the HPA activity. Likewise, nicotine, after peripheral administration, easily penetrates and stimulates the hypothalamic brain structures involved in the HPA axis stimulation (25).

In the experiment reported here both carbachol, a cholinergic muscarinic receptor agonist and nicotine, nicotinic receptor agonist, increased the secretion of ACTH and corticosterone 1 h after administration. Corticosterone pretreatment considerably diminished, but did not abolish, the carbachol-induced increase in ACTH and corticosterone secretion 24 and 48 h later. Corticosterone pretreatment evoked an equally potent inhibition of nicotine-induced ACTH and corticosterone secretion. These inhibitory effects of corticosterone, 24 and 48 h after its administration, seem to result from feedback inhibition of the HPA axis via glucocorticoid receptors. On the other hand, tolerance to nicotine responsiveness may be induced by acute administration of corticosterone or by exposure to a novel environment which elevated corticosterone levels (26, 27). Adrenal corticoids may modulate the responsiveness of rats and mice to nicotine, perhaps by a rapid and reversible reduction of nicotine receptor function in addition to glucocorticoid receptors desensitization (26).

In summary, the results of this study indicate that a single corticosterone (25 mg/kg s.c.) pretreatment induces almost total, lasting at least for 48 h, functional inhibition of the HPA axis stimulated by adrenergic or cholinergic receptor agonist. Although this inhibition was measured for 48 h, a much longer refractory period is very likely. None of the known adrenergic or cholinergic antagonists is able to exert a comparative suppressive effect. The present results indicate that feedback inhibition of the HPA axis, via glucocorticoid receptors, fast in the onset and prolonged in duration, can be achieved by a single corticosterone administration.

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