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Rapid Communication

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EFFECT OF CORTICOTROPIN RELEASING HORMONE ON THE PITUITARY-ADRENOCORTICAL ACTIVITY UNDER BASAL AND SOCIAL STRESS CONDITIONS

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The effect of social crowding stress on the CRH-induced hypothalamic--pituitary-adrenocortical (HPA) responsiveness was assessed in rats crowded for 3 days, when the HPA response to neurotransmitter receptors stimulation was powerfully reduced. CRH given systemically dose-dependently increased the secretion of corticosterone. The increase was not affected by pretreatment with prazosin or propranolol, an α_1-or β-adrenergic receptor antagonist, indicating the lack of involvement of adrenergic receptors in that stimulation. In the corticosterone response to CRH administered icv, a moderate involvement of hypothalamic α_1-adrenergic receptors and neuronal noradrenaline seems possible. The corticosterone responses to CRH given by either route to rats exposed to social crowding stress were identical with the responses of unstressed controls. Our results for the first have time shown that social crowding stress does not impair the HPA responsiveness to CRH stimulation.

K e y w o r d s: *Corticotropin releasing hormone, social stress, corticosterone, adaptation.*

INTRODUCTION

The secretion of hormones by the anterior pituitary gland is regulated by neurohormones released into hypophysial portal blood from nerve terminals in the median eminence. Neurons delivering corticotropin releasing hormone (CRH) to hypophysial portal vessels in the median eminence are concentrated in a discrete zone of the parvocellular division of the paraventricular nucleus of the hypothalamus. Corticotropin releasing hormone is established as a primary physiologic regulator of the hypothalamo-pituitary-adrenal (HPA) axis, though various other neuropeptides can stimulate ACTH secretion in response to

neural and humoral stimuli. Specific binding sites for CRH were found in the anterior lobe while no receptors for CRH were present in the posterior pituitary lobe (1). The widespread distribution of CRH and CRH receptors throughout the central nervous system suggests that this peptide may subserve functions apart from its hypophysiotropic role. Indeed, icv administration of CRH induces autonomic, behavioral and visceral effects resembling those observed in stress (2—5). These effects of CRH are independent of the activation of the HPA axis because hypophysectomy did not attenuate the CRH-induced responses, however, systemic administration of CRH stimulates only the pituitary-adrenal axis (6). Thus CRH may be critical for the integration of endocrine and behavioral responses to stressors.

The HPA axis of intact rats is extremly sensitive to exogenous corticosterone. Small tonic increases above normal in plasma corticosterone provoke a decreased endogenous corticosterone secretion (7). Physiological mechanisms which are involved in regulation of the HPA axis during stress are not clear. The stress-induced increase in the CRH and corticosterone secretion may down-regulate pituitary corticotroph CRH receptors (8—9). Chronic immobilization stress significantly reduces the number of CRH receptors in the anterior pituitary but not the responsiveness of corticotrophs, which suggests participation of other ACTH secretagogues (10). Sustained central delivery of CRH over several days attenuates the ACTH-corticosterone release (11).

We have currently demonstrated that chronic social crowding stress in rats elicits adaptation of the HPA responsiveness to stimulation of the central adrenergic, cholinergic muscarinic and histaminergic systems (12—15). The most potent impairment of the HPA axis, up to 90%, was observed to central β-adrenergic receptor stimulation in rats crowded for 3 days (12—13).

The aim of the present study was to determine whether the responsiveness of the HPA axis to exogenous CRH was impaired in rats exposed to social crowding stress. We have also examined whether the CRH-induced stimulation of the HPA axis involves central adrenergic mechanisms.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 190—230 g. The animals were housed in groups of 7 per a cage and kept in a room at $21 \pm 2°C$ on a diurnal light cycle, and were given free access to commercial food and tap water. They were arbitrarily assigned to one of the two experimental groups: control and social stress of crowding. The control rats were housed in groups of 7 to a cage ($52 \times 32 \times 20$ cm) and they remained in their home cages until scheduled for treatment. The stressed rats were crowded in groups of 21 to a cage of the same size for 3 days, since after that time we found the most potent and significant impairment of the HPA responsiveness to central adrenergic and cholinergic receptor stimulation.

Required doses of the drugs were dissolved in saline immediately before use and injected ip in volume of 0.2 ml/kg, or they were administered into the right lateral cerebral ventricle in a volume

of 10 µl to rats whose skulls were prepared 24 h earlier, under light ether anesthesia, for free-hand icv injections. Prazosin and propranolol, adrenergic receptor antagonists, were injected 15 min before CRH by either route. One hour after CRH administration, the rats were killed by rapid decapitation, and their trunk blood was collected. Control rats were injected ip or icv with saline and were decapitated concurrently with experimental animals to obtain control corticosterone levels. After centrifugation, aliquots were frozen at −70°C until the assay. The concentration of corticosterone was measured fluorometrically. To avoid corticosterone fluctuations due to the circadian rhythm, all experiments were performed between 9—11 h, and all decapitations took place between 11—12 h.

For a HPLC assay the brains were quickly removed and the hypothalami were dissected on a cooled plate and immediately frozen on dry ice. Frozen tissue samples were placed into approx. 10 vol. ofice-cold 0.1 M $HClO_4$ containing 5 mM of ascorbic acid and 25 µg/l of 3,4 dihydrobenzylamine (internal standard), weighed and homogenized with an Ultra-Turrax homogenizer (10s at 20000 rpm). The homogenates were centrifuged at 14000 × g and the supernatants were subsequently filtrated through 0.22 µm RC-58 membranes (BAS MF-1 centrifugal microfilters). Filtrates were injected into the HPLC system. A BAS 400 liquid chromatograph was used (BAS, USA), equipped with an LC4B/17AT electrochemical detector and a 3 µm C_{18} Phase 2 analytical column (100 mm × 3 mm) which was coupled with a 7 µm C_{18} guard column (15 mm × 3 mm). The mobile phase (36 mM citrate — 28 mM phosphate buffer pH 3.5, containing 0.77 mM of EDTA and 5% methanol) was pumped at 0.9 ml/min. through the column thermostatted at 32°C. Separated sample component of noradrenaline was detected at the oxidation potential of 0.8 V. All reagents were of analitical grade (Merck, Germany and Sigma, USA).

The drugs used were: Corticotropin releasing hormone (CRH), propranolol (Sigma) and prazosin (Pfizer).

All data are presented as means ±SEM. Statistical significance of differences between groups was assayed by an analysis of variance, followed by individual comparisons with Duncan test.

RESULTS

Corticosterone response to CRH in normal rats

After systemic administration CRH (0.2—2 µg/kg ip) elicited a significant, dose-related rise in the serum corticosterone levels 1 h later (*Fig. 1*). Given by this route, CRH should directly reach its receptors on anterior pituitary corticotrops and stimulate ACTH secretion. Systemic pretreatment of rats with prazosin (0.1 mg/kg) or propranolol 0.1 mg/kg), an α_1- or β-adrenergic receptor antagonist, respectively, did not change the corticosterone response to CRH (*Fig. 2*). By that route of administration, CRH did not induce any change in the hypothalamic level of noradrenaline (*Fig. 3*). This indicates that CRH injected ip does not interfere with adrenergic α- or β-receptors in the anterior hypophysis or external zone of the median eminence of the hypothalamus, or with the hypothalamic noradrenergic system.

Rats that were injected icv with CRH (1 µg) showed a significant increase in the serum corticosterone level 1 h later, as compared with saline-treated controls. To determine whether the CRH-induced activation of the HPA axis

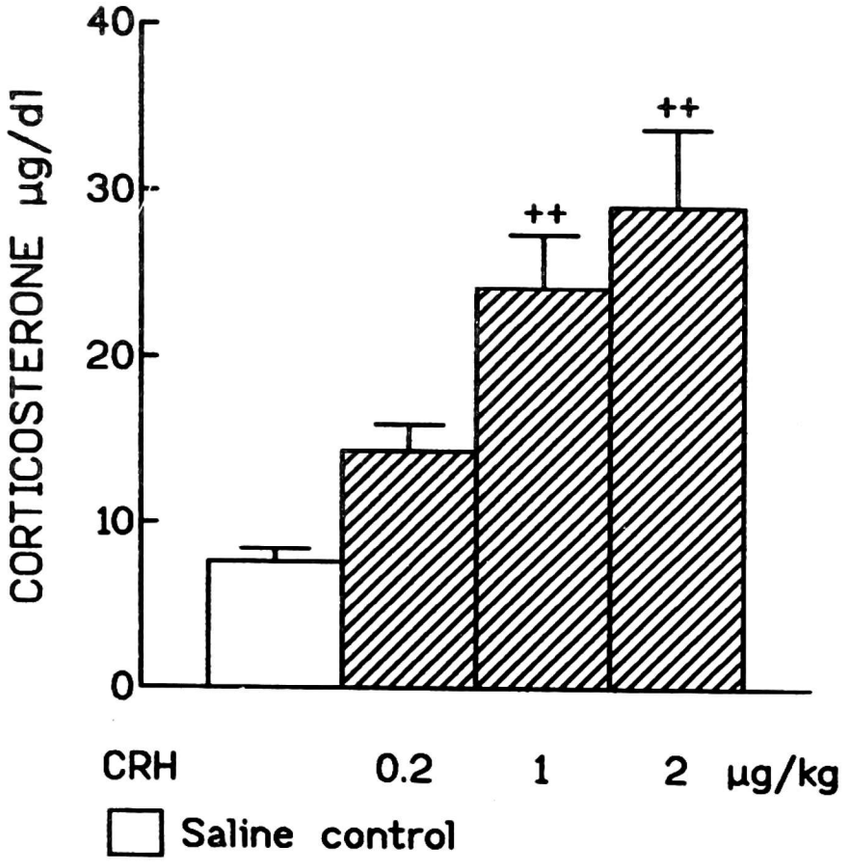

Fig. 1. Effect of CRH on the serum corticosterone levels in rats. CRH was injected ip and 1 h later the rats were decapitated. In *Fig. 1—5* values represent the mean ±SEM of 6 rats. $^{++}p < 0.001$ vs. saline-treated group.

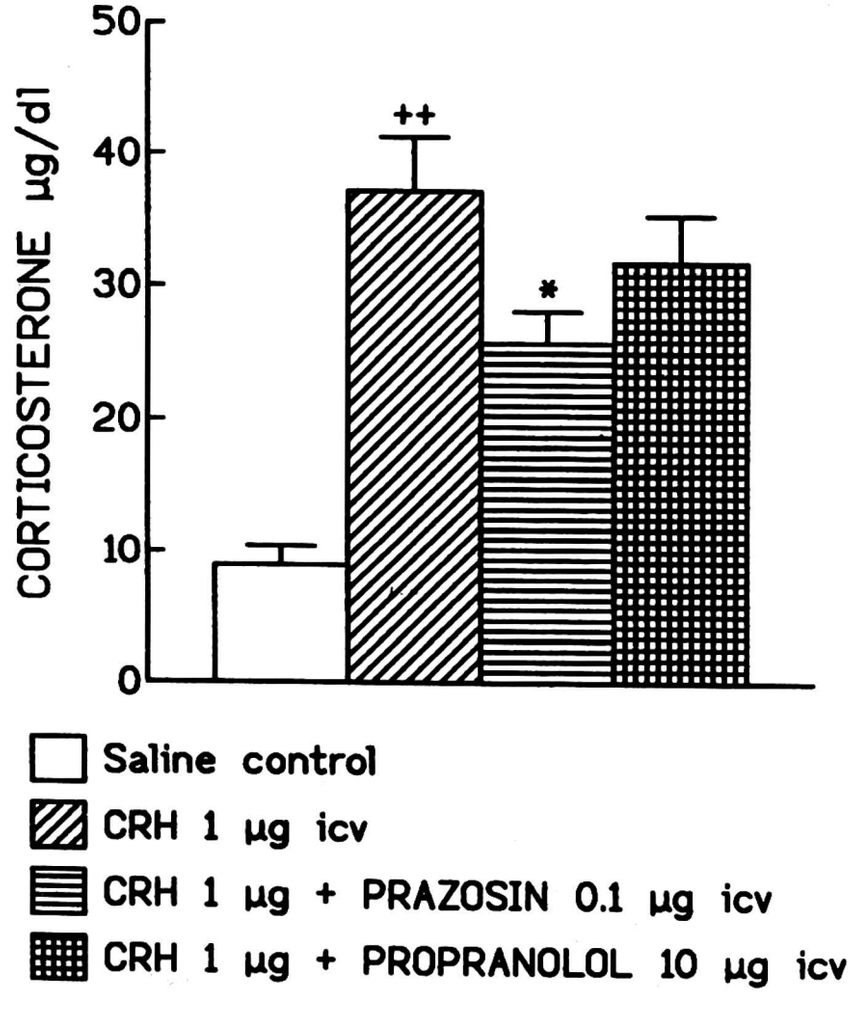

Fig. 2. Effect of prazosin and propranolol on the CRH-induced corticosterone response. All drugs were given ip, prazosin and propranolol 15 min. before CRH. $^{++}p < 0.001$ vs. saline treated controls; *p < 0,05 vs. CRH-treated group.

was associated with stimulation of the central noradrenergic system, hypothalamic noradrenaline was measured. In rats treated icv with CRH the hypothalamic NA level moderately decreased, by 15% (*Fig. 3*). Although the change was statistically not significant, at reflects moderate stimulation of the hypothalamic noradrenergic system which, in addition to a direct effect of CRH, may influence stimulation of the HPA axis.

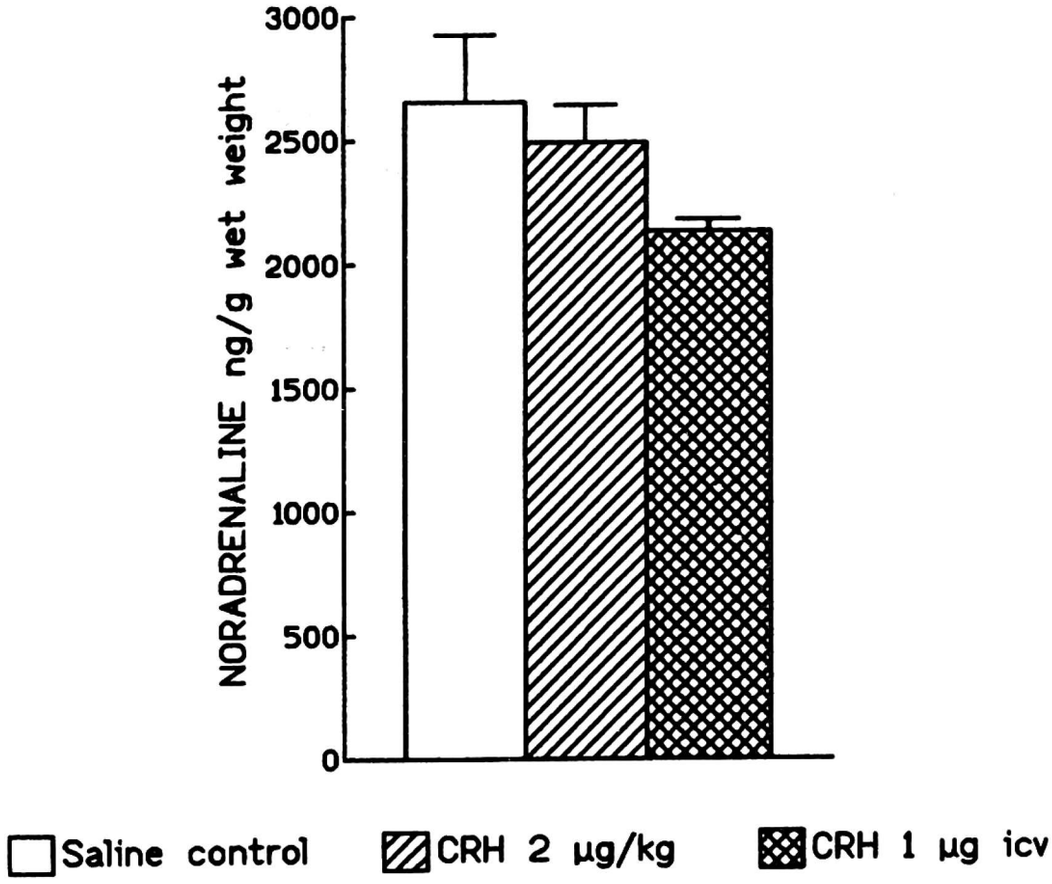

Fig. 3. Hypothalamic noradrenaline concentration after ip and icv treatment with CRH.

To further evaluate the role of a central noradrenergic component in the icv CRH-induced corticosterone response, the rats were pretreated icv with adrenergic receptor antagonists. Prazosin (0.1 µg), an α_1-adrenergic receptor blocker, given in its most effective dose (16), significantly decreased, by 40%, the icv CRH-induced corticosterone response. Propranolol (10 µg), a β-adrenergic receptor antagonist, only slightly diminished the corticosterone response to icv administered CRH (*Fig. 4*). This suggests that, centrally given CRH may, in part, elicit the pituitary-adrenocortical hormone secretion by stimulation of α_1-adrenergic receptors.

Effect of CRH on corticosterone secretion in crowded rats

In order to determine whether chronic crowding stress induced adaptation of the CRH system, the effect of CRH on the corticosterone secretion was

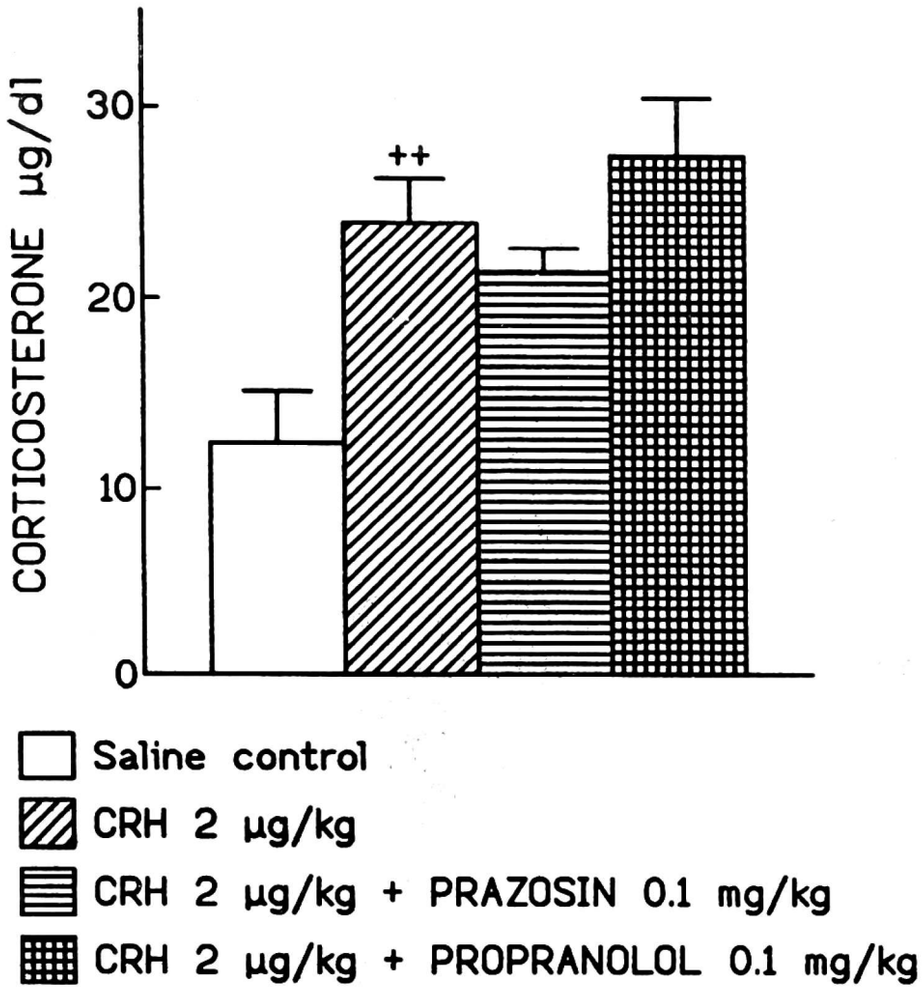

Fig. 4. Effect of prazosin and propranolol on the CRH-induced corticosterone response. All drugs were given icv, prazosin and propranolol 15 min. before CRH. $^{++}$ $p < 0.001$ vs. saline treated group.

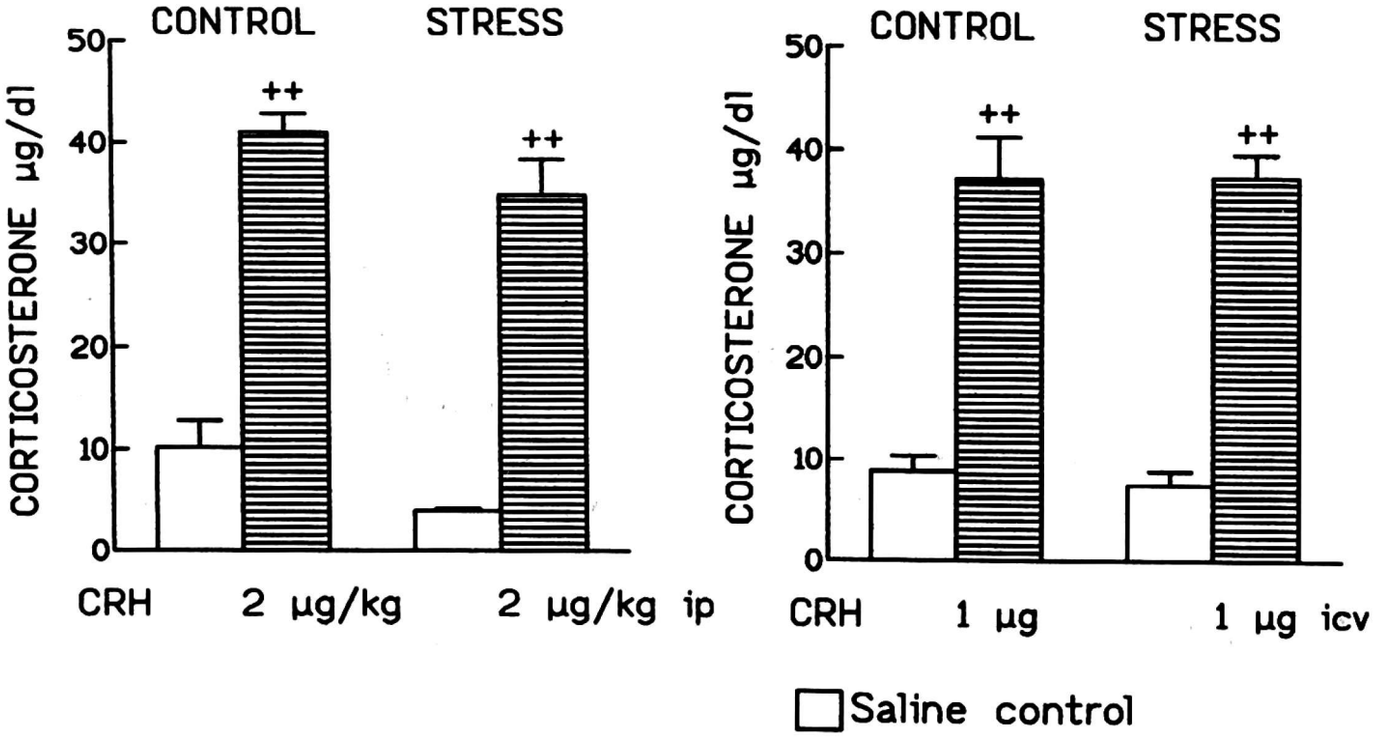

Fig. 5. The corticosterone response to CRH given ip and icv in control rats and in animals crowded for 3 days. $^{++}$ $p < 0.001$ vs. saline-treated group.

compared in crowded and control rats. At the time of maximum reduction of corticosterone response to neurotransmitters, i e after 3 days of crowding, the increases in the corticosterone secretion elicited by CRH given systemically or icv were identical in crowded and control rats (*Fig. 5*). Those data show that social crowding stress did not affect the HPA responsiveness to CRH stimulation.

DISCUSSION

The present results show that social crowding stress does not al all affect the HPA responsiveness to exogenous CRH. In the rats crowded for 3 days, when we observed dramatic impairment of the HPA responsiveness to the central β-adrenergic, cholinergic muscarinic and histamine H_2-receptor stimulation (12—15), the corticosterone response after systemic or icv CRH administration was identical with the response in control, unstressed animals. Our results indicate that systemic treatment with CRH causes direct stimulation of pituitary corticotroph CRH receptors and ACTH and corticosterone secretion. Using the later route of CRH administration we did not observe any adrenergic involvement in the pituitary-adrenocortical stimulation. Intraperitoneal pretreatment of rats with prazosin or propranolol, an α_1- or β-adrenergic antagonist, in effective doses (16) did not substantially change the corticosterone response to the subsequently given CRH. Also hypothalamic level of noradrenaline was not affected by ip injected CRH. This indicates that partial leak of CRH from systemic circulation into the hypothalamus and central activation of the HPA axis, via noradrenergic stimulation, is not likely.

It is known that icv administered CRH may elicit stres-like responses of both the HPA axis and the sympathetic nervous system. Stress induces release of catecholamines from axons projecting to the paraventricular nucleus, activation of α_1-adrenergic receptors and secretion of CRH in the portal capillary plexus (17). Intraventricular administration of CRH dose-dependently increases the release of NA from the rat hypothalamus (18). Our results suggest that, after icv CRH administration, α_1-adrenergic receptors may be involved in the corticosterone secretion, since icv pretreatment with prazosin significantly attenuates the CRH-induced hormone response. In addition, the hypothalamic level of noradrenaline substantially declines. However, it is not clear at present to what extent noradrenaline liberated by CRH during stress is involved in stimulation of the HPA axis, since the CRH antagonist significantly attenuates increases in levels of major metabolite of NA, but not in stress-induced increases in plasma corticosterone (19). CRH antagonist also blocks behavioral effects of CRH (20) and reduces emotionality in socially defeated rats via a pituitary-adrenal independent mechanism (21).

Neither our present results nor earlier data (12, 13, 15) suggest any significant role of the central noradrenergic system in stimulation of the HPA axis, since in rats crowded for 3 days, the HPA activity was almost totally resistant to the central adreneregic receptor stimulation (12, 13) whereas in the present experiment it fully responded to the CRH stimulation. Therefore we assume that a major part of the pituitary-adrenocortical response elicited by icv administered CRH, is induced by its penetration, via portal circulation, of the anterior pituitary corticotrophs and direct stimulation of the ACTH and corticosterone secretion. The present results show that after systemic administration CRH selectively activates the homologous anterior pituitary corticotroph receptors and stimulates ACTH and corticosterone secretion.

In contrast to the reported down-regulation of pituitary CRH receptors by the stress-induced increase in CRH secretion, our results for the first time have shown that social crowding stress does not at all impair the HPA responsiveness to CRH. The mechanisms of the fully retained responsiveness of the CRH system and of the almost complete desensitization of central neurotransmitter systems, involved in the HPA axis stimulation in rats exposed to short-term social stress, need further elucidation.

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