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SOCIAL CROWDING STRESS DIMINISHES THE PITUITARY-ADRENOCORTICAL AND HYPOTHALAMIC HISTAMINE RESPONSE TO ADRENERGIC STIMULATION.

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Social stress of crowding almost totally reduced the rise in serum corticosterone elicied by intracerebroventricular administration of isoprenaline, a β -adrenergic receptor agonist, after 3 and 7 day of crowding and substantially diminished that response after 14 and 21 days. Crowding stress totally abolished the increase in hypothalamic histamine induced by isoprenaline in control rats. Crowding also significantly diminished the increase in serum corticosterone evoked by clonidine, an α_2 -adrenergic agonist, and abolished the clonidine-induced elevation in hypothalamic histamine levels. The stimulatory effect of phenylephrine, an α_1 -adrenergic agonist, on corticosterone secretion was only moderately diminished in crowded rats. Neither phenylephrine nor crowding stress changed significantly the hypothalamic histamine levels.

These results indicate that social stress of crowding considerably impairs the hypothalamic-pituitary-adrenocortical responsiveness to central β - and α_2 -adrenergic receptor stimulation. Crowding also abolishes the rise in hypothalamic histamine induced by β - and α_2 -adrenergic agonist, suggesting a role of hypothalamic histamine in the HPA adaptation to the social stress of crowding.

Key words: *Crowding stress, adrenergic stimulation, receptors desensitization, corticosterone, hypothalamic histamine.*

INTRODUCTION

Important role in transforming psychological influences into different functional changes play the neuroendocrine systems. Psychological stressors, like handling, crowding and novelty, are generally accepted to activate the hypothalamic-pituitary-adrenal (HPA) system. Social stress may induce changes in both central monoaminergic and neurohormonal systems. Chronic exposure of animals to a stressor induces adaptation, as reflected by a decreased adrenocortical response to the stimulus (1, 2). The synthesis and release of hypophysiotropic hormones by neurons in the hypothalamus is

regulated mostly by monoaminergic neurotransmitters. Most forms of stress considerably alter the brain noradrenergic neuronal activity by either enhanced synthesis or release of catecholamines (3).

Numerous neuroendocrine and pharmacological findings support the hypothesis that adaptive changes induced by chronic stress include monoaminergic receptors. Repeated stress is known to induce both reduction in the density of β -adrenoceptors in several regions of the brain and their desensitization (4, 5). This is considered one of the biochemical factors underlying a form of receptor adaptation in order to prevent some dangerous effects of the persisting high levels of catecholamines. As regards adrenergic receptors, only desensitization of β -adrenergic receptors has been extensively studied (1, 6, 7). We have recently found that social stress of crowding considerably affects the responsiveness of the HPA system to some monoaminergic and cholinergic stimulation (8, 9).

The release and metabolism of histamine is changed in animals under stressful situations. Histamine is suggested to alter the binding sites of β -adrenoceptors, and it may influence the interaction between isoprenaline and β -adrenoceptors (9). However, a possible interaction of central histamine and monoaminergic receptors in adaptation of the HPA axis in animals exposed to social stress is still unknown.

The purpose of the present study was to determine how chronic social stress of crowding influences the functional adaptability of central adrenergic receptors known to be involved in mediation of the hypothalamic-pituitary-adrenal activity under basal and stress conditions, and to assess the role of hypothalamic histamine in the HPA responsiveness during social stress of crowding.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 180–200 g at the beginning of experiment. The rats were housed in standard cages on a diurnal light cycle at a room temperature of 18–21°C for one week prior to experimentation. They were fed on standard laboratory diet with tap water ad libitum. The rats were randomly assigned to one of the two experimental groups: control and social stress of crowding. Control rats were housed 7 per cage (52 × 32 × 20 cm) and remained in their home cages until scheduled for treatment. Stressed rats were crowded in groups of 21 per cage of the same size for 3, 7, 14 and 21 days. The effects of adrenergic receptor agonists were examined on days 3, 7, 14 and 21st of crowding and were compared with the effects in control animals. All drugs were injected in a volume of 10 μ l into the right lateral cerebral ventricle of conscious rats. Control rats were injected with the same volume of saline. One hour after drug administration the rats were killed by rapid decapitation, and their trunk blood was collected and the hypothalami isolated. The control animals were decapitated simultaneously with each experimental group to obtain control serum corticosterone and hypothalamic histamine levels. The serum corticosterone was determined spectrofluorometrically (11) and expressed as μ g/100 ml.

To minimize circadian variability, all experiments were performed between 10.00 and 11.00 hours and the animals were decapitated between 11.00 and 12.00 hours, when plasma corticosterone is at a relatively low level.

For histamine determinations the rats were decapitated at the required time, their brains were quickly removed and placed on ice; the cerebella were discarded and the hypothalami were isolated and stored at -80°C until further use. For the determination of histamine concentration, a 10 or 20% (w/v) homogenate of the tissue was made in 0.4 M perchloric acid. The homogenate was centrifuged and the supernatant was adjusted to pH 5–6 with 0.2 M KOH. Isolation and analysis of histamine was then carried out by modification of the procedure described by Kremzner and Pfeiffer (12). A 0.5 ml aliquot was passed through a Cellex P column (5×30 mm) and washed sequentially with 0.5 ml of 0.03 and 0.1 M sodium phosphate buffer (pH 6.2). Histamine was eluted with 1.5 ml of 0.07 M hydrochloric acid and, after condensation with O-phthalaldehyde, it was estimated fluorometrically at 360/450 nm (13).

The drugs used were: L-Phenylephrine hydrochloride, D-L-Isoproterenol HCl (Sigma) and Clonidine (Boehringer). The drugs were dissolved in a 0.9% NaCl solution immediately before use.

All data are presented as mean \pm SEM. Statistical significance of differences between groups was assessed by an analysis of variance, followed by individual comparisons with the Duncan test.

RESULTS

Effect of crowding on corticosterone response to adrenergic agonists

Phenylephrine ($30 \mu\text{g}$ icv), an α_1 -adrenergic agonist, induced a slightly diminished rise in serum corticosterone levels in rats crowded for 3–7 days as compared with the response in control rats. The corticosterone response to phenylephrine after 3–21 days did not differ significantly from the control response (*Fig. 1*).

The significant increase in serum corticosterone levels induced in control rats by clonidine, an α_2 -adrenergic agonist ($10 \mu\text{g}$ icv), was considerably reduced after 3 days, and moderately diminished after 14 and 21 days of crowding (*Fig. 2*).

The corticosterone response to isoprenaline ($10 \mu\text{g}$ icv), a β -adrenergic agonist, was almost totally abolished in rats crowded for 3 and 7 days and also substantially diminished after 14 and 21 days of social crowding (*Fig. 3*).

Effect of adrenergic agonists on serum corticosterone and hypothalamic histamine levels

In non-stressed rats, the adrenergic agonists phenylephrine, clonidine and isoprenaline given icv significantly raised by 214, 339 and 312%, respectively, the serum corticosterone levels 1 h later, the relatively lowest rise being observed after phenylephrine, an α_1 -adrenergic agonist (*Fig. 4*). At the same time, the hypothalamic histamine content increased in the adrenergic agonist-treated rats by 109, 135, and 125%, respectively; however, that

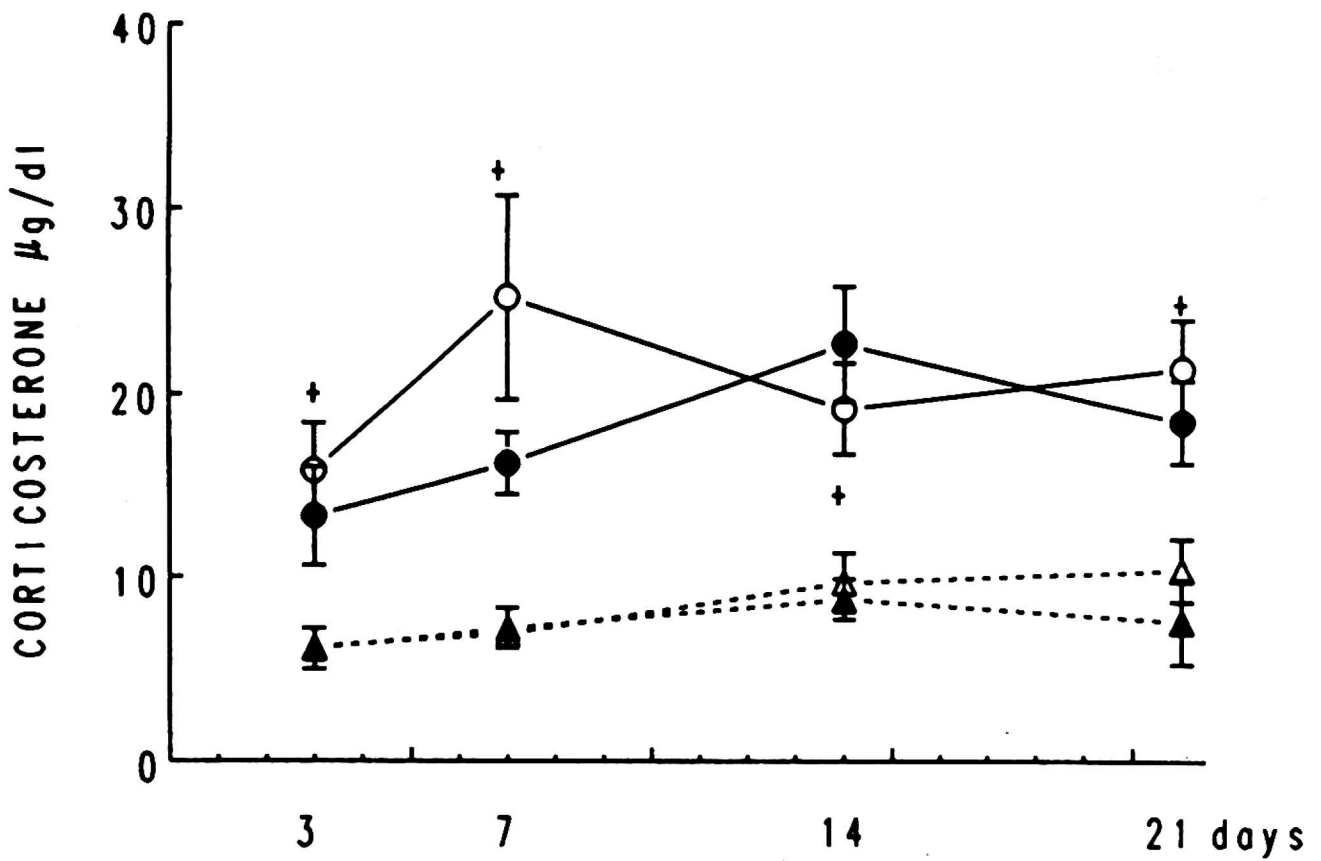


Fig. 1. Effect of crowding stress on serum corticosterone levels induced by phenylephrine given icv. Values represent the mean \pm SEM of 7 rats. Δ - Saline control, \blacktriangle - Saline+stress, \circ - Phenylephrine 30 μ g icv control, \bullet - Phenylephrine 30 μ g icv+stress; + $p < 0.05$ vs. saline controls.

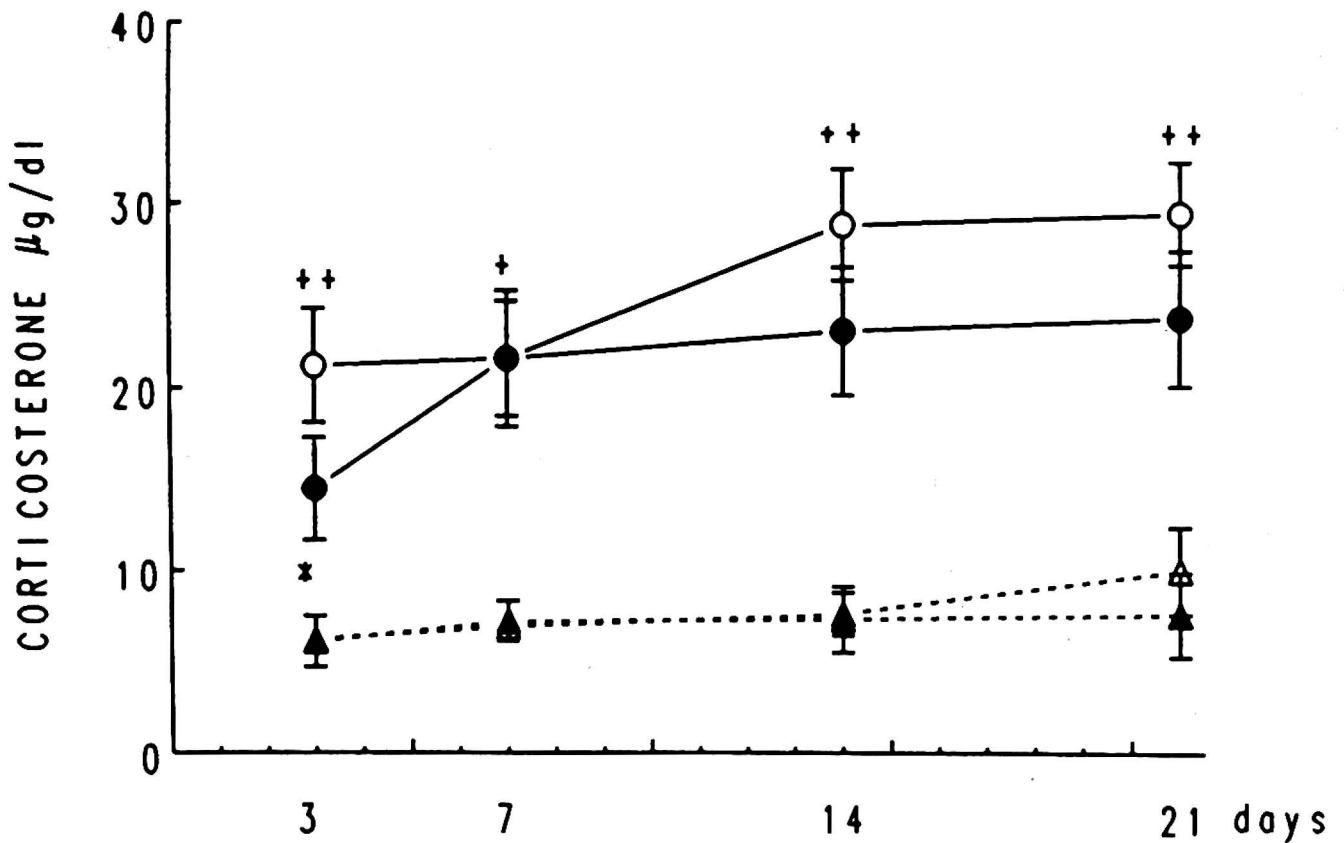


Fig. 2. Effect of crowding stress on serum corticosterone levels induced by clonidine given icv. Values represent the mean \pm SEM of 7 rats. Δ - Saline control, \blacktriangle - Saline+stress, \circ - Clonidine 10 μ g icv control, \bullet - Clonidine 10 μ g icv+stress; + $p < 0.05$ and + + $p < 0.001$ vs. saline controls; * $p < 0.05$ vs. clonidine treated group.

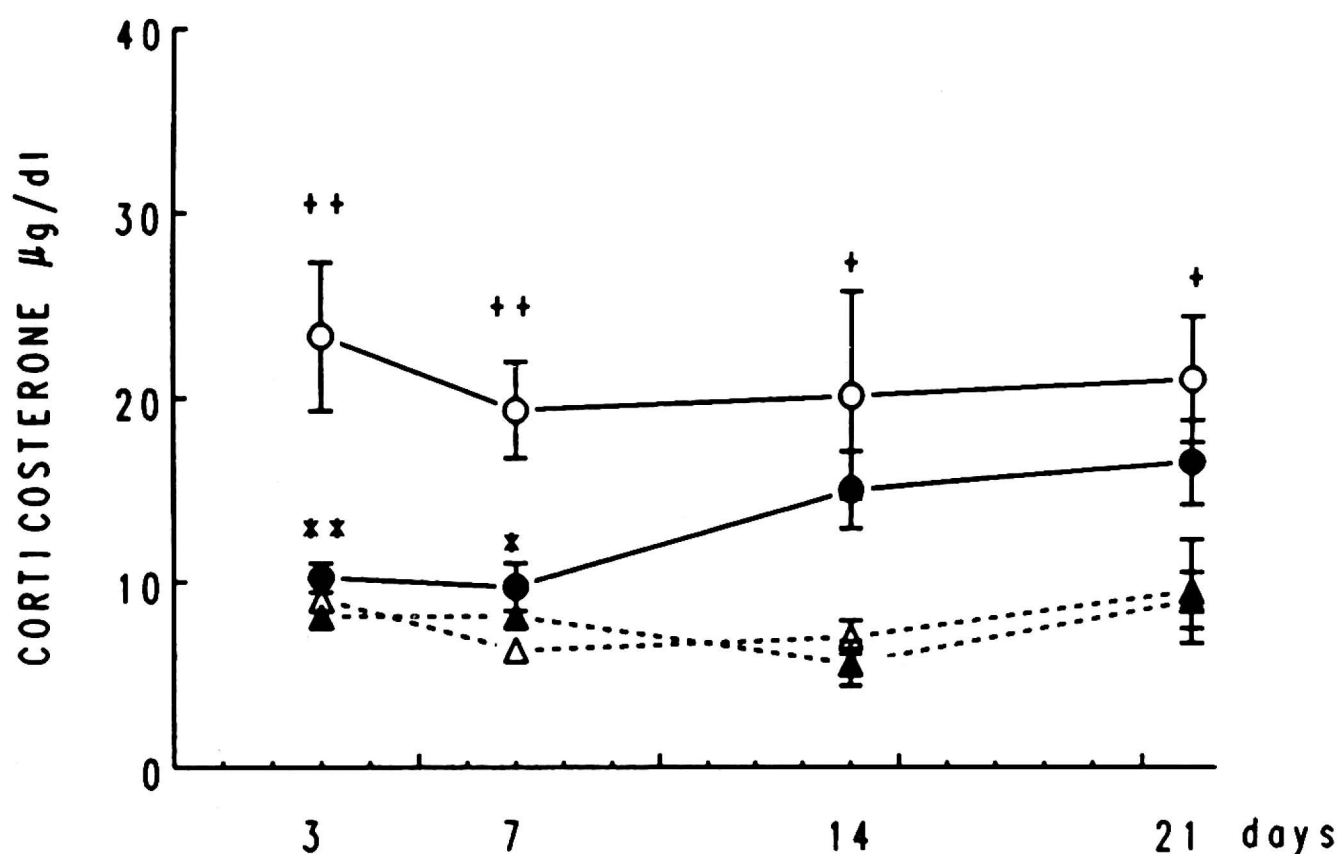


Fig. 3. Effect of crowding on serum corticosterone levels induced by isoprenaline given icv. Values represent the mean \pm SEM of 6–7 rats. Δ – Saline control, \blacktriangle – Saline + stress, \circ – Isoprenaline 10 μ g icv control, \bullet – Isoprenaline 10 μ g icv + stress. + $p < 0.05$ and ++ $p < 0.01$ vs. saline controls, * $p < 0.05$ and ** $p < 0.001$ vs. isoprenaline treated group.

increase was significant only after clonidine and isoprenaline (Fig. 4). The above finding suggests that hypothalamic histamine may mediate the α_2 - and β -adrenergic receptor induced increase in corticosterone secretion.

Effect of crowding on hypothalamic histamine after adrenergic stimulation

The levels in hypothalamic histamine induced by phenylephrine in rats crowded for 3, 7 and 14 days were moderately, but non-significantly lower from those in control rats. Social crowding for 3 and 7 days totally abolished and after 14 days slightly diminished the increase in hypothalamic histamine content evoked by clonidine in control rats. Similarly, crowding stress also totally reduced the increase in hypothalamic histamine levels induced by isoprenaline in control rats throughout the whole observation period (Table 1).

DISCUSSION

In agreement with previous reports (8, 9), we observed that rats exposed to chronic social crowding stress showed diminished responsiveness of the HPA axis to central adrenergic stimulation. A maximum reduction in corticosterone

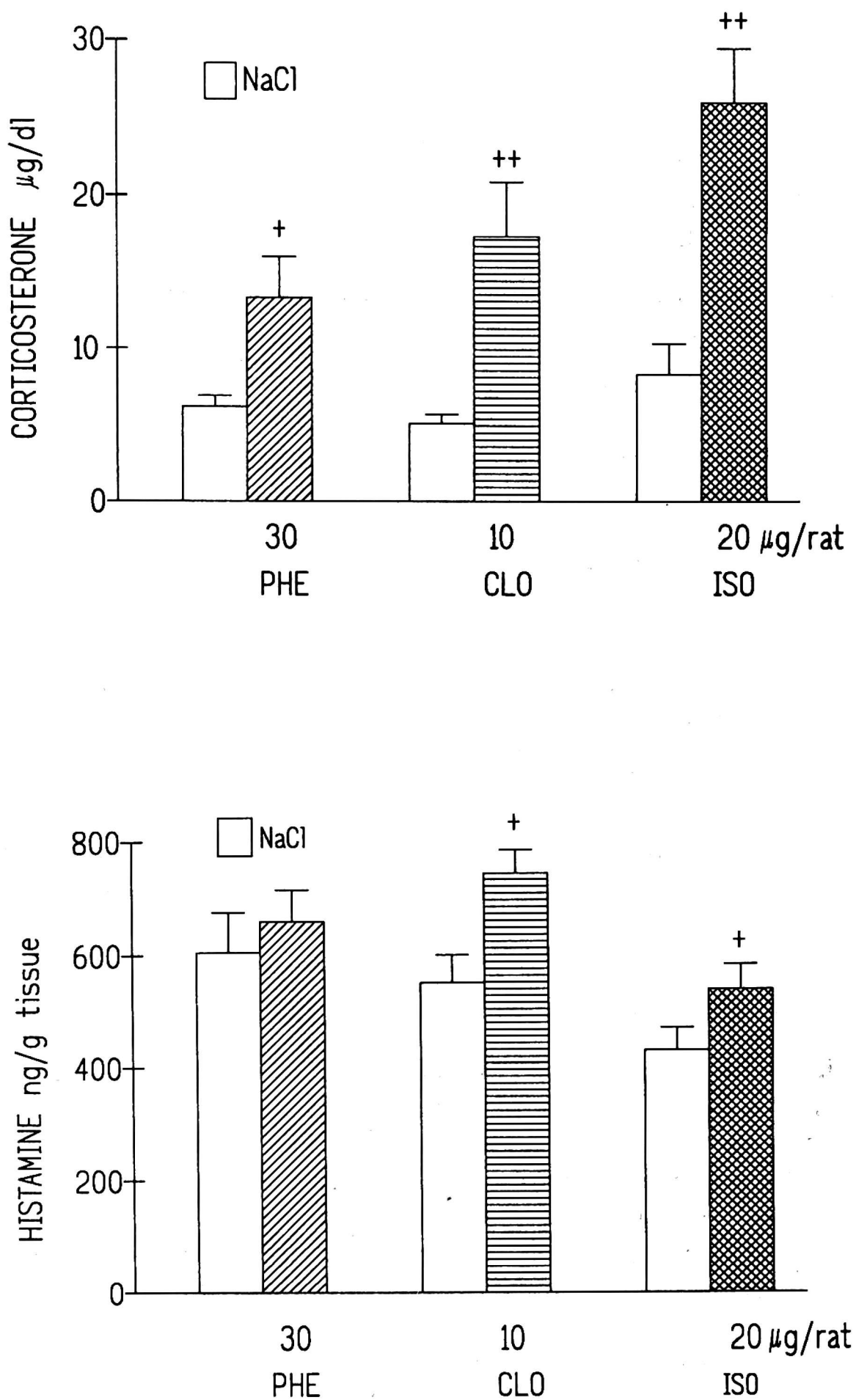


Fig. 4. Effect of phenylephrine (PHE) 30 µg icv, clonidine (CLO) 10 µg icv and isoprenaline (ISO) 20 µg icv on serum corticosterone and hypothalamic histamine levels in non stressed rats. Values represent the mean \pm SEM of 6–7 rats. + p <0.05 and ++ p <0.001 vs. saline controls.

Table 1. The changes in hypothalamic histamine content after central adrenergic receptors stimulation in control and crowded rats (% values)

Treatment	Days		
	3	7	14
Phenylephrine vs. saline-control	109	128	109
Phenylephrine vs. saline-stressed	94	99	76
Clonidine vs. saline-control	146 ⁺⁺	136 ⁺	137 ⁺
Clonidine vs. saline-stressed	101*	100*	117
Isoprenaline vs. saline-control	125 ⁺	104	131 ⁺
Isoprenaline vs. saline-stressed	105*	93	72

The mean hypothalamic histamine content in control rats was 415–756, in control crowded rats 402–653 ng/g tissue; in agonist control groups 542–908 and in agonist crowded groups 290–754 ng/g tissue. ⁺p<0.05; ⁺⁺p<0.01 vs. saline control and *p<0.05 vs. saline stressed group.

response by 85 and 88% after the 3rd and 7th day of crowding, respectively, was caused by icv administration of isoprenaline. This finding indicates that during social crowding stress the strongest functional adaptation of the HPA axis appears to β -adrenergic receptors stimulation and it occurs on the first days of crowding. This adaptation gradually diminished in rats crowded for 14 and 21 days, when the reduction in corticosterone response to isoprenaline is 35 and 25%, respectively, of the control response.

A considerable diminution of the HPA responsiveness to stimulation of central β -adrenergic receptors, observed in the present experiment, may be caused by a decrease in the receptor density on hypothalamic CRH secreting neurons or by reduction in their sensitivity. Although chronic stressors have been shown to reduce the density of β -adrenoceptors in various regions of the brain including hypothalamus (4), this effect is relatively transient, being absent 24 h following stressor termination (14). Also icv infusion of isoproterenol, a β -adrenergic agonist, causes reduction of central β_2 -adrenoceptors (7).

The dramatic decrease in the HPA response to icv administered isoprenaline, observed during social stress of crowding, may be related to diminution in cAMP responses to β -adrenergic stimulation. Repeated stress is known to reduce cAMP responses to catecholamines (15) and cAMP in both the pituitary corticotrophs and hypothalamic CRH containing neurons appears to be involved in release and synthesis of pituitary hormones (16).

It is known that cAMP responses in the brain are also mediated by α -adrenergic receptors which potentiate the response to β -receptor stimulation (17). Stress can also induce desensitization of α_1 -adrenoceptors (6). In the

present experiment the corticosterone response to phenylephrine, an α_1 -adrenergic agonist was moderately, though not significantly, diminished after 3 and 7 days of crowding, concurrently with the maximum reduction in the corticosterone response to isoprenaline, a β -adrenergic receptor agonist. Central α_1 -adrenergic mechanisms are important components in regulation of the hypothalamic CRH secretion during stress (18). Thus it is possible that in the present experiment a moderate α_1 -receptor desensitization is partly involved, in a dramatic reduction of the HPA response to β -adrenergic receptor stimulation.

The rise in corticosterone secretion induced by clonidine was significantly blocked after 3 days and moderately diminished after 14 and 21 days of crowding. We have shown that clonidine stimulates the HPA axis by acting on α_2 - and α_1 -adrenoceptors (19), and that it may stimulate both postsynaptic and presynaptic α_2 -adrenoceptors (20). These receptors undergo desensitization after a short-term exposure to the agonist epinephrine in vitro (21); moreover, chronic stress induces changes in hypothalamic pre-synaptic α_2 -adrenoceptors, which generally reflect noradrenergic subsensitivity (22). The inhibition by crowding stress of the clonidine-induced rise in corticosterone secretion may be mainly caused by the action of clonidine on postsynaptic α_2 -adrenoceptors. These receptors were characterized autoradiographically on CRH-secreting neurons, and both α_2 - and α_1 -receptors may mediate stimulation of the HPA axis during stress (7).

In the present experiment on non-stressed rats, clonidine and isoprenaline significantly raised both serum corticosterone and hypothalamic histamine levels whereas phenylephrine, markedly increased serum corticosterone, but induced only a modest elevation in hypothalamic histamine levels. We have recently found that under basal conditions a substantial part of the stimulatory effect of clonidine on the HPA axis is mediated by histaminergic mechanisms, including hypothalamic histamine and brain histamine H_1 - and H_2 -receptors (23, 24).

Crowding stress considerably diminishes responsiveness of the serum corticosterone and abolishes the rise in hypothalamic histamine levels induced by clonidine and isoprenaline. This observation suggests a role of brain histamine in inducing activation of the HPA axis after icv administration of clonidine and isoprenaline under both basal and chronic social crowding stress conditions. In our recent experiment crowding stress considerably diminished the functional HPA responsiveness to central histamine H_2 -receptors stimulation (data not shown) which further confirms the significance of brain histaminergic mechanisms in adaptation of the HPA axis activity during social stress of crowding.

Another possibility of reducing the HPA responsiveness to adrenergic stimulation during crowding stress is desensitization of CRH receptors on

pituitary corticotrophs. These receptors may desensitize following either a continuous in vitro exposure to CRH or repeated in vivo administration of exogenous CRH. Chronic immobilization stress reduces the number of CRH receptors in the anterior pituitary (26), and these receptors appear to be down-regulated during an increased secretion of the hypothalamic hormone (27).

Our present data do not support this concept, since all centrally administered adrenergic receptor agonists stimulate to a similar extent the secretion of CRH and should induce similar desensitization of pituitary CRH receptors. Nonetheless, inhibition of HPA responses to adrenergic agonists by crowding stress has been found to be receptor specific.

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