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CENTRAL HISTAMINERGIC MECHANISMS IN THE CORTICOSTERONE RESPONSE TO CLONIDINE

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Involvement of central histaminergic mechanisms in stimulation of the hypothalamic-pituitary-adrenal (HPA) axis by clonidine was investigated in conscious rats. Clonidine as well as adrenergic and histamine receptor antagonists were administered intracerebroventricularly (icv), the antagonists always 15 min prior to clonidine, and 1 h later the trunk blood was collected for corticosterone determination.

α-Fluoromethylhistidine (α-FMH), a neuronal histamine synthesis inhibitor, was given ip 2 h before clonidine. Immediately after decapitation, brains were exposed and hypothalami were isolated on ice and frozen for further spectrofluorimetric histamine determination.

The clonidine-induced increase in the serum corticosterone level was considerably, but not totally, reduced by icv or ip pretreatment with yohimbine, an α_2-adrenergic receptor antagonist. The rise in the corticosterone level induced by clonidine was significantly diminished by mepyramine, a histamine H_1-receptor antagonist, and moderately lowered by cimetidine, a histamine H_2-receptor antagonist. Clonidine significantly augmented the histamine content in the hypothalamus and rest of the brain. The clonidine-induced increase in hypothalamic histamine might be the cause of an increased corticosterone secretion via stimulation of central H_1-histamine receptors. On the other hand, α-FMH injected 2 h before clonidine considerably diminished both the histamine content in the hypothalamus and the corticosterone secretion induced by clonidine.

These results indicate that clonidine given centrally stimulates the HPA activity via not only α-adrenergic but also histaminergic mechanisms. Clonidine augments the hypothalamic histamine which, in turn, stimulates the corticosterone secretion, predominantly via histamine H_1-receptors. Neuronal histamine is considerably involved in the stimulatory action of clonidine since inhibition of the neuronal histamine synthesis by α-FMH significantly depresses the corticosterone response to clonidine.

Key words: *corticosterone, clonidine, central histaminergic mechanisms, neuronal histamine, histamine antagonists, α-fluoromethylhistidine.*

INTRODUCTION

Clonidine, an α-adrenergic drug, given centrally is known to stimulate the hypothalamic-pituitary-adrenal axis, expressed by an increase in corticosterone secretion. We have demonstrated that clonidine increases corticosterone secretion by stimulating central α_2-and α_1-adrenergic receptors (1-2). Although widely used as an α_2-adrenergic agent, clonidine can no longer be considered as a specific agonist of α-adrenoceptors (3). Some effects of clonidine are not shared by endogenous α-adrenergic agonists, nor are they inhibited by α-adrenoceptor blocking drugs. In our experiment, the α_2-adrenergic antagonist yohimbine given centrally or systemically was not able to completely inhibit the rise in corticosterone secretion evoked by central administration of clonidine (1-2).

Some evidence indicates that clonidine is able to activate histamine H_2-receptors in both peripheral and cerebral tissues (3). Central histamine H_2-receptors are involved in the hypothermic effect of clonidine (4). However, clonidine does not seem to act as a partial agonist at H_2-receptors in peripheral tissues (5).

In our preliminary experiments diminution of the central neuronal histamine synthesis by α-FMH, a specific irreversible inhibitor of histidine decarboxylase (6), decreased the corticosterone response to clonidine (7). It is known that single administration of α-FMH decreases the histamine content only of non-mast cells in the brain (8). This inhibitor induces the maximal decrease in the histidine decarboxylase activity in the hypothalamus and other brain structures 2 h after systemic administration (9-11).

It is evident that histamine affects the secretion of ACTH and corticosterone and seems indispensable to the normal functioning of the corticotropinergic system (12-14).

The aim of the present study was to find out whether central histaminergic components are involved in the stimulatory action of clonidine on the HPA axis in conscious rats. We examined the effect of the centrally administered clonidine on histamine level in the hypothalamus, a structure closely involved in stimulation of the HPA axis, as well as the effects of blockade of the neuronal histamine synthesis by α-FMH and histamine H_1- and H_2-receptor antagonists on the clonidine-induced corticosterone secretion.

MATERIALS AND METHODS

Male Wistar rats weighing 200—230 g were used in all the experiments. They were housed in groups of 7 to a standard cage on a diurnal light cycle at a room temperature of 18—21°C for one week prior to experimentation. The animals had free access to food and water. The rats were

arbitrarily assigned to one of the experimental groups. The indicated doses of clonidine and histamine receptor antagonists were injected in 10 µl of saline into the right lateral cerebral ventricle of naive non-anesthetized rats. The histamine antagonists were injected 15 min before clonidine. α-FMH was dissolved in a volume of 1 ml/kg of saline and injected intraperitoneally 2 h before clonidine. After injection of the drugs, the animals were placed back in their cages and 1 h after the last injection they were killed by a rapid decapitation and their trunk blood was collected. Control rats received 10 µl or 0,2 ml of saline, respectively, and were decapitated simultaneously with the experimental group to obtain resting serum corticosterone levels. After centrifugation, serum aliquots were frozen until the assay. The serum corticosterone was determined spectrofluorometrically (15) and expressed as µg/100 ml. One analysis was performed in each rat's serum, but 6—12 animals were used for each point. In order to avoid interference by the circadian rhythm in corticosterone levels all decapitations were caried out between 10.00 and 11.00 h, i.e. when the serum corticosterone concentration is low in the normal diurnal rhythm.

For histamine determinations the rats were decapitated at the required time, their brains were quickly removed, placed on ice, the cerebellum were discarded and the hypothalami were isolated and stored at –80°C until use. For determination of the 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 (16). 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 M 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-phtaldialdehyde, it was estimated fluorometrically at 360/450 nm (17).

The following drugs were used: clonidine (Boehringer), yohimbine (Sigma), mepyramine maleate (May and Baker), cimetidine (Smith Kline and French), histamine diphosphate (Sigma), (S)-α-fluoromethylhistidine hydrochloride (Kollonitsch, Merck Sharp and Dohme).

The data are presented as arithmetical means and SEM. The significance of differences between groups was assessed by analysis of variance, followed by individual comparisons with the Duncan test.

RESULTS

1. Effect of clonidine and yohimbine on serum corticosterone levels

Clonidine (10 µg) injected icv induced a dose-related increase in the resting serum corticosterone levels measured 1 h after its injection, whereas after systemic administration no increase in the hormone levels was observed (2). The increase in the serum corticosterone levels induced by clonidine (10 µg icv) was significantly, but not totally, reduced by icv pretreatment with yohimbine (0,01-0,1 µg), an α_2-adrenergic receptor antagonist. Also systemic pretreatment with yohimbine (0,001-2 mg/kg) considerably attenuated the rise in the serum hormone levels (*Fig. 1*). This finding may suggest that yohimbine influences both pre- and post-synaptic adrenergic receptors involved in the clonidine-induced stimulation of pituitary-adrenocortical axis. Moreover, the obtained results suggest that not only adrenergic system is involved in the stimulatory action of centrally administered clonidine.

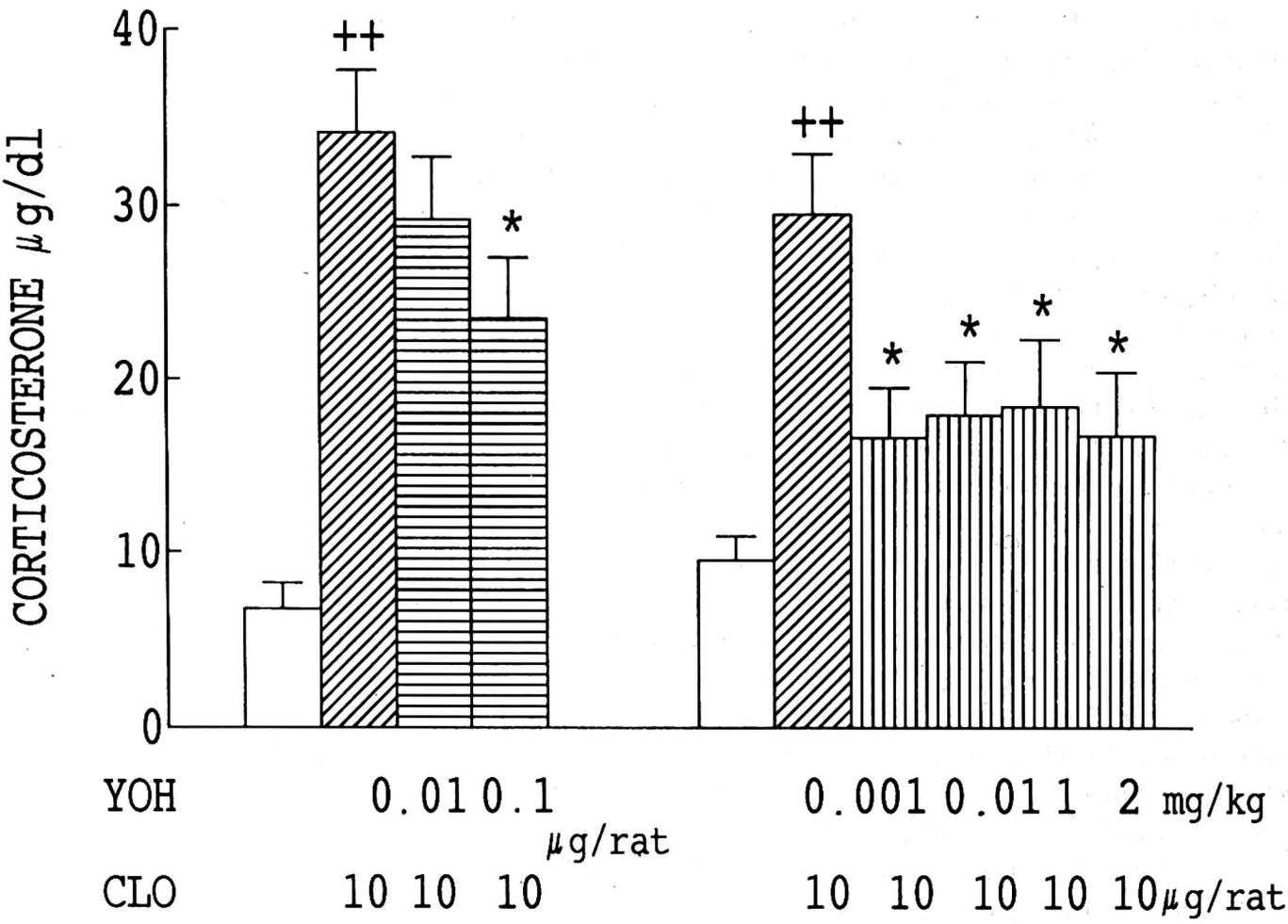

Fig. 1. Effect of yohimbine (YOH) given icv or ip on serum corticosterone levels induced by clonidine (CLO) given icv. Yohimbine was injected 15 min before clonidine. Values represent the mean ± SEM of 7 rats. ++ $p<0.001$ vs. saline controls; * $p<0.05$ vs. clonidine treated group.

2. Effect of clonidine on brain histamine levels

Clonidine (10 μg), administered icv in a dose which 1 h later considerably elevated the serum corticosterone levels, significantly increased the histamine content in both the hypothalamus and rest of the brain. The basal histamine content of 380 ng/g in control rats reached a significantly higher level of 500 ng/g 1 h after icv clonidine administration (*Fig. 2*).

3. Effect of α-FMH on the clonidine-induced corticosterone secretion

Pretreatment of rats with α-FMH (20 mg/kg ip), a histamine synthesis inhibitor, 2 h before icv clonidine considerably impaired the corticosterone secretion induced by clonidine (*Fig. 3*). This finding suggests that neuronal histamine is involved in the clonidine-induced central stimulation of the HPA axis.

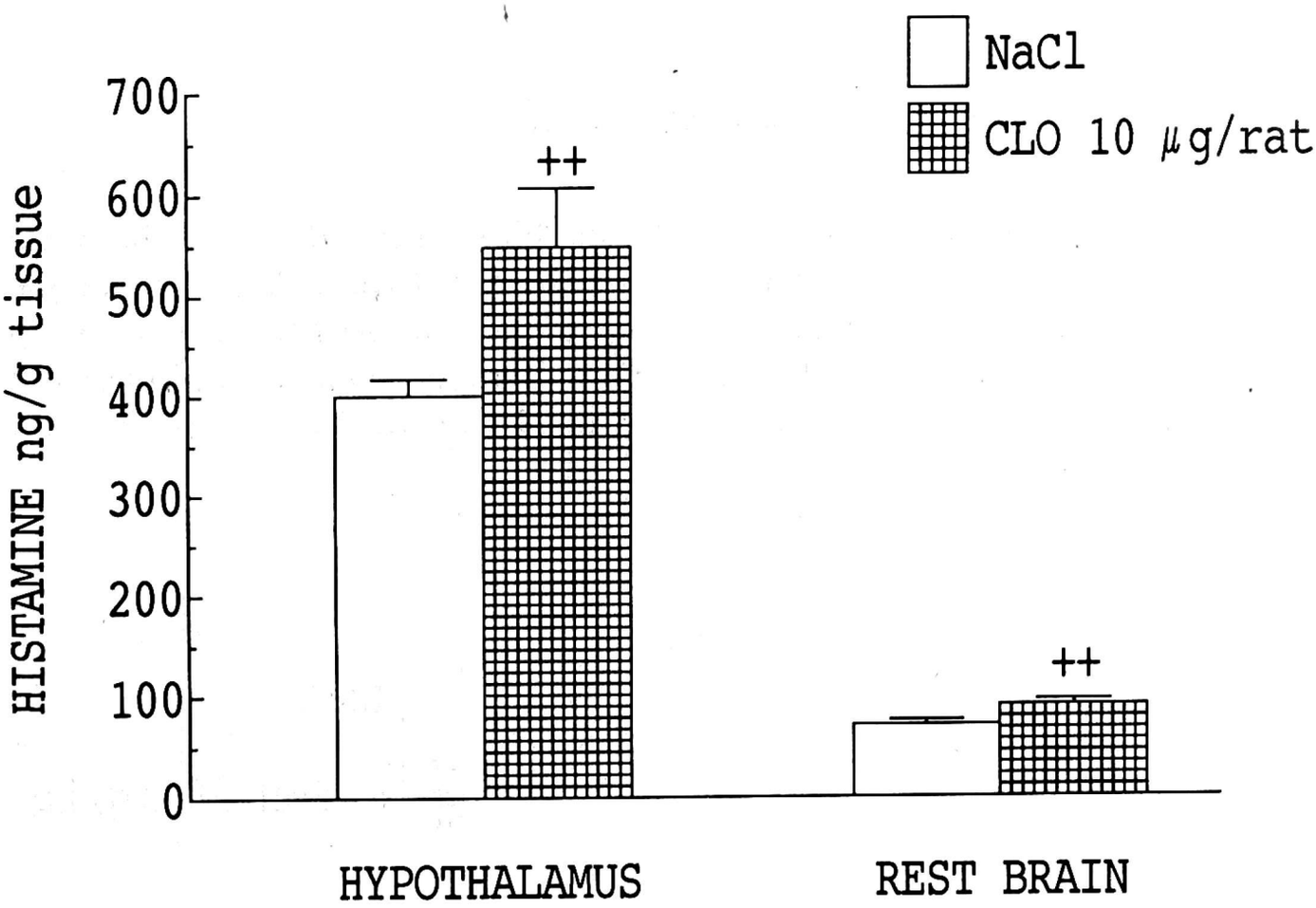

Fig. 2. Effect of clonidine (CLO) given icv on histamine content in the hypothalamus and rest of brain. Rats were decapitated 1 h after clonidine administration. + + $p < 0.001$ vs. saline controls.

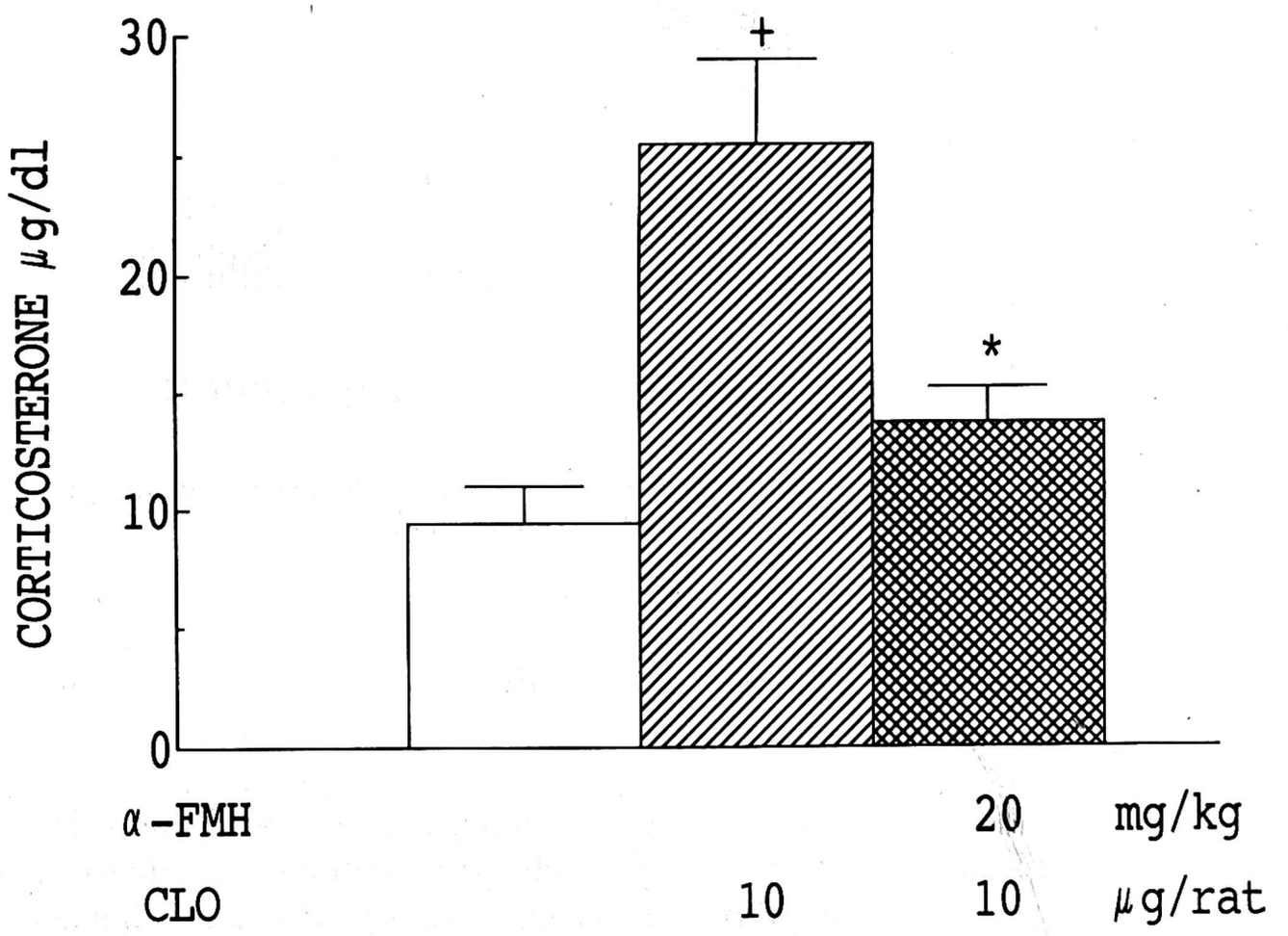

Fig. 3. Effect of α-FMH on clonidine-induced corticosterone secretion. α-FMH was injected ip 2 h before clonidine and 1 h later the rats were decapitated. + $p < 0.05$ vs. saline controls; * $p < 0.05$ vs. clonidine treated group.

4. Effect of α-FMH on brain histamine levels

Alpha-FMH (20 mg/kg ip), a specific inhibitor of histidine decarboxylase 3 h later induced a significant decrease in the hypothalamic histamine content: from 375 ng/g in control rats to 264 ng/g in α-FMH treated group (*Fig. 4*). A still stronger depletion of the histamine content was observed in the whole brain: from 92 ng/g in control rats to 34 ng/g in α-FMH treated group.

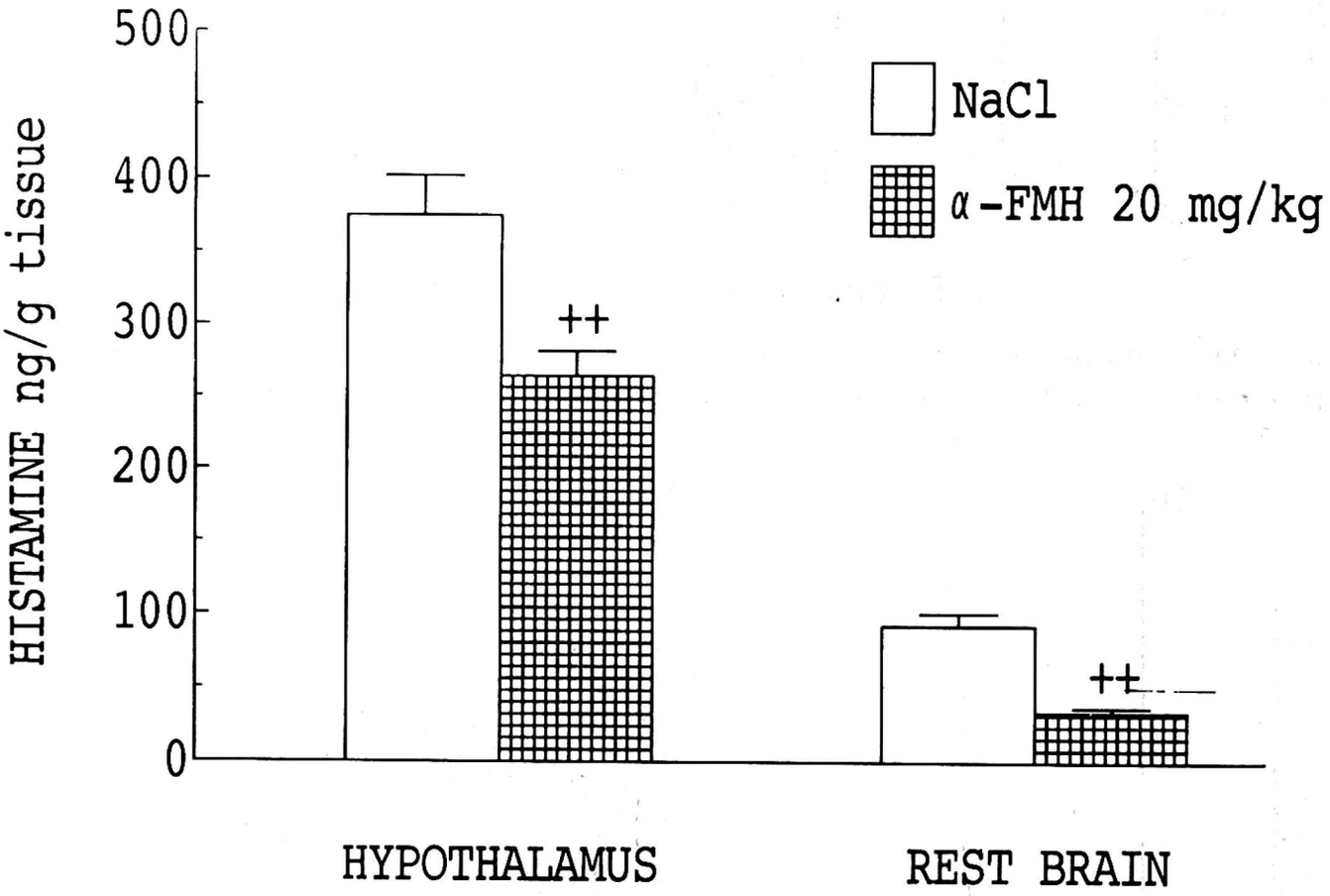

Fig. 4. Histamine content in the hypothalamus and rest of brain 3 h after ip administration of α-FMH. ++ $p<0.001$ vs. saline controls.

5. Effect of histamine receptor antagonists on the clonidine-induced corticosterone secretion.

Mepyramine, a histamine H_1-receptor antagonist, given icv 15 min prior to clonidine significantly diminished the clonidine-induced corticosterone response, whereas a considerably weaker and statistically non significant diminution of that response was produced by cimetidine, a histamine H_2-receptor antagonist (*Fig. 5*).

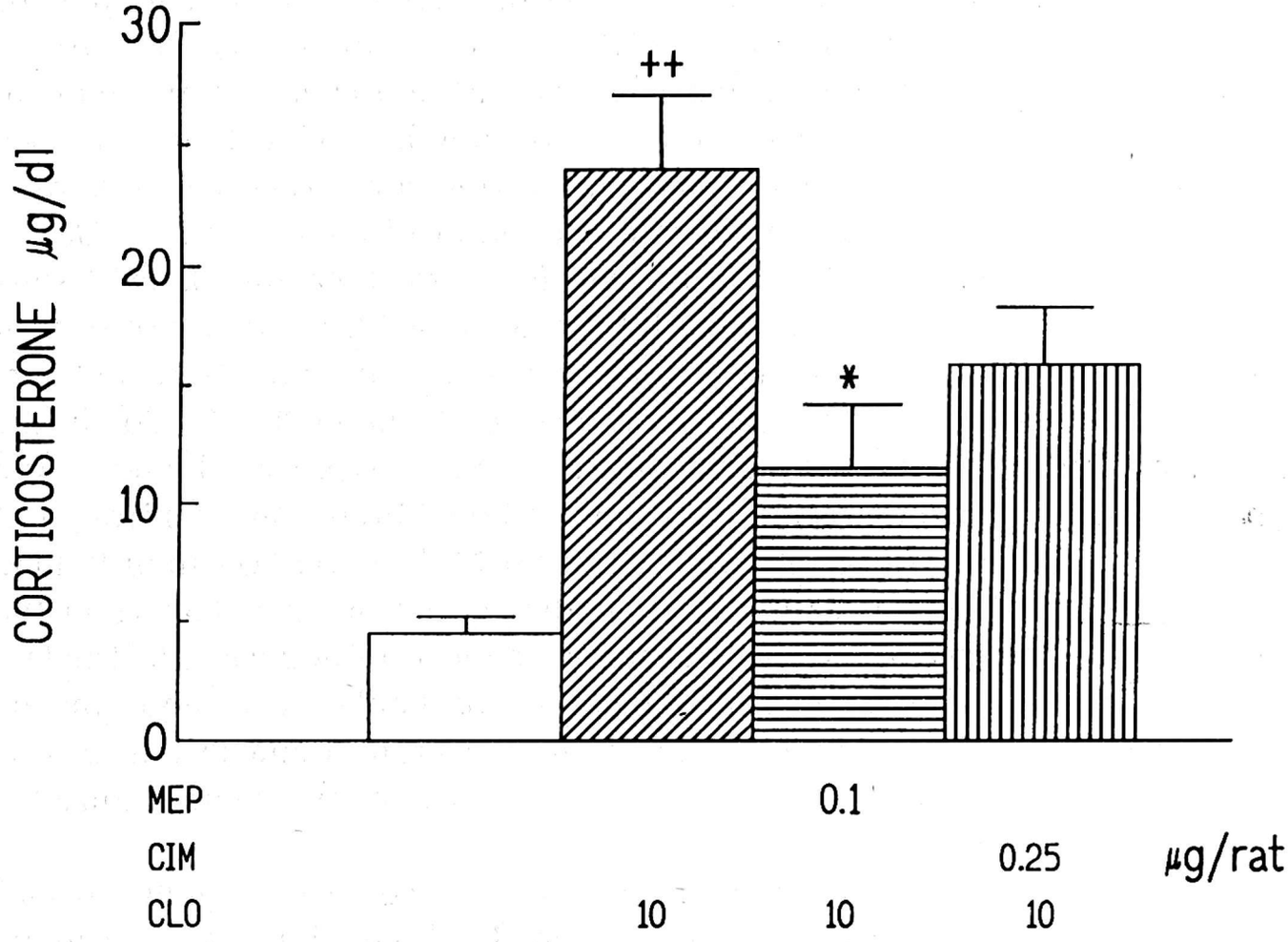

Fig. 5. Effect o mepyramine (MEP) and cimetidine (CIM) on serum corticosterone levels induced by clonidine (CLO). The drugs were injected icv, mepyramine and cimetidine 15 min before clonidine. ++ $p<0.001$ vs. saline controls; * $p<0.05$ vs. clonidine treated group.

DISCUSSION

The present results suggest that clonidine given icv increases the corticosterone secretion mainly by stimulation of central α-adrenergic receptors. In our experiment, low doses of yohimbine (0.01—0.1 µg icv and 0.001—0.01 mg ip) inhibited the corticosterone response to clonidine, suggesting an involvement of α_2-adrenergic receptors. Clonidine seems to act on hypothalamic α_2-adrenoceptors, since these receptors have not been found as yet, on pituitary corticotrops. However, similar inhibition of the clonidine-induced corticosterone secretion by higher doses of yohimbine (1-2 mg/kg) given systemically suggests also an action of clonidine on postsynaptic α-adrenergic receptors in the hypothalamus, since yohimbine at contrentrations about 100 times higher than those necessary to block presynaptic α-receptors can produce postsynaptic α-receptor blockade (18). Nevertheless, neither α_1-nor α_2-adrenergic blockers were able to completely abolish the corticosterone secretion induced by clonidine (1,2).

Our preliminary data showed that a histaminergic component may be involved in central stimulation of the HPA axis by clonidine (7). The present results clearly indicate significant participation of brain neuronal histamine in the HPA response to centrally administered clonidine. One hour after icv administration of clonidine, at the time when serum corticosterone levels were considerably elevated, the hypothalamic and brain histamine concentrations were significantly increased. It is not known, as yet, whether this change results from an increased rate of synthesis, or from a decreased histamine turn-over in histaminergic neurons. Histamine immunoreactive neuronal fibers and terminals are the most numerous in different hypothalamic nuclei (19, 20); in the present experiment, about 6 times higher histamine levels were found in the hypothalamus in comparison with the whole brain in control and clonidine-injected rats. In rats pretrated with α-FMH the changes in histamine levels of different brain structures were unidirectional but the changes in the hypothalamus, the cortex and the striatum were most pronounced (9). The last two structures, due to their relative weight, are probably most important for average changes in histamine levels in the rest of brain found in our experiment. However, both the cortex and the striatum are of minor importance for basal and stimulated HPA activity.

The elevated brain histamine levels were associated with a significant and persistent increase in the plasma corticosterone levels (10, 21). Also exogenous histamine injected icv to conscious rats considerably stimulated the HPA axis and raised the serum corticosterone levels (12).

A significant reduction, by 60%, in the clonidine-induced corticosterone response in rats pretreated 2 h earlier with α-FMH observed in our experiments is consistent with the hypothesis that neuronal histamine is involved in stimulation of the HPA axis by clonidine. This inhibitor of histidine decarboxylase is known to depress the rapidly turning-over pool of brain histamine in neurons, but not in mast cells (8, 22). Single administration of α-FMH in a dose of 20 mg/kg induced a complete loss of the histidine decarboxylase activity in the cerebral cortex and hypothalamus after 2 h (9), whereas a parallel decrease in the brain histamine content was less pronounced (10, 11, 23). This phenomenon may be explained by the fact that histamine synthesis in the rat hypothalamus is not acutely regulated via histidine decarboxylase (24). Although part of the hypothalamic histamine may also be contained in the non-neuronal pool, e. g. in mast cells or vascular epithelia, the present results clearly indicate that the neuronal pool of hypothalamic histamine is of strategic significance for stimulation of the HPA axis by clonidine.

In the present experiment the increase in corticosterone secretion evoked by clonidine was significantly attenuated by icv pretreatment with mepyramine, a H_1-receptor antagonist, whereas only a moderate decrease was induced by cimetidine, a H_2-receptor antagonist. This difference may be due to the

presence of abundant H_1 postsynaptic receptors, correlated with distribution of histaminergic nerve fibers in the hypothalamus. In addition, H_1-receptors are more efficient in stimulating the CRH and ACTH release (14, 25). However, the present results do not suggest that clonidine acts as a partial agonist at histamine H_2-receptors in the hypothalamus.

We demonstrated earlier that clonidine given icv stimulated the HPA axis and corticosterone secretion, mainly by acting on central α-adrenergic receptors. Our present results show that, in this stimulation clonidine also utilizes central neuronal histaminergic mechanisms. This assumption is supported by the facts that clonidine significantly increases histamine levels in the hypothalamus, and the clonidine-induced corticosterone secretion is considerably impaired by pretreatment with α-FMH, as well as by the histamine H_1-receptor antagonist, mepyramine.

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