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EFFECT OF COMPOUND 48/80 ON THE THALAMIC MAST CELLS, SEROTONIN LEVEL AND CORTICOSTERONE SECRETION IN RATS

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The effect of thalamic mast cells (MCs) degranulation and serotonin liberation by compound 48/80 on the hypothalamic-pituitary-adrenocortical (HPA) activity, measured indirectly through corticosterone secretion, was investigated in conscious rats. All drugs were given intracerebroventricularly (icv), the serotonin antagonists 15 min prior to compound 48/80. One hour after administration, compound 48/80 (1 and 5 μg) caused a significant increase in degranulated MC number in the thalamus, from control value of 20% up to 58%, and a considerable rise in the serum corticosterone level, but only minor diminution of the thalamic serotonin content. Pretreatment with methysergide, a serotonin receptor antagonist, only slightly dimished the compound 48/80-induced corticosterone response, while pretreatment with cyproheptadine, an antagonist of serotonin-histamine and cholinergic-receptors, significantly decreased the compound 48/80-elicited corticosterone response.

These results show for the first time that thalamic mast cells contain a very small amount of serotonin, which may play only a minor role in increasing the HPA activity by compound 48/80. These findings also suggest that other mediators liberated from mast cells by compound 48/80 are responsible for the çonsiderable increase in the HPA activity.

K e y w o r d s: *mast cells, thalamus, serotonin, pituitary-adrenocortical activity, corticosterone.*

INTRODUCTION

Growing body of evidence consistently indicates involvement of brain histamine and serotonin in regulation of the hypothalamic-pituitary-adrenocortical (HPA) activity (1, 2). A significant part of brain histamine is localized in both neurons and mast cells (MCs). Mast cells, first observed in the connective tissues and in gastrointestinal mucosa, were later demonstrated in the brain by a number of histologic techniques (3—6). The brain MCs are perivascular and are particular abundant in the thalamus and hypothalamus and they contribute up to 90% of the thalamic histamine and up to 50% of the

whole brain histamine levels (7). It is now well established that brain MCs resemble the connective tissue MCs and have a higher histamine content than mucosal MCs (8). In rodents serotonin is also present in peripheral MCs. Peritoneal MCs from Wistar rats contain histamine in a 5-fold excess of serotonin while MCs from the mesentery and lung contain serotonin in an excess of histamine (9). In rats MC granules serotonin is bound to an enzymatic site of the chymase enzyme. Some evidence suggests that both histamine and serotonin share the same granular storage site (10). Compound 48/80, a mast cells degranulating agent, was described as a more effective liberator of histamine than of serotonin from some peripherial MCs (9) or as devoid of differential effect on that secretion (11). Serotonin can also be released in the absence of substantial histamine secretion, and in the absence of demonstrable exocytosis of secretory granules (12). In the rat thalamus and hypothalamus, compound 48/80 applied in a perfusion system induced a dose dependent and parallel release of serotonin, histamine and β-hexosaminidase (13).

We have shown recently that in the compound 48/80-induced increase in corticosterone secretion histamine liberated from brain MCs, particularly from thalamic MCs may be moderately involved (14, 15). In contrast to the well known role of neuronal serotonin in regulation of the HPA activity, the significance of the brain MCs serotonin in this regulation has not yet been defined. Therefore the purpose of the present study was to examine a potential role of serotonin contained in the thalamic mast cells in stimulation of the HPA axis.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 180—230 g. The animals were housed in groups of 7 per cage and were maintained with commercial food and drinking water *ad libitum*, having been kept on a diurnal light cycle one week prior to the experiment. The animals were arbitrarily assigned to one of the experimental groups. The indicated doses of the drugs were injected in 10 μl of saline into the right lateral cerebral ventricle (icv) of non-anesthetized rats. Control rats received 10 μl of a 0.9% NaCl solution. Serotonin antagonists were administered icv 15 min before compound 48/80. One hour after the last injection, the rats were decapitated and their trunk blood was collected. Control animals were decapitated concurrently with the experimental rats. Serum samples were separated by centrifugation and were frozen for a subsequent corticosterone determination. The serum corticosterone concentration was determined fluorometrically. All experiments were performed between 9 and 11 a.m. and all decapitations between 11 and 12 a.m. to avoid corticosterone level fluctuations due to diurnal rhythm.

The drugs used in those studies were: compound 48/80, a product of condensation of N-methyl-p-methoxy-phenethylamine with formaldehyde (Sigma), methysergide maleate (Sandoz) and cyproheptadine hydrochloride (Merck Sharp and Dohme, Research Dohme). The drugs were dissolved in saline immediately before use.

A microscopic analysis of the thalamic and hypothalamic mast cells were based on 7 animals from intact, icv saline controls and injected icv with compound 48/80 in doses of 1 and 5 μg, 1 h before decapitation. The brains were immediately removed, the hypothalami and thalami were

isolated and fixed with 50—100% ethanol, embedded in paraffin and sectioned. Frontal sections were cut at 4—5 μm thickness and were stained with toluidine blue at pH 4.5. The number of mast cells, intact or at different stages of degranulation was counted with a light microscope. One hundred microscopic fields from 10—14 sections, 47.15 mm^2 for each of 7 rat brain structures were counted.

For an HPLC assay the brains were quickly removed and the hypothalami and thalami were dissected on a chiled plate and immediately frozen on dry ice. Frozen tissue samples were placed into approx. 10 vol. of ice-cold 0.1 M $HClO_4$ containing 5 mM of ascorbic acid and 25 μg/l of 3,4 dihydroxybenzylamine (internal standard), weighed and homogenized with an Ultra-Turrax homogenizer (10s at 20000 rpm). The homogenates were centrifuged at 14000 × g and supernatants were subsequently filtered through 0.22 μm RC-58 membranes (BAS MF-1 centrifugal microfilters). The 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 octane sulfonate, 0.27 mM EDTA and 5% methanol) was pumped at 0.9 ml/min. through the column thermostatted at 32°C. The separated sample components of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were detected at an oxidation potential of 0.8 V. All reagents were of an analytical grade (Merck, Germany and Sigma, USA).

The data were statistically evaluated by analysis of variance, followed by a specific comparision with the Duncan test.

RESULTS

Effect of compound 48/80 on thalamic mast cells

In the present experiment the majority of MCs in the rat brain were found to occur in the thalamus while the hypothalamus contained lesser amount of MCs. Therefore changes in the MCs number and degranulation after treatment with compound 48/80 were examined in the thalamus. In control, icv saline pretreated rats, the majority, 80% of MCs were intact, while 20% of them were partly degranulated (*Fig. 1—2*). Compound 48/80 (1 and 5 μg) 1 h after icv administration, caused a significant increase in the number of degranulated MCs, up to 58 and 54%, respectively (*Table 1*).

Effect of compound 48/80 on serum corticosterone levels

Compound 48/80 given icv in doses of 1—5 μg increased serum corticosterone levels in a dose dependent manner. One hour after administration, the serum corticosterone levels rose from the resting values of 7.5—10.5 μg/dl up to 17.6 and 36.4 μg/dl after doses of 1 and 5 μg, respectively (*Fig. 3*).

Compound 48/80-induced changes in thalamic serotonin level

In order to examine possible involvement of serotonin from the thalamic MCs in the observed HPA stimulation by compound 48/80, levels of

Fig. 1. Metachromatic mast cells associated with the vessels in the thalamus. Toluidine blue staining. Magnification 600× approx.

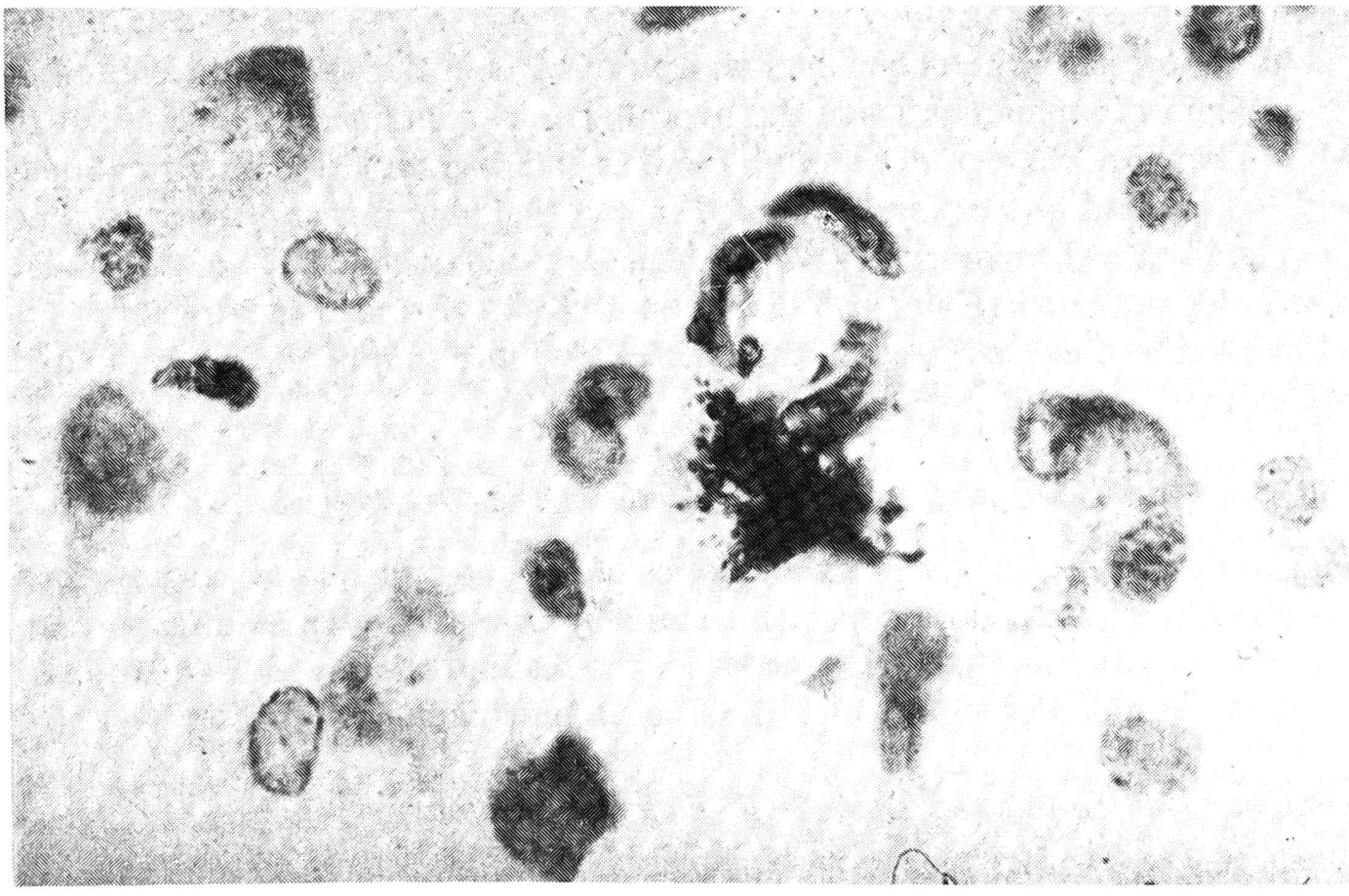

Fig. 2. Extra capillary location of thalamic mast cells degranulated by compound 48/80, 1 μg icv. Magnification 600× approx.

Table 1. Effect of compound 48/80 on thalamic mast cells

Mast cells mean number from 7 rats per 100 microscopic fields				
		intact	degranulated	total
saline control	10 µl icv	13±6.3	4.6±3	17.6±8.1
48/80	1 µg icv	2.9±1.3^{+}	4±2.7^{+}	6.9±3.9^{+}
saline control	10 µl icv	8.4±3.2	1.8±1	10.3±3.8
48/80	5 µg icv	4.4±3.1	5.1±3.4	9.6±6.5

The rats were decapitated 1 h after icv administration of compound 48/80. Each value represents the mean±SEM of 7 rats. $^{+}$ $p < 0.05$ vs. saline control group.

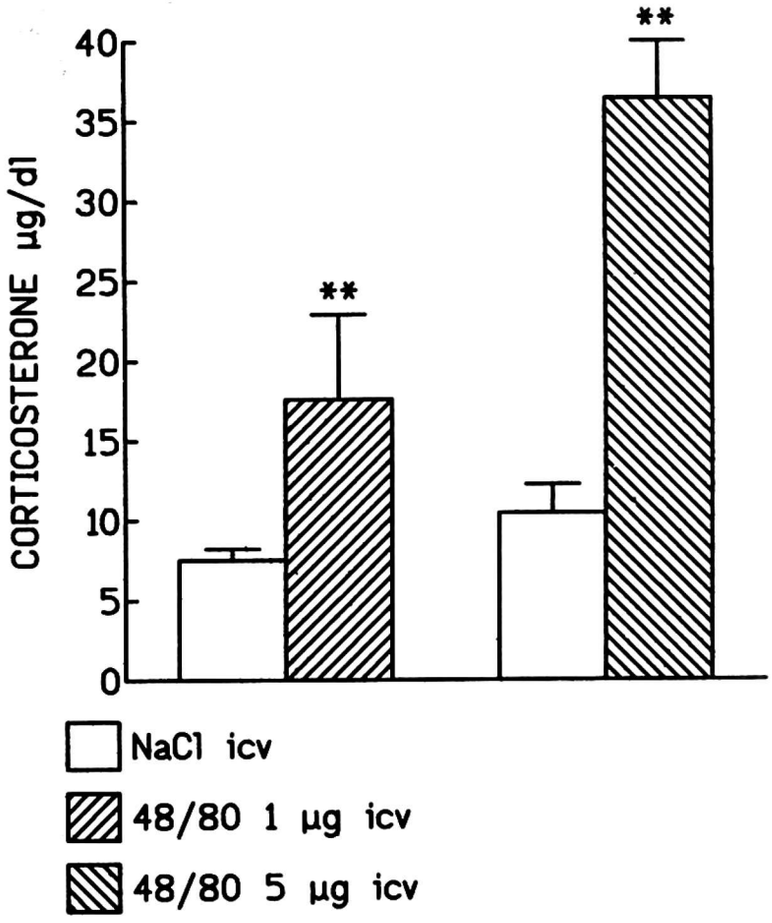

Fig. 3. Changes in serum corticosterone levels induced by compound 48/80, given icv 1 h before decapitation. Each value represents the mean ±SEM of 7 rats. ** $p < 0.001$ vs. saline treated controls.

serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were measured in that brain structure. One hour after administration of compound 48/80 in doses of 1 and 5 µg, the thalamic 5-HT and 5-HIAA levels did not substantially differ from the levels of control, saline-treated animals (*Figs 4—5*), indicating that the thalamic MCs do not contain any marked amount of 5-HT.

Effect of methysergide on coticosterone response to compound 48/80

To determine a possible functional effect of 5-HT liberated from the thalamic MCs on the observed rise in the corticosterone secretion, serotonin receptor antagonists were given 15 min. prior to compound 48/80.

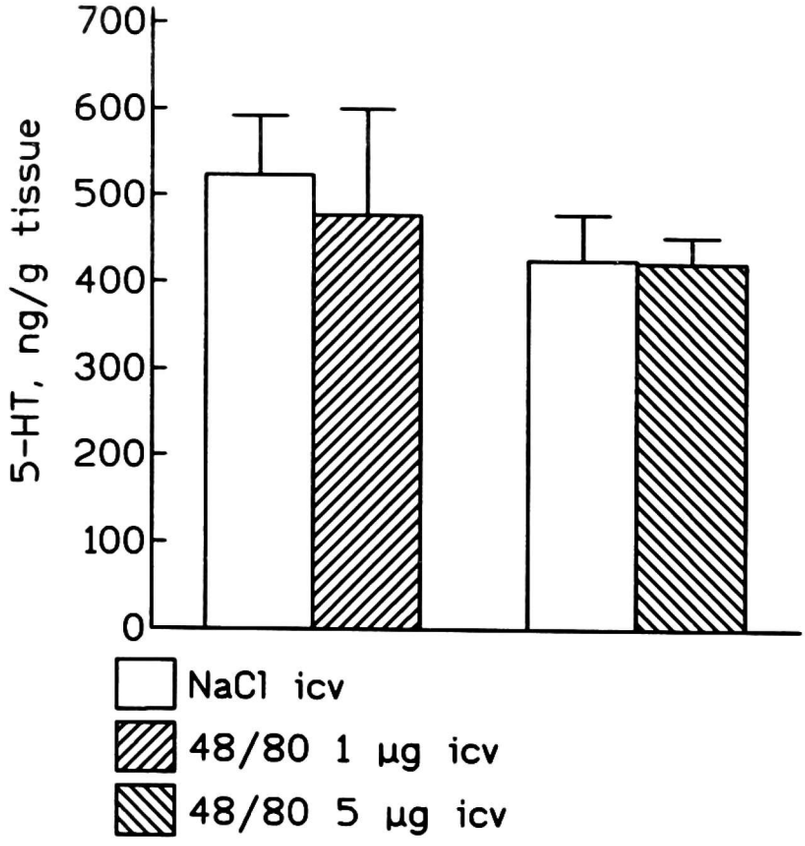

Fig. 4. Effect of compound 48/80 on the thalamic serotonin (5-HT) content Compound 48/80 was given icv 1 h before decapitation. Each value represents the mean ± SEM of 7 rats.

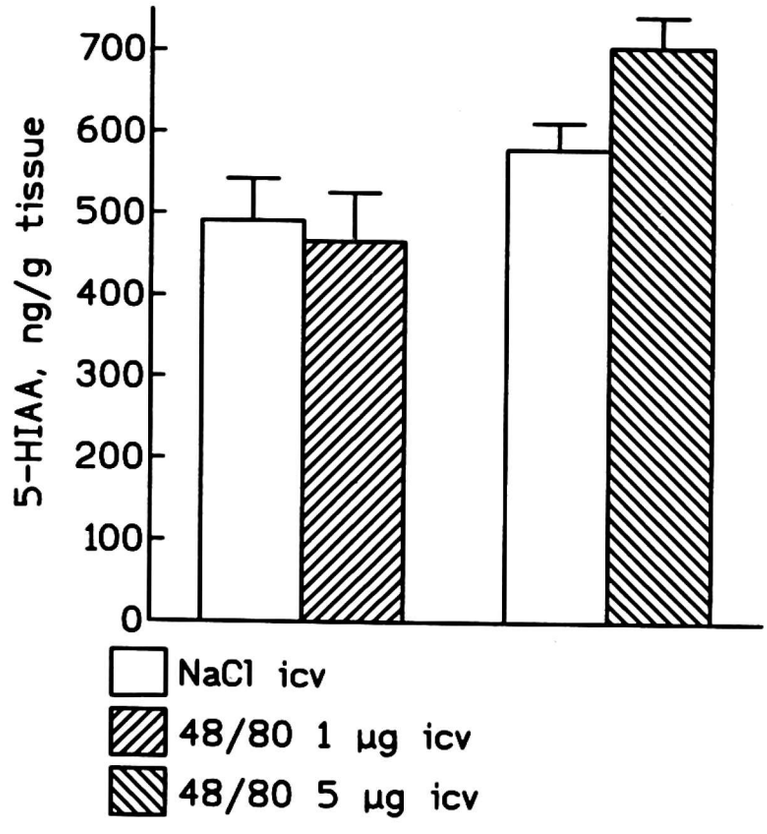

Fig. 5. Effect of compound 48/80 on the thalamic 5-hydroxyindoloacetic acid (5-HIAA). Compound 48/80 was given icv 1 h before decapitation. Each value represents the mean ± SEM of 7 rats.

Methysergide and cyproheptadine were given icv in doses which themselves did not markedly affect the resting serum corticosterone levels (*Tab. 2*).

Intraventricular pretreatment with metysergide (0.1 µg) moderately, diminished by 25%, the rise in the serum corticosterone levels elicited by compound 48/80 (*Fig. 6*).

Table 2. Effect of methysergide and cyproheptadine on serum corticosterone levels

Dose µg/rat icv	Corticosterone µg/dl	Dose µg/rat icv	Corticosterone µg/dl
Saline control 10 µl	11.3±2.1	Saline control 10 µl	9.8±2.3
Methysergide 0.01	11.1±1.7	Cyproheptadine 0.1	12.4±2.7
Methysergide 0.1	10.7±2.1	Cyproheptadine 1	9.5±2.1
Methysergide 1	12.2±1.9	Cyproheptadine 10	7.2±1.5
		Cyproheptadine 20	11.0±3.2

The drugs administrated icv 1 h before decapitation.
Each value represents the mean±SEM of 7 rats.

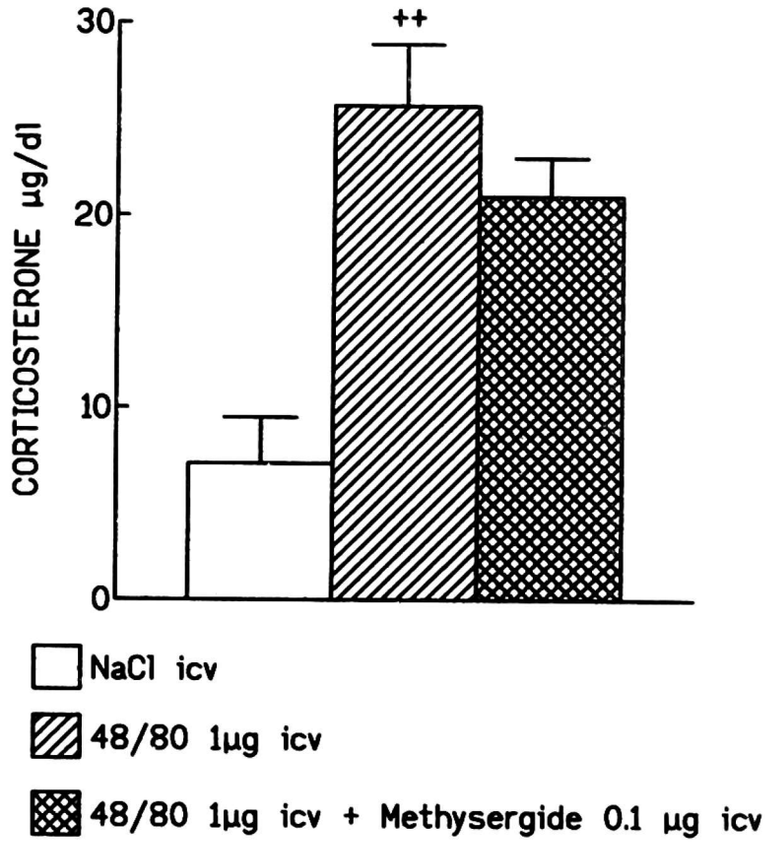

Fig. 6. Effect of methysergide on the compound 48/80-induced corticosterone response. Both drugs were given icv, methysergide 15 min before compound 48/80. Each value represents the mean ±SEM of 7 rats. $^{++}$ $p < 0.001$ vs. saline treated controls.

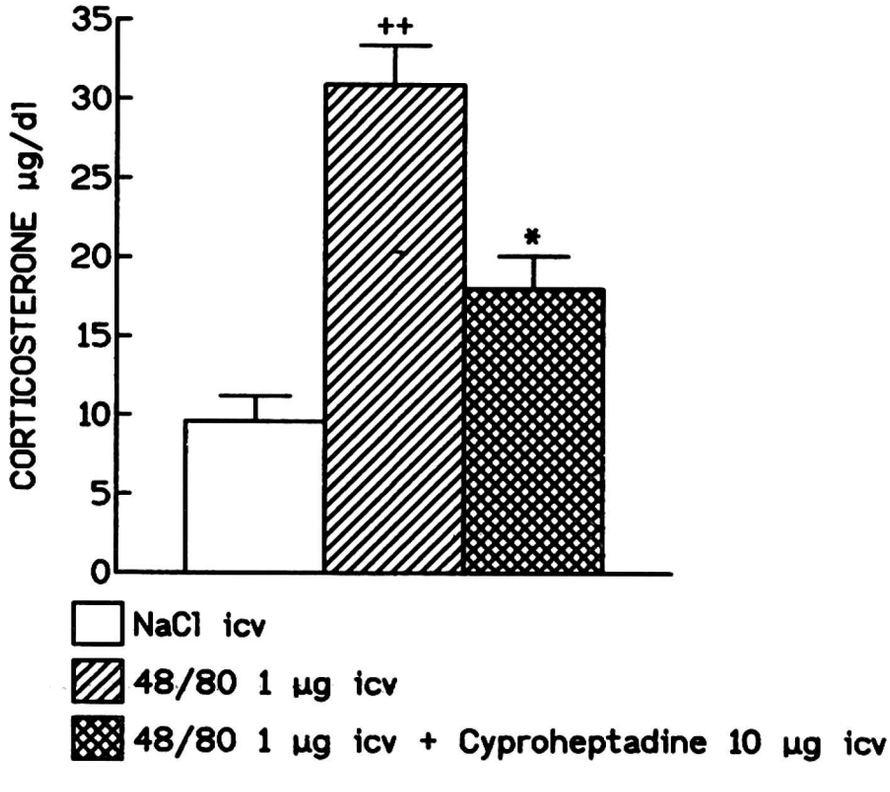

Fig. 7. Effect of cyproheptadine on the compound 48/80-induced corticosterone response. Both drugs were given icv, cyproheptadine 15 min before compound 48/80. $^{++}$ $p < 0.001$ vs. saline treated controls, and * $p < 0.05$ vs. compound 48/80 treated group.

Effect of cyproheptadine on corticosterone response to compound 48/80

Cyproheptadine, a drug with a serotonin-, histamine- and acetylcholine receptor blocking activity, given icv significantly, reduced by 61%, the compound 48/80 induced corticosterone response (*Fig. 7*).

DISCUSSION

The results of the present experiment, in agreement with earlier reports, clearly show that compound 48/80 given centrally increases the HPA activity, measured indirectly through corticosterone secretion. Compound 48/80 is known to degranulate MCs and liberate histamine and serotonin from both the peripheral (9—12, 16) and brain MCs (13), which share histochemical characteristics with the connective tissue MCs (17). Compound 48/80 given systemically elicits circulatory shock which may be significantly prevented by both histamine- and serotonin- antagonists (18). Also in the rat thyroid gland MCs are present and they contain histamine and serotonin whose levels show circadian variations linked changes in the gland activity (19). Brain MCs and their mediators, histamine and serotonin, released by compound 48/80 administered to 3rd brain ventricle, were shown to parcipate in the neuroendocrine control of thyreotropin secretion (20).

There is no reliable information available on the probable content and potential role of the brain MCs serotonin in stimulation of the HPA axis. In line with ealier reports, our present results show a significantly higher MCs number in the thalamus as compared with the hypothalamus. In contrast to the considerable depletion of the thalamic histamine found by us after icv administration of compound 48/80 (15), in the present experiment no marked changes in the thalamic content of serotonin or its metabolite, 5-hydroxyindoleacetic were observed. This result indicates that the rat thalamic MCs contain a significant amount of histamine, up to 60% of the total histamine in that structure (15), but only less than 10% of the total serotonin content. Our present data show that a proportion of histamine to serotonin in the thalamic MCs is similar to that found in the peritoneal MCs (9).

Consequently, we did not find any significant impairment by methysergide of the compound 48/80-induced corticosterone response. Methysergide, a fairly selective serotonin receptor antagonist, inhibited only slightly, by 25%, the corticosterone response elicited by compound 48/80. On the other hand, cyproheptadine which in addition to its anti-serotoninergic action, possesses also histamine-, acetylcholine- and dopamine-blocking actions (21, 22), significantly diminished, by 61% the corticosterone response to compound

48/80. In a perfusion system for the rat hypothalamus and anterior hypophysis, cyproheptadine had a direct inhibitory effect on both the serotonin-induced CRH and CRH-induced ACTH release (23). Since in the present experiment the amount of serotonin liberated from the thalamic MCs by compound 48/80 was negligible, the selective antiserotoninergic action of cyproheptadine in inhibiting the HPA axis stimulation should be weaker than that observed in the present experiment. A substantial part of the combined inhibitory effect of cyproheptadine, found in the present experiment may be caused by its anti-histaminic action. We found recently that the histamine receptor antagonists mepyramine and cimetidine diminished the compound 48/80-induced corticosterone response by ca 25% (14).

Our present results for the first time show that the thalamic mast cells contain only minor part of the total serotonin in that structure. Consequently, serotonin released from MCs does not markedly participate in the compound 48/80-elicited corticosterone response. Since also histamine released from the brain mast cells only moderately stimulates the HPA activity (14, 15), some other mediators liberated by compound 48/80 from the brain mast cells (24) may be responsible for the considerable stimulation of the HPA axis after brain mast cells degranulation.

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