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EFFECT OF COMPOUND 48/80 ON MAST CELLS AND BIOGENIC AMINE LEVELS IN BRAIN STRUCTURES AND ON CORTICOSTERONE SECRETION

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The effect of compound 48/80, given intracerebroventricularly, on mast cells (MC) and histamine, serotonin and noradrenaline levels in the hypothalamus, thalamus and hippocampus as well as on corticosterone secretion was investigated in rats. A relatively high amount of mast cells was found in the thalamus, a very low in the hypothalamus and almost none in the hippocampus. Compound 48/80 (1 and 5 μg) induced MC degranulation, a significant increase in corticosterone secretion and diminution of the thalamic histamine level. The drug also elicited small biphasic changes in histamine level in the hypothalamus and moderately increased serotonin turnover in the brain structures studied, but did not affect noradrenaline level in those structures. These results indicate that brain MC contain a significant amount of histamine, but not serotonin. Compound 48/80 releases histamine from MC, but does not markedly influence neuronal biogenic amines in the brain structures involved in regulation of the hypothalamic-pituitary-adrenal axis.

Key words: *Mast cells, hypothalamus, thalamus, hippocampus, compound 48/80, biogenic amines.*

INTRODUCTION

Described as a potent histamine liberator, compound 48/80 is now considered to release various mediators, including histamine and serotonin, from mast cell (MC) granules (1-3). A biochemical mechanism of this effect remains unknown. As a basic secretagogue, compound 48/80 interacts with the negatively charged region of membrane Giα and seems to activate MC by direct G-protein activation rather than *via* a receptor-depending pathway (4). It is now well established that both histamine and serotonin are able to release corticotropin releasing hormone (CRH) from hypothalamic neurons and to stimulate the hypothalamic-pituitary-adrenal (HPA) axis (5,6). A significant

part of brain histamine is contained not only in neurons, but also in MC (7,8). The highest density of MC is encountered in the thalamus (2).

We have recently shown that degranulation of thalamic MC by compound 48/80 given intracerebroventriculary (icv) is accompanied with a considerable reduction of histamine in this structure and an increased corticosterone secretion (9). We have also shown that pretreatment with the histamine receptor antagonists mepyramine and cimetidine moderately diminishes the compound 48/80-induced corticosterone response (10). The thalamic MC degranulation elicits only minor diminution of the thalamic serotonin level, while the serotonin receptor antagonists methysergide and cyproheptadine slightly decrease the corticosterone response to compound 48/80 administered icv (11).

Histamine released by compound 48/80 may induce stimulation of the HPA axis through activation of histamine H_1- or H_2-receptors (5). The latter receptor stimulation elicits release of endogenous noradrenaline (NA) in the rat hypothalamus (12) and NA is also known to stimulate CRH secretion from parvocellular paraventricular hypothalamic neurons (13). Therefore histamine liberated by compound 48/80 from brain MC may indirectly stimulate the HPA axis activity *via* catecholamine release in the hypothalamus. Since the majority of brain MC are contained in the thalamus, changes in both histamine, serotonin and noradrenaline levels may indirectly affect the CRH-containing neurons.

The hippocampus is known to be significantly involved in regulation of the HPA axis through its projection to the hypothalamus (14). This structure may serve as a site for feedback inhibition of the corticosterone secretion mediated by glucocortical receptors (15-17) which may also be directly regulated by the noradrenergic system (18).

Although generally regarded as an MC degranulator and histamine and serotonin releaser, compound 48/80 given centrally may affect catecholaminergic, histaminergic and serotonergic neurons in brain structures and in this way modulate the activity of the HPA axis.

The aim of the present study was to determine whether compound 48/80 given icv affects the content of the biogenic amines histamine, serotonin and noradrenaline, contained in MC and/or in neurons of such brain structures as the hypothalamus, thalamus and hippocampus which are known to be involved in regulation of the HPA axis activity.

MATERIALS AND METHODS

Male Wistar rats weighing 180-230 g were used for these studies. One week before experimentation, the animals were housed under standard laboratory conditions on a day light cycle, with food and tap water available *ad libitum*. Compound 48/80 (1 and 5 μg) was administered in a volume of 10 μl into the right lateral cerebral ventricle to rats whose skulls were prepared 24 h earlier, under light ether anesthesia, for free-hand icv injections. Control animals were injected with

10 µl of saline. After injection of compound 48/80, the rats were placed back in their cages. One hour after the injection, the rats were decapitated and their trunk blood was collected in small centrifuge tubes. 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 by a fluorometric method. To avoid corticosterone fluctuations due to the circadian rhythm, all experiments were performed between 9 and 11 a.m., and all decapitations took place between 11 and 12 a.m.

A microscopic analysis of brain mast cells was based on 6 rats, injected icv with saline or compound 48/80 1 h before killing. The brains were immediately removed, their structures were isolated and fixed with a 50—100% ethanol or Persinger's fixative. Then they were embedded in paraffin and sectioned. Frontal sections were cut at 4—5 µm in thickness and were stained with a 0.5% toluidine blue at pH 4.5. The number of cells, intact or at different stages of degranulation, was counted on coded slides with a light microscope at a magnification of 400. One hundred microscopic fields from 10—14 sections, 47.15 mm^2 for each of 6 the rats brain structure were counted.

For an HPLC assay, the brains were quickly removed, placed on glass plates kept on ice and washed with ice-cold saline. Hypothalami, thalami and hippocampi were isolated on a chilled plate and immediately frozen on dry ice. The frozen tissue samples were placed in approx. 10 vol. of ice-cold 0.1 M $HClO4_4$ containing 5 mM ascorbic acid and 25 µg/l 3,4 dihydroxybenzylamine (internal standard), weighed and homogenized with an Ultra-Turrax homogenizator (10 s at 20000 rpm). The homogenates were centrifuged at 14000 × g and the supernatants were subsequently filtrated through 0.22 mm RC-58 membranes (BASF MF-1 centrifugal microfilters). The filtrates were injected into the HPLC system. A BAS 400 liquid chromatograph (BAS, USA), equipped with an LC4B/17AT electrochemical detector and 3 µm C_{18} Phase 2 analytical column (100 mm × 3 mm) which was coupled with 7 µm C_{18} quard column (15 mm × 3 mm) was used. A mobile phase (36 mM citrate-28 mM phosphate buffer pH 3.5, containing 0.77 mM octane sulfonate, 0.27 mM EDTA and a 5% methanol) was pumped at 0.9 ml/min. through a column thermostatted at 32°C. The separated sample components 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and noradrenaline (NA) were detected at an oxidation potential of 0.8V. All the reagents were of analytical grade (Merck, Germany and Sigma, USA).

For determination of the histamine concentration, a 10 or 20% (w/v) homogenate of the tissue was prepared in 0.4 M perchloric acid. The homogenate was centrifuged and the supernatant was adjusted to pH 5-6 with 0.2 M KOH. 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-phtaldialdehyde, was estimated fluorometrically at 360/450 nm.

The drug used in the present study was compound 48/80, a product of condensation of equimolar concentrations of formaldehyde and p-methoxy-N-methylphenyletylamine (Sigma). The drug was dissolved in a 0.9% NaCl solution immediately before use.

All the data are presented as means ± SEM. The statistical significance of differences between groups was assessed by an analysis of variance, followed by individual comparisions with Duncan's test.

RESULTS

Compound 48/80-induced changes in mast cells in brain structures

In accordance with previous reports (10, 19), we observed considerable variations in the distribution and number of MC within the same experimental group. The majority of MC were found to occur perivascularly and along

blood vessels. In the present experiment, the highest quantity of MC in the rat brain was observed in the thalamus, while the hypothalamus contained only a trace amount of MC. In control, icv saline-pretreated rats, the majority (74—86%) of MC were intact, while 14—26% were partly degranulated. At 1 h after icv administration, compound 48/80 (1 and 5 μg) caused a significant increase in the number of degranulated MC, up to 46 and 58%, respectively (*Table 1*).

Table 1. Effect of compound 48/80 on mast cells degranulation in the thalamus.

	Mean number of mast cells		
	intact	degranulated	total
Saline control 10 μl	13.0±6.3	4.6±3.0	17.6±8.1
48/80 1 μg	2.9±1.3^{+}	4.0±2.7^{+}	6.9±3.9^{+}
Saline control 10 μl	41.7±16.2	6.7±2.3	48.4±18.1
48/80 5 μg	20.2±4.9	17.3±3.9	37.5±8.5

The rats were decapitated 1 h after icv administration of compound 48/80. Each value represents the mean ± SEM of 100 microscopic field from 7 rats. $+p<0.05$ vs. saline control group.

Effect of compound 48/80 on corticosterone secretion.

Compound 48/80 administered icv in doses of 1 and 5 μg increased the serum corticosterone levels in a dose-dependent manner. One hour after administration, the serum corticosterone concentration rose from the resting values of 8.9 and 12.8 μg/dl up to 22.7 and 36.4 μg/dl after doses of 1 and 5 μg, respectively (*Fig. 1*).

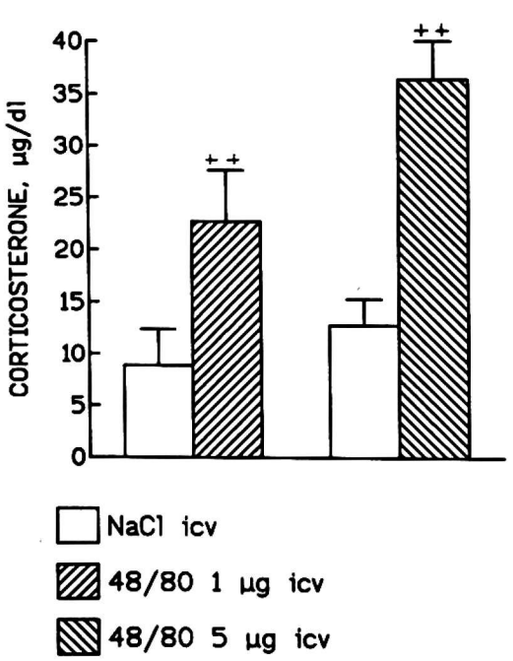

Fig. 1. Effect of compound 48/80 given icv on corticosterone secretion. In *Figs. 1—5* values represent the mean ± SEM of 6 rats. $+p<0.05$ and $++p<0.01$ vs saline control.

Effect of compound 48/80 on histamine level in brain structures

Compound 48/80 used in a lower dose (1 μg icv) moderately, but insignificantly diminished (by 17%) the hypothalamic histamine level; when given in a higher dose (5 μg icv), it substantially elevated that level (by 32%) as measured 1 h after administration (*Fig. 2*).

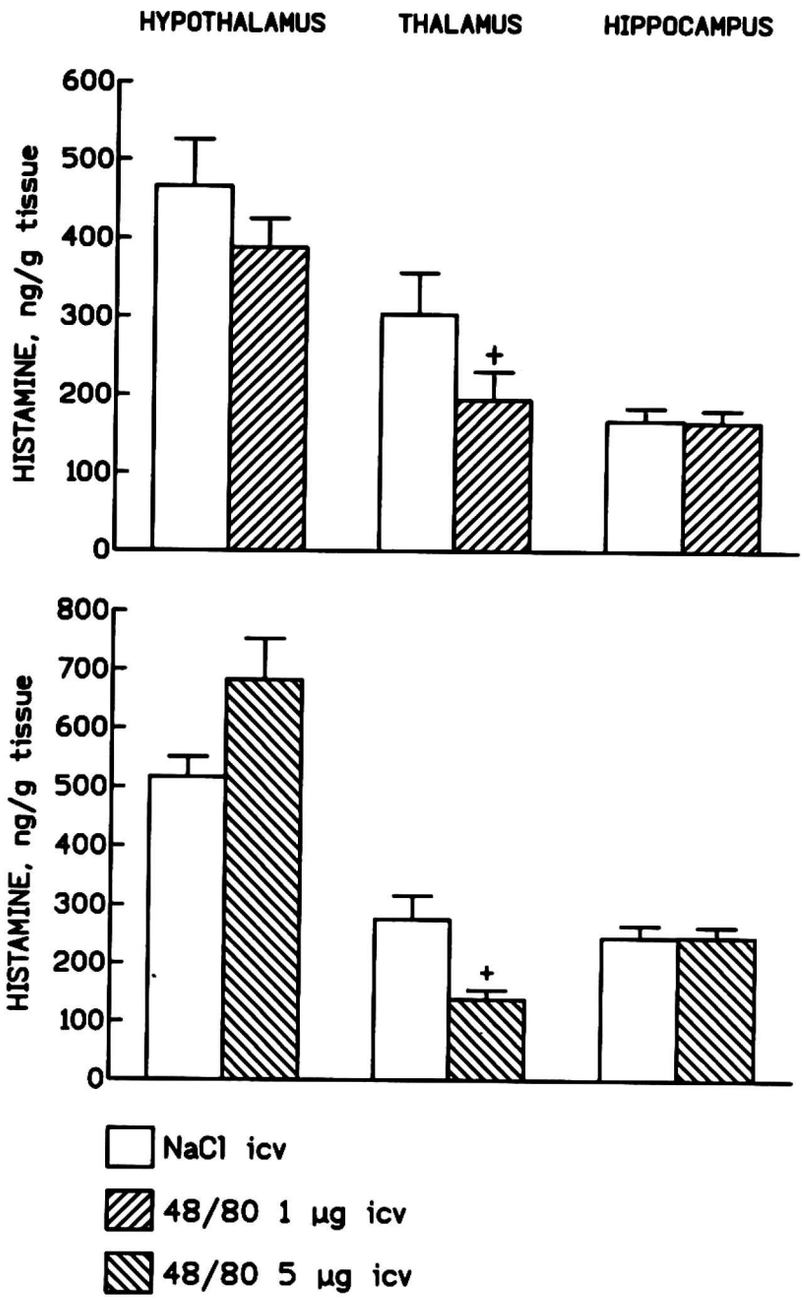

Fig. 2. Histamine level in brain structures of rats pretreated icv with compound 48/80. See legend to *Fig. 1.*

A significant dose-related diminution of the histamine content was observed in the thalamus, by 37% after a lower dose and by 50% after a higher one of compound 48/80. Neither dose of compound affected the histamine level in the hippocampus (*Fig. 2*).

Effect of compound 48/80 on serotonin level in brain structures

In agreement with previous results compound 48/80 given icv in a dose of 1 μg induced only a slight decrease (by 12%) in the hypothalamic serotonin level and a parallel increase in the level of its metabolite 5-HIAA (by 5%) at

1 h after administration. Given in a higher dose of 5 μg, compound 48/80 increased, in a non-significant manner both the serotonin and the 5-HIAA levels in the hypothalamus, by 31 and 10%, respectively, at 1 h after administration (*Figs. 3-4*).

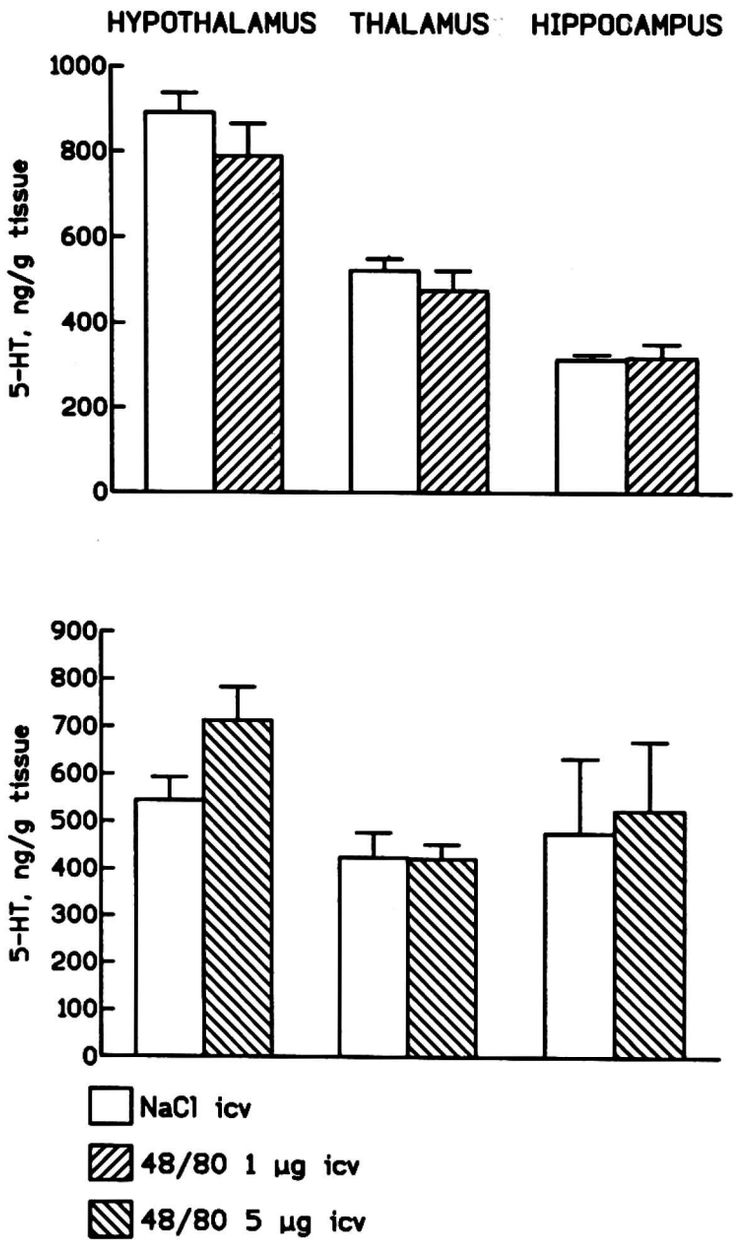

Fig. 3. Serotonin (5-HT) level in brain structures of rats pretreated icv with compound 48/80. See legend to *Fig. 1.*

Only minor decreases were observed in the thalamic serotonin and 5-HIAA levels, by 9 and 5%, respectively, after a lower dose (1 μg); following a higher dose (5 μg), only the 5-HIAA level was increased by 22% in that brain structure.

The hippocampal levels of serotonin and its metabolite were not affected by compound 48/80 used in a lower dose (1 μg), but were higher than control ones, by 10 and 94% ($p<0.05$), respectively, after a higher dose (*Figs. 3—4*).

Changes in noradrenaline level

Compound 48/80 (5 μg icv) did not induce any marked changes in noradrenaline levels in the hypothalamus, thalamus or hippocampus (*Fig. 5*).

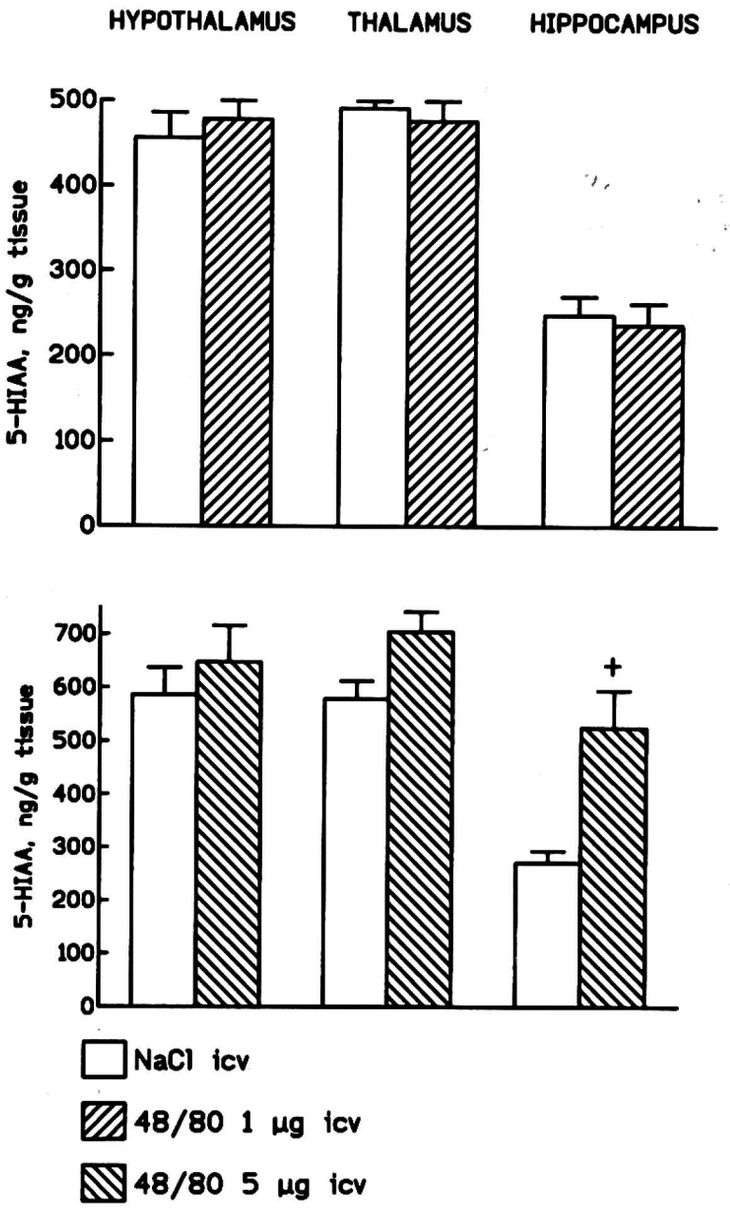

Fig. 4. Effect of compound 48/80 given icv on 5-hydroxyindoleacetic acid (5-HIAA) in brain structures. See legend to *Fig. 1.*

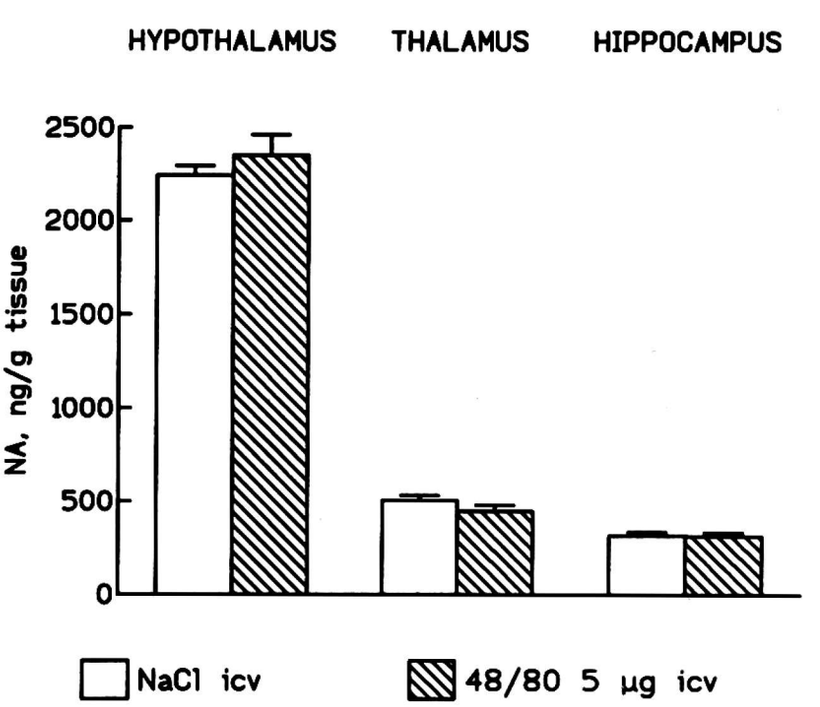

Fig. 5. Noradrenaline (NA) level in brain structures of rats pretreated icv with compound 48/80. See legend to *Fig. 1.*

DISCUSSION

The present data clearly show that the graded increase in corticosterone secretion elicited by compound 48/80 (1 and 5 μg icv) is accompanied with a parallel, significant decline in the number of MC and in histamine level in the thalamus. Our results indicate that at least a half of the total thalamic histamine is contained in MC, since after MC degranulation by compound 48/80 its level fell by 50%. In accordance with earlier reports (9, 11) we did not find any significant number of MC in the hypothalamus. In the present experiment the hypothalamus contained only a trace amount of MC. A moderate decline in the hypothalamic histamine level (17%), evoked by a lower dose (1 μg) of compound 48/80, and a substantial rise produced by its higher dose (5 μg) cannot be exclusively related to changes in the histamine content due to MC degranulation. Compound 48/80 is also known to exert additional effects which are not connected with MC degranulation. It is an antagonist of calmodulin-regulated functions which are distinct from histamine release (20, 21). The complex effects of compound 48/80 may participate in final HPA and histamine responses.

A significant part of hypothalamic histamine is contained in neurons, since α-fluoromethylhistidine, an inhibitor of histidine decarboxylase, i.e. of neuronal histamine synthesis, reduced the hypothalamic histamine level by 60% in our earlier experiments (22).

Our present results corroborate some earlier data that the major part of histamine in the hypothalamus is contained in neurons, whereas in the thalamus ca 50% of histamine represent the MC pool (2, 7, 23). These results also indicate that compound 48/80 selectively liberates histamine from the degranulated MC, but not from histaminergic neurons.

Only single MC were encountered in the hippocampus of both saline-injected and compound 48/80-treated rats. Hence the whole amount of the hippocampal histamine appears to be located in histaminergic neurons. Compound 48/80 did not influence the hippocampal histamine level, which indicates that the neuronal histamine pool was not affected, nor was the hippocampal histamine involved in corticosterone response to that compound. Although compound 48/80 used in a lower dose (1 μg) significantly enhanced corticosterone secretion, it did not substantially alter the serotonin levels in the three structures investigated. In the thalamus, where significant degranulation of MC was observed, only a slight decrease in 5-HT and 5-HIAA levels was observed, by 9 and 5%, respectively. When given in a higher dose (5 μg), compound 48/80 moderately increased the serotonin level in the hypothalamus and significantly elevated the levels of 5-HIAA in the thalamus and hippocampus, by 22 and 94%, respectively, which indicates an increased serotonin turnover in these structures. The latter change suggests possible involvement of the serotonin of these structures in the compound 48/80-elicited stimulation of the HPA axis. The observed increases in the serotonin level may

also be caused by inhibition of its release by the histamine concurrently liberated from MC or from histaminergic neurons (24). However, only small changes in the serotonin levels in the structures under study indicate a weak serotonin component in the central action of compound 48/80.

We did not observe any marked changes in the noradrenaline content in brain structures in the compound 48/80-treated as compared to saline control rats. Noradrenergic systems in the paraventricular nucleus of the hypothalamus (13) and hippocampus (25, 26) are known to play a key role in regulation of the HPA axis. The present results indicate that these systems are not substantially activated by compound 48/80.

In conclusion, our data show that the rat MC are present in a relatively high amount in the thalamus where they contain 50% of the total brain histamine. Very few MC occur in the hypothalamus, and they are practically absent in the hippocampus. The mast cells degranulator compound 48/80 significantly decreases the thalamic histamine level and enhances the turnover of serotonin, but it does not markedly influence the level of noradrenaline in the brain structures involved in regulation of the HPA axis. The histamine released from the thalamic MC by compound 48/80 may be involved in stimulation of the HPA axis. Compound 48/80 does not significantly activate the central noradrenergic system in the hypothalamus, thalamus and hippocampus. Compound 48/80 can therefore be regarded as a selective brain MC degranulator and histamine releaser and it does not markedly affect the biogenic amines involved in regulation of the HPA axis activity in the hypothalamus, thalamus and hippocampus.

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