Effect of modified brain histamine contents on prolactin and thyrotropin secretion in male rats

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Effects of modified brain histamine contents on thyrotropin and prolactin secretion were studied in male rats. Under basal conditions the histamine content in the hypothalamus was approximately 8–10-fold higher than that in the striatum and the rest of the brain. L-histidine (1000 mg/kg, ip), a histamine precursor, and metoprine (20 mg/kg, ip), an inhibitor of histamine N-methyltransferase, elevated histamine content in the brain by 65% and 167%, respectively. When the treatments were given together an additive effect (119–250% increase) on brain histamine was observed. Metoprine significantly decreased serum prolactin levels, while L-histidine had no effect. This effect of metoprine was not modified by treatment with L-histidine. Thus, metoprine has an inhibitory effect on prolactin secretion that is not related to elevated brain histamine contents. The increased brain histamine content after L-histidine treatment had no effect on prolactin secretion. Basal levels of serum thyrotropin were decreased by both L-histidine and metoprine. L-histidine being more potent. In rats treated with a-fluoromethylhistidine, an inhibitor of L-histidine decarboxylase, the cold-induced (rats kept for 60 min at +4°C) thyrotropin secretion was increased while the stress-induced prolactin secretion was decreased. In these rats, metoprine did not affect thyrotropin release but blunted the prolactin response. In conclusion, endogenous histamine inhibits thyrotropin secretion but does not affect prolactin release. Owing to its other effects, metoprine is not suitable as a tool to elevate endogenous histamine contents in the brain, at least when the regulation of anterior pituitary hormone release is being studied.

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Histamine modifies the release of anterior pituitary (AP) hormones in rats (1). The site of action of histamine is generally not in the AP gland but in the hypothalamus, where histamine can modulate the function of other neurotransmitter systems or neurosecretory cells (2, 3). In the hypothalamus, most of which is inside the blood brain barrier (BBB), histamine is stored in nerves and can be released upon nerve stimulation (for a review, see Ref. 3). Histamine does not cross the BBB, and therefore its central effects on AP hormone release have been studied using microinjections of the amine directly into the brain tissue or into the brain ventricles (1).

Histamine, given peripherally by intra-arterial infusion (4) or centrally either by injection to the brain ventricles (5) or into the rostral hypothalamus (6), increases prolactin secretion in male rats. Elevated basal prolactin levels in serum have also been measured in rats with portocaval anastomosis (7). These rats have a very high level of endogenous histamine in the brain (8, 9). Histamine has not been found to modify the release of prolactin from AP in vitro (10). Histamine injected intracerebroventricularly or into anterior hypothalamus (11) inhibits cold-induced (rats kept for 30 min at +4°C) thyrotropin (TSH) secretion in male rats. Histamine does not modify the release of TSH from superfused AP cells (12), indicating a central site of action for histamine.

In nervous tissue, histamine is synthesized from L-histidine by histidine decarboxylase (HDC) (EC 4.1.1.22) and degraded by histamine N-methyltransferase (HMT) (EC 2.1.1.8) (13). L-Histidine, given intraperitoneally (ip), can be used to elevate brain histamine levels (14), because L-histidine easily crosses the BBB and is decarboxylated in the brain to histamine by HDC. Metoprine, an HMT inhibitor (15), has been used to increase brain histamine contents as well. Brain histamine can be depleted by using a-fluoromethylhistidine (α-FMH), which is a suicide substrate and a specific kcat-inhibitor of HDC (16). α-Fluoromethylhistidine inhibits stress-induced prolactin surges (17, 18) and morphine-induced prolactin secretion (19), but has no effect on basal prolactin secretion (18, 20) in conscious male rats. Large doses of L-histidine given ip (21, 22) inhibit cold-stimulated TSH secretion. The effects of acute elevation of endogenous histamine content on either prolactin or basal TSH

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secretion in male rats have not been studied before. Also, no data are available regarding depletion of brain histamine and TSH secretion. Therefore, we have used L-histidine either alone or in combination with metoprine to elevate brain histamine contents, and a-FMH to deplete brain histamine contents. The changes in serum TSH and prolactin have been measured. The effect of metoprine on prolactin release from primary cultured AP cells was also studied.

Materials and methods

Animals

Male outbred Wistar rats (Han:Wist) weighing 200–250 g were used. The rats were kept in individual plastic cages in an adaptation room (+30°C) with a light/dark cycle of 12/12 h, respectively (lights on from 07:00 h to 19:00 h) for at least 7 days before the experiment. The rats were fed with standard laboratory chow (iodine content 0.5–1 mg/kg) and tap water ad libitum. The use of animals was approved by the local committee for animal experimentation.

Experimental design

All experiments were performed in conscious rats. In order to increase brain histamine contents, the rats were treated either with metoprine or L-histidine. Metoprine was dissolved in a minimal quantity of 10% lactic acid and diluted with 0.9% saline to a final concentration of 20 mg/10 ml. Control rats were given an equal volume (1 ml/100 g body weight) of 10% lactic acid/0.9% saline mixture. Metoprine (20 mg/kg) or the vehicle was administered ip 4 h before decapitation. L-Histidine was dissolved in 0.9% saline in a final concentration of 1000 mg/10 ml. Corresponding control animals were given an equal volume (1 ml/100 g body weight) of saline. L-Histidine (1000 mg/kg) or saline was administered ip 1 h before decapitation. In order to decrease brain histamine contents, a-FMH was given ip in two doses of 100 mg/kg and 50 mg/kg 24 and 4 h before decapitation, respectively. Control animals were given equal volumes of 0.9% saline, which was used as the vehicle. The rats were removed from the adaptation room to an adjacent room just before decapitation. Some of the rats were exposed to cold stress (+4°C) for 60 min before sacrifice. Trunk blood was collected and the sera were kept frozen (−20°C) until the hormone concentrations were determined.

For determination of histamine contents, the skulls of the rats were opened within 30 s and immersed in liquid nitrogen for 5 s. Then the brains were removed from the skulls, and the hypothalamus, striatum and the rest of the brain (= whole brain) were dissected on ice and kept in liquid nitrogen until stored in a freezer at −80°C. All decapitations were done between 13.00 h and 15.00 h.

Anterior pituitary cell culture

For each experiment, 20 Han:Wist rats weighing 200–250 g were decapitated and the APs dissected and sliced on ice. The AP fragments were stirred in a trypsinizing flask for 30 min at +37°C in DMEM/F12 medium (50 ml) containing 5 mg/ml of collagenase (Sigma Type II) and 0.1 mg/ml of DNase (Sigma Type I). The isolation of AP cells was completed by repeated trituration with a plastic pasteur pipette. The cells were centrifuged at 1000 g for 10 min through a buffer containing 10% (w/v) bovine serum albumin. The pellet was resuspended in DMEM/F12 medium containing 10% fetal calf serum. The cells were plated on 24-well plates (Costar, MA) at a density of 10⁵ cells per well. The experiments were performed 3 days after plating in serum-free conditions.

Radioimmunoassays for prolactin and TSH

Thyrotropin and prolactin concentrations of the sera and the samples from AP cell cultures were measured from duplicate samples (0.1 ml) by specific radioimmunoassays. The rat TSH and prolactin kits were gifts from the NIH. The results of TSH are expressed in ng/ml of NIDDK-TSH-RP-2 standards. The biological potency is 35 USP bovine units of TSH per milligram in the McKenzie assay. The prolactin results are expressed in ng/ml of NIDDK-rPRL-RP-2 standards, which has a biological potency of 30 IU/mg in the pigeon local crop sac assay of Nicoll. The intra-assay and interassay coefficients of variation of the radioimmunoassays were less than 15% and 20%, respectively.

Histamine contents

The brain samples and AP cells were homogenized by sonication. A protein-free supernatant was obtained by precipitating the homogenates with 2% perchloric acid and centrifugation at 10 000 g for 30 min at 4°C. Determination of histamine contents of the brain samples and AP cells was done by using an HPLC method with fluorimetric detection (23).

Drugs

Histamine·HCl, L-histidine, DMEM/F12 medium and TRH were purchased from Sigma (St Louis, MO). Metoprine and a-FMH were a generous gift from Dr J Kollonitsch (Merck Sharp and Dohme, Rahway, NJ).

Statistics

One-way analysis of variance was used to test the overall statistical significances between groups. Fisher’s least significance test was used as a post hoc test. p < 0.05 was considered statistically significant.
Table 1. Effect of metoprine, 20 mg/kg ip, and L-histidine, 1000 mg/kg ip, or both treatments together, on histamine contents in the hypothalamus, striatum and the rest of the brain.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hypothalamus (pmol/g)</th>
<th>Striatum (pmol/g)</th>
<th>Rest of the brain (pmol/g)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (control group)</td>
<td>3350 ± 250</td>
<td>330 ± 22</td>
<td>410 ± 27</td>
<td>10</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>5480 ± 500&quot;</td>
<td>620 ± 64&quot;</td>
<td>720 ± 42&quot;</td>
<td>10</td>
</tr>
<tr>
<td>Metoprine</td>
<td>5520 ± 420&quot;</td>
<td>880 ± 57&quot;</td>
<td>840 ± 65&quot;</td>
<td>7</td>
</tr>
<tr>
<td>L-Histidine + metoprine</td>
<td>7340 ± 480&quot;</td>
<td>1150 ± 70&quot;</td>
<td>980 ± 53&quot;</td>
<td>11</td>
</tr>
</tbody>
</table>

L-Histidine and metoprine were given 1 and 4 h before decapitation, respectively. Control animals were given equal volumes (1.0 ml/kg, ip) of corresponding vehicles. Values are means ± sem; ** p < 0.01 vs corresponding control; *p < 0.05 and †p < 0.01 vs either metoprine or L-histidine-treated animals. N = number of animals.

Results

Effect of L-histidine and metoprine on brain histamine contents

In control rats, with no drug treatment, 8–10-fold higher histamine contents were found in the hypothalamus as compared to the striatum or the rest of the brain (whole brain) (Table 1). L-Histidine (1000 mg/kg, ip) and metoprine (20 mg/kg, ip) were equally effective in increasing the histamine content in all brain areas studied (Table 1). When L-histidine was given to metoprine-pretreated animals, an additional increase in histamine levels was observed as compared to either treatment alone (Table 1).

Effect of L-histidine and metoprine on serum TSH and prolactin levels under basal conditions

L-Histidine (1000 mg/kg, ip) did not affect serum prolactin concentration (Fig. 1A) but significantly lowered serum TSH levels (Fig. 1B) in male rats. Metoprine (20 mg/kg, ip) significantly decreased both prolactin and TSH levels in serum (Fig. 1A, B). Neither the inhibitory effect of metoprine on prolactin levels nor the inhibitory effect of L-histidine on TSH levels was modified when L-histidine was administered to metoprine-pretreated rats (Fig. 1A, B).

Effect of α-FMH and metoprine on serum TSH and prolactin levels after cold stress

After α-FMH (100 mg/kg + 50 mg/kg, ip) there is a tendency to increase cold-induced (rats kept for 60 min at +4°C) TSH secretion although this is not significant (p = 0.08) (Fig. 2). Metoprine (20 mg/kg, ip) had no effect. After the 60-min cold exposure, serum prolactin levels were high (Fig. 2). α-Fluoromethylhistidine and especially metoprine dramatically decreased the stress-induced prolactin secretion (Fig. 2).

![Fig. 1](https://example.com/fig1.png)

Fig. 1. (A) Serum prolactin and (B) thyrotropin concentrations in male rats treated with saline (Ctr), L-histidine (L-hist, 1000 mg/kg, ip), metoprine (Meto, 20 mg/kg, ip) or L-histidine (1000 mg/kg) + metoprine (20 mg/kg) (L-hist + Meto). L-Histidine and metoprine were given 1 and 4 h before decapitation, respectively. Values are means ± sem (N = 7–11); *p < 0.05 and **p < 0.01 vs corresponding control.
Effect of metoprine on histamine contents and prolactin release in primary cultured rat AP cells

In primary cultured rat AP cells, the histamine content was 2.42 ± 0.25 pmol/l/10^5 cells. In control cells, with no TRH exposure, metoprine (1, 10 and 100 µmol/l, 60 min) did not have any effect on AP cell histamine contents (Fig. 3A). In metoprine (1 and 10 µmol/l, 60 min)-pretreated cells, a 10-min exposure to TRH (0.2 µmol/l) slightly decreased the histamine contents. However, a high concentration of metoprine (100 µmol/l) did not change the amine contents in these cells (Fig. 3A).

During a 60-min incubation period, the AP cells released 15% of their prolactin contents into the incubation medium (Fig. 3B). Thyrotropin-releasing hormone (0.2 µmol/l, 10 min) increased prolactin release, but this effect was not significant. However, TRH increased significantly the prolactin release in metoprine (1 µmol/l)-treated cells (Fig. 3B). In the other cases, metoprine had a non-significant inhibitory effect on the prolactin release.

Discussion

L-Histidine (1000 mg/kg) and metoprine (20 mg/kg) were equally effective in elevating histamine contents in the brain. When the treatments were given together, an additive effect on brain histamine was observed. Hence, the tools seemed to work with regard to the brain histamine contents. The effects of the two treatments on AP hormone secretion were, however, conflicting.

In spite of elevated brain histamine contents, L-histidine did not have any effect on serum prolactin concentrations. This finding is not in line with several
previous reports showing that exogenous histamine elevates serum prolactin levels in rats irrespective of whether given centrally, i.e. into brain ventricles (4, 24, 25) and into the preoptic anterior hypothalamus (6), or peripherally by an intra-arterial infusion (4). In both cases, the effect of histamine apparently is at a suprapituitary level, because histamine does not liberate prolactin from primary cultured pituitary cells (10) or AP fragments (4). In male rats there is evidence of a histamine-induced inhibitory regulation of prolactin secretion arising from the amygdala (6). It is possible, therefore, that an increase in endogenous brain histamine contents by L-histidine affects at the same time both stimulatory and inhibitory nerves regulating prolactin secretion. For instance, intracerebroventricularly administered histamine modifies differentially the activity of various dopaminergic neurons of the hypothalamus (26).

Metoprine increased brain histamine contents in a similar manner to L-histidine. Metoprine decreased basal levels of serum prolactin while L-histidine had no effect. When metoprine and L-histidine were administered together, an additional increase in brain histamine was observed. However, no additional decrease in serum prolactin was observed. Thus, it is apparent that the effect of metoprine on serum prolactin is not related to elevated brain histamine contents.

Prolactin secretion from the anterior pituitary is under inhibitory control by dopamine (27, 28). Thus, metoprine-induced release of dopamine into the pituitary portal blood would reduce serum prolactin levels. This may not be the case because the prevailing effect of metoprine on dopamine release seems to be an inhibition rather than a stimulation (29).

Histamine-induced prolactin secretion is mediated by increased vasopressin activity either in the hypothalamus or in the AP gland (30–32). In male rats, metoprine increases water intake and urine volumes (33) dose dependently by inhibiting vasopressin secretion (34). Therefore, the effect of metoprine on prolactin secretion might be due to, or mediated by, reduced vasopressin activity.

In the cold-stressed rats, serum prolactin levels were high. Histamine is one of the mediators of stress-induced prolactin secretion (2, 35–37). In rats treated with a-FMH, which depletes brain histamine, the cold-stress-induced prolactin secretion was significantly inhibited, which may indicate a role for endogenous histamine in this phenomenon. a-Fluoromethylhistidine also inhibited restraint stress-induced prolactin secretion (17, 18) but did not affect basal prolactin levels (18).

Metoprine, which increases brain histamine contents, also abolished the cold-stress induced prolactin response. Hence, it is apparent that the effect of metoprine on prolactin secretion at low ambient temperature is not related to increased brain histamine contents. Again, an inhibitory effect of metoprine on vasopressin activity would explain this result. Restraint stress-induced prolactin secretion can be antagonized with vasopressin V-1 receptor antagonists (31).

Metoprine did not increase significantly the AP cell histamine contents. The lack of effect of metoprine may be due to degradation of AP cell histamine by diamine oxidase (EC 1.4.3.6), which oxidizes histamine to imidazole acetic acid in peripheral tissues (38, 39). Under normal conditions in the brain, however, HMT (EC 2.1.1.8) (13) is the only histamine-metabolizing enzyme. Metoprine had no effect on basal release of prolactin from AP cells. The rather modest effect of TRH on prolactin release from the AP cells may be due to the male sex of the rats (40). There is a sex difference in the ability of TRH to induce prolactin secretion. The TRH-induced prolactin surges in male rats are weak and variable (1, 40). Interestingly, however, 1 µmol/l of metoprine increased the TRH response in the AP cells.

Basal levels of serum TSH were decreased by both L-histidine and metoprine. However, L-histidine was more potent in reducing the TSH levels, although both treatments strongly increased the brain histamine contents. This may reflect an additional effect of metoprine on TSH secretion, which now is exceeded by the inhibitory effect of endogenous histamine. Indeed, when L-histidine and metoprine were given together the TSH levels were at the same level as after L-histidine alone. Pretreatment with a-FMH, an inhibitor of HDC, tended to increase the cold-induced (rats kept for 60 min at +4°C) TSH secretion while metoprine had no effect. These results indicate that endogenous histamine has an inhibitory effect on basal and cold-stress-induced (21) TSH secretion. The present results give further evidence for the suprapituitary site of action of histamine. We have found previously in superfused AP cells that histamine did not have any effect on TSH release (12).

Taken together, the present results indicate that:

(i) treatment of rats with either L-histidine or metoprine increases brain histamine contents;

(ii) L-histidine does not affect basal levels of serum prolactin;

(iii) metoprine decreases both basal and cold-stress-stimulated prolactin release but these effects are not related to increased endogenous histamine levels;

(iv) in L-histidine-treated rats, basal TSH secretion is reduced;

(v) endogenous histamine has an inhibitory effect on TSH secretion, but may have both stimulatory and inhibitory regulation on prolactin release;

(vi) metoprine is not a suitable drug for increasing endogenous histamine contents in hormonal studies.

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