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# HISTAMINE LIPOLYTIC ACTIVITY AND SEMICARBAZIDE-SENSITIVE AMINE OXIDASE (SSAO) OF RAT WHITE ADIPOSE TISSUE (WAT)

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**Abstract**—Histamine has previously been described as a possible substrate for the semicarbazide-sensitive amine oxidase activity (SSAO) of rat white adipose tissue (WAT). We report here on a histamine function in this tissue which concerns the activity of this deaminating system distinct from the classical diamine oxidase. Our results show that: (1) histamine plays a role in controlling rat adipose tissue lipolysis with the contribution of $H_1$ and $H_2$ receptors that participate in histamine lipolysis in an opposite way. Both $H_1$ and $H_2$ roles can be differentiated using selective agonists (2- and 4-methyl histamine) and antagonists (pyrilamine and cimetidine); (2) histamine might also control rat lipolysis induced by noradrenergic agonists; (3) the SSAO present in rat WAT controls histamine levels at the receptor sites as shown by the modification of histamine lipolytic potency obtained when inhibitors of this enzyme are used.

Histamine is an almost ubiquitous amine mostly linked to mast cell populations of tissues where it is stored in granules and released under physiopathological conditions [1–5], exerting the function of a local hormone. Non-mast cell histamine has also been described in many other cells, where it is available in an unstored diffusable form [6]. Moreover, because histamine synthesis is induced in rapidly growing tissue [7], it has also been suggested that histamine plays a role as growth and differentiating factor [8–10]. Regardless of its physiological role, histamine turnover is crucial to the maintenance of active amine concentrations at the receptor level and to the control of cell functions. Although enzymatic mechanisms involved in histamine synthesis [histidine decarboxylase (HDC†) EC 4.1.1.22] and intracellular degradation [oxidative deamination by diamine oxidase (DAO) EC 1.4.3.6, and N-methylation by *N*-methyltransferase (HMT) EC 2.1.1.8, followed by monoamine oxidase (MAO) EC 1.4.3.4., oxidation] have been extensively studied, less is known about further possible control of histamine concentration due to the activity of some amine oxidases which are distinct from the classical DAO for substrate specificity and inhibitors sensitivity. These tissue-bound enzymes have benzylamine, a non physiological amine, as preferential substrate. For this reason they should be called "benzylamine oxidases", but are conventionally referred to as semicarbazide-sensitive amine oxidases (SSAOs) (EC 1.4.3.6) because of their common sensitivity to carbonyl reagents. We have previously described the presence and the histaminase activity of an SSAO in rat white adipose tissue (WAT) [11] and in the same tissue of other animal species [12]. This enzyme is concentrated in adipocytes, mainly located on membranes and expressed during fat cell differentiation [13].

Due to the high levels of SSAO in adipocytes and of its occurrence only in differentiating cells, of the presence of histamine in rat WAT [14], of the histaminase activity of WAT SSAO, we have put forward the hypothesis that SSAO can control the levels of amines regulating cell function and maturation. We thus thought it important to study the role of histamine in rat WAT and how its function could be altered by the inhibition of its degradation. In fact, although many histamine functions have been described, its role on WAT seems different among species [15]. The lipid mobilizing potency of histamine has been characterized in dog and in human whereas its activity in rat remains controversial [16, 17]. In canine the cAMP rising in histamine-stimulated adipocytes is susceptible to $H_2$ antagonists, suggesting the direct involvement of $H_2$ receptors in histamine lipolysis [18].

In order to clarify the possible function of adipocyte SSAO we have examined the hypothesis that a modification of the histamine effect would be measurable when histamine degradation in WAT is blocked by the use of SSAO inhibitors. To this end, we decided to characterize histamine lipolytic activity *in vitro* and to study the effect of semicarbazide and B24 [19], two inhibitors of WAT SSAO with different mechanisms of action, on histamine lipolytic potency.

## MATERIALS AND METHODS

### *Materials*

Male Wistar rats came from the Morini breeding colony (San Polo D'Elsa, Reggio Emilia, Italy).

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† Abbreviations: WAT, white adipose tissue; SSAO, semicarbazide-sensitive amine oxidase activity; DAO, diamine oxidase; HDC, histidine decarboxylase; HMT, *N*-methyltransferase; MAO, monoamine oxidase; KRB, Krebs bicarbonate buffer; BSA, bovine serum albumin; AD, adenosine deaminase.

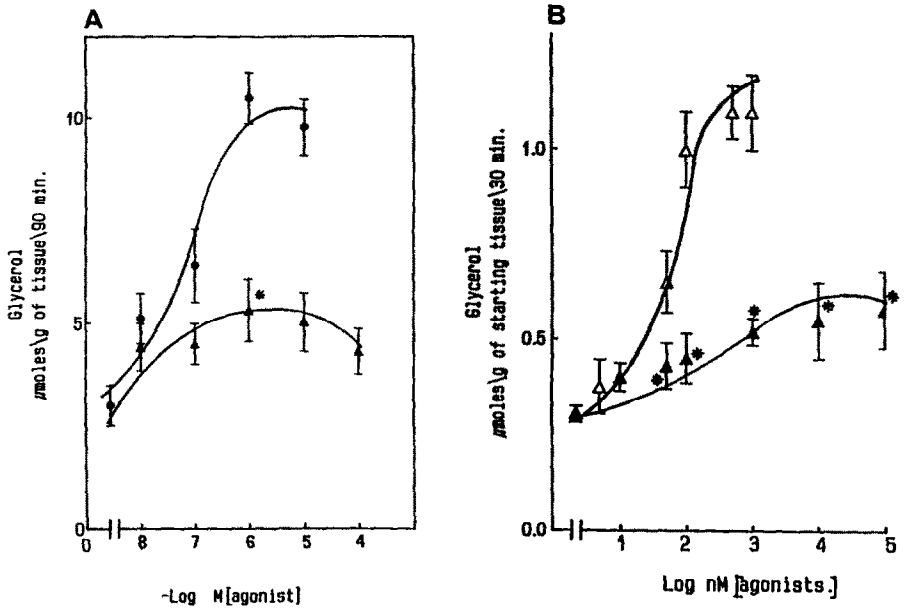


Fig. 1. Histamine lipolytic effect on WAT (A) and on isolated adipocytes (B). (A) The effect of histamine (from 0.001 to 0.1 mM) on WAT lipolysis was measured in comparison to noradrenaline (from 10 nM to 10 μM). Experiments were run on WAT pieces as described in the Materials and Methods. Glycerol released by the agonists in 90 min at 37° in an atmosphere of 5% $CO_2$ and 95% $O_2$ was taken as a measure of lipolysis. Results are means ± SE of 7–10 experiments run in duplicate. *$P < 0.05$ (Student's *t*-test for paired data), significant compared to basal value. (●) Noradrenaline; (▲) histamine. (B) Cells were obtained and treated as described in the Materials and Methods. Glycerol release was measured after 30 min of incubation at 37° in an atmosphere of 5% $CO_2$ and 95% $O_2$ from the addition of 10, 50, 100, 500 and 1000 nM noradrenaline and of 100, 10, 1, 01, 0.01 and 0.005 μM histamine. The incubation was carried out for 30 min at 37°. Results are means ± SE of five different experiments run in duplicate. *$P < 0.05$ (Student's *t*-test for paired data), significant compared to basal value. (△) Noradrenaline; (▲) histamine.

Bovine serum albumin fraction V (BSA), collagenase type II, 1(−)-arterenol, pyrilamine hydrochloride, histamine hydrochloride, semicarbazide hydrochloride, 1(−)-isoproterenol hydrochloride, adenosine deaminase and pargyline hydrochloride were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Horse radish peroxidase and the glycerol UV determination Kit were from Boehringer (Mannheim, Germany). Homovanillic acid was from Merck (Darmstadt, Germany). *O*-Phthalaldehyde was from Carlo Erba (Milan, Italy).

2- and 4-Methyl histamine and cimetidine were kindly supplied by the Smith Kline and French Lab. Ltd (Welwyn Garden City, U.K.).

*Methods*

Male Wistar rats were used as a source of WAT. The epididymal portion of WAT was removed, washed in saline solution and used for lipolysis measurements.

*WAT lipolysis.* WAT was cut into small pieces (10–30 mg) and placed in cell-culture multiwell boxes in the presence of 1 mL of Krebs-bicarbonate buffer (KRB), pH 7.4, of the following composition (mM): 118.4 NaCl, 4.7 KCl, 1.5 $KH_2PO_4$, 1.25 $CaCl_2$, 10 glucose, 24.6 $NaHCO_3$, 1.5 $MgSO_4$. BSA (3%) was added to complete the buffer, and tissue pieces were placed at 37° in a humidified atmosphere of 95% $O_2$ and 5% $CO_2$ for 15–30 min. After that time, lipolysis was induced by the addition of noradrenaline or histamine and measured after 90 min of incubation at the same temperature and oxygenation. Tissue was then removed, blotted, dried and weighed. Lipolysis was assessed by measuring the glycerol content of the medium by an enzymatic method [20]. Results were expressed as μmoles of glycerol released in 90 min/g of tissue.

*Preparation of mature adipocytes.* Adipocytes were prepared according to Rodbell [21]. WAT was weighed, minced, oxygenated, digested in KRB plus BSA in the presence of 0.5 mg/mL of type II collagenase at 37° for 40–60 min. After digestion, the material was filtered through a nylon mesh (250 μm) in order to remove any undigested tissue, and mature adipocytes were allowed to float to the top of the tube. The infranatant was aspirated by suction and new fresh buffer added. Cells were gently resuspended by inversion of the tube and again washed as described above. Usually a series of three to four washings was performed before using the cells.

Mature adipocytes were finally diluted in the appropriate volume of KRB pH 7.4 with 3% BSA and used for lipolysis measurement (0.5–1 × $10^6$ cells/mL).

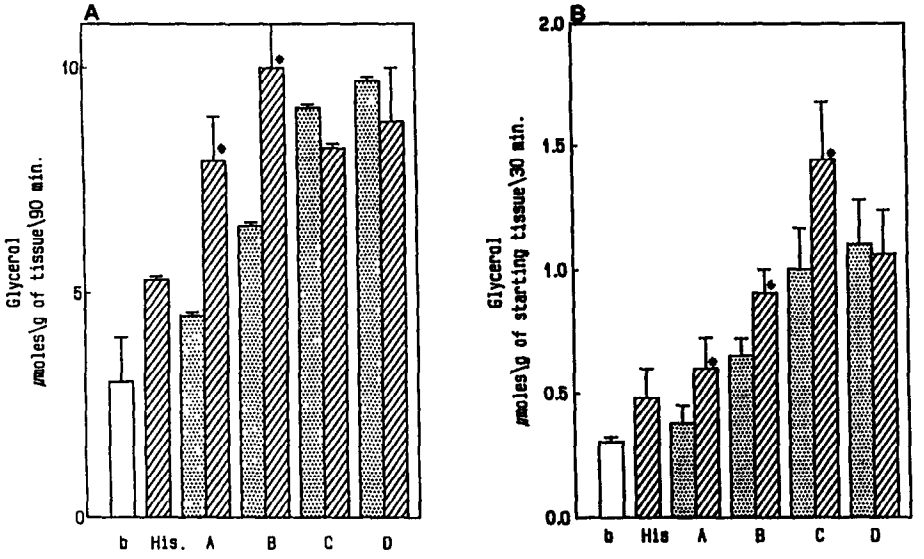


Fig. 2. Histamine produces an additive effect on noradrenaline stimulated WAT (A) and isolated adipocyte lipolysis (B). (A) Histamine produces an additive lipolytic effect on WAT pieces as compared to that obtained by noradrenaline alone. Histamine (1 μM) was added simultaneously to noradrenaline (0.01, 0.1, 1 and 10 μM) and the glycerol released was measured enzymatically as described in the Materials and Methods, after a further 90 min of incubation in the presence of the two agonists. *$P < 0.05$ (Student's *t*-test for paired data), significant compared to the values obtained with noradrenaline alone. Results are means ± SE of five experiments run in duplicate. b (□) Basal release of glycerol; His (▨) histamine 1 μM; A (▩) noradrenaline 10 nM, (▨) noradrenaline 10 nM + 1 μM histamine; B (▩) noradrenaline 100 nM, (▨) noradrenaline + 1 μM histamine; C (▩) noradrenaline 1 μM, (▨) noradrenaline 1 μM + 1 μM histamine; D (▩) noradrenaline 10 μM, (▨) noradrenaline 10 μM + 1 μM histamine. (b) Histamine (1 μM) was added to different noradrenaline concentrations. Results are means ± SE of five different experiments run in duplicate. *$P < 0.05$ (Student's *t*-test for paired data), significant compared to the values obtained with noradrenaline alone. b (□) basal glycerol release; His (▨) histamine 1 μM; A (▩) noradrenaline 10 nM, (▨) noradrenaline 10 nM + histamine 1 μM; B (▩) noradrenaline 50 nM, (▨) noradrenaline 50 nM + histamine 1 μM; C (▩) noradrenaline 0.10 μM, (▨) noradrenaline 0.10 μM + histamine 1 μM; D (▩) noradrenaline 0.5 μM; (▨) noradrenaline 0.5 μM + 1 μM histamine.

*Adipocyte lipolysis.* Glycerol release induced by noradrenaline, isoprenaline, histamine, 2- and 4-methyl histamine was measured by incubating mature adipocytes (usually 200 μL of cell suspension) in plastic tubes containing 1 mL of complete KRB, in an atmosphere of 5% $CO_2$ and 95% $O_2$ at 37°. Cells were first preincubated for 30 min at the same temperature and oxygenation and then lipolytic stimuli added for a further 30 min. Trichloroacetic acid (5%) was added to stop the reaction; the tubes were then mixed by vortexing and centrifuged at 10,000 *g* for 15 min at room temperature. Clear supernatant was used for glycerol content measurement. When the histamine receptor antagonists, pyrilamine and cimetidine, or the SSAO inhibitors, semicarbazide and B24, were used, they were preincubated for 30 min before the addition of histamine.

Noradrenaline solution was prepared in 1 mg/mL of ascorbic acid for a final concentration of ascorbic acid in the cell suspension of 10 μg/mL. This concentration did not affect basal lipolysis. Results were expressed as μmoles of glycerol released in 30 min/g of starting tissue. Glycerol release was linear up to 60 min and the further addition of adenosine deaminase (AD) (0.2 mU/mL) did not modify histamine lipolysis.

*SSAO determination.* The SSAO content of adipocytes was measured using benzylamine and histamine hydrochloride according to Matsumoto *et al.* [22] in the condition of MAO inhibition by 1 mM pargyline.

Kinetic constants for the two amines were determined in cell homogenates obtained in KRB (without BSA), in the presence of 1 mM pargyline preincubated for 30 min at 37°, using benzylamine and histamine from 1 to 0.001 mM. Substrates were incubated for 30 min and the reaction stopped by the addition of NaOH 0.1 N.

The degree of SSAO inhibition reached during a typical lipolysis experiment was evaluated in intact cells incubated with or without SSAO inhibitors (0.1 mM) and in the KRB plus 3% BSA as control. After the incubation, cells were washed with KRB to remove SSAO inhibitors, homogenized in the same medium and an aliquot (100 μL) used for SSAO measurement in the condition of MAO inhibition using benzylamine and histamine as substrate.

It is already known that commercial preparations

Table 1. Apparent kinetic constants of WAT SSAO and BSA for benzylamine and histamine oxidative deamination

| Substrate | $K_m$ ($\mu$M) | $V_{max}$ (nmol/mg of protein/min) |
|---|---|---|
| Benzylamine | 37.8 ± 4.9 | 8.5 ± 0.5 |
| | 537 ± 84* | 0.064 ± 0.005* |
| Histamine | 319.5 ± 21.5 | 5.3 ± 0.2 |
| | ND* | ND* |

Results are means ± SE of five experiments run in duplicate.

Adipocyte SSAO affinity towards benzylamine and histamine were measured as described in Materials and Methods. A suspension of 3% BSA in KRB, without cells, was subjected to the same procedure and the kinetic constants for benzylamine (*) and histamine (*) oxidation were also measured as described in Materials and Methods.

ND, not measurable activity on histamine oxidation up to 2 mM concentration.

of BSA still contain a residual amine oxidase activity. To evaluate whether this enzymatic activity could oxidize histamine and interfere with our observations, we measured the kinetic constants of BSA amine oxidase activity toward benzylamine and histamine. Samples of buffer plus 3% BSA were assayed for amine oxidase activity fluorimetrically according to Matsumoto *et al.* [22], using benzylamine concentrations from 1 to 0.04 mM and histamine from 2 to 0.125 mM. Results were processed by the method of Wilkinson [23].

*Protein content.* Protein levels of samples were assayed according to Lowry *et al.* [24]. The protein content of samples used for enzymatic activity determination ranged from 0.3 to 0.5 mg/mL.

*Histamine measurement.* Histamine levels in WAT were measured by Shore's [25] method. Tissue pieces (100 mg) were homogenized in perchloric acid (0.1 M), then centrifuged at 10,000 *g* for 15 min. The supernatant was treated for butanol and subsequent heptane extraction as described in the original method. Histamine was conjugated with the *o*-phthalaldehyde (1% in methanol) and fluorescence measured in a Zeiss fluorimeter at 450 nm emission and 360 nm excitation, in comparison with a calibration curve obtained with histamine concentrations ranging from 300 to 10 ng/mL.

## RESULTS

To verify the exclusive participation of adipocyte SSAO in histamine degradation we preliminarily measured apparent kinetic constants for benzylamine and histamine oxidation by the amine oxidases present in our system.

As shown in Table 1, BSA (3%) does contain benzylamine oxidase activity but it is completely inactive on histamine concentrations up to 2 mM. Adipocyte SSAO, on the other hand, is able to degrade histamine although with a lower affinity than benzylamine (Table 1).

The investigation of histamine lipolytic activity started with the measurement of glycerol release in intact WAT. Noradrenaline-induced lipolysis was first measured in WAT pieces and compared to the effect obtained with increasing histamine concentrations. The concentration–response curve obtained with histamine showed weak lipolytic activity of this autacoid ($pD_2$ 8 for noradrenaline and 8.82 for histamine), reaching a maximum at 1 $\mu$M concentration. At this value it represents 49 ± 5% of the activity reached by maximum effective concentrations of noradrenaline (Fig. 1A).

Moreover, the lipolytic effect of 10 and 100 nM noradrenaline was additive to that of 1 $\mu$M histamine. At higher noradrenaline concentrations (from 1 to 10 $\mu$M) the addition of 1 $\mu$M histamine to the system did not increase further the noradrenaline effect (Fig. 2A). In order to assess the nature of the lipolytic activity of histamine measured in WAT we confirmed our observations on isolated adipocytes.

As shown in Fig. 1B, histamine also exerts its effect on glycerol release in isolated cells. In these experimental conditions histamine lipolytic activity is followed, as in the total WAT, in comparison with that of noradrenaline. Histamine concentration–responses were measured from $10^{-8}$ to $10^{-4}$ M ($pD_2$ 7.1 for noradrenaline and 7.05 for histamine). At the highest concentration the effect of histamine was 58 ± 5% of that obtained with the maximum effective noradrenaline concentration. In isolated adipocytes, the addition of 1 $\mu$M histamine to submaximal

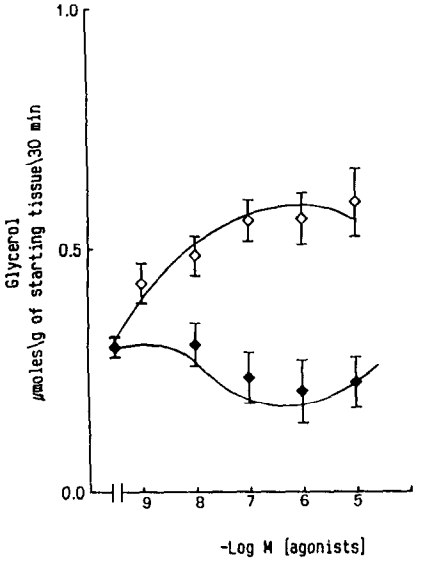


Fig. 3. The effect of the two histaminergic agonists, 2- and 4-methyl histamine, on adipocyte lipolysis. The lipolytic potencies of 2- and 4-methyl histamine were evaluated in isolated adipocytes preincubated for 15 min in the conditions described in the Materials and Methods and then for a further 30 min in the presence of the agonists. 2- and 4-Methyl histamine (10, 1, 0.1 and 0.01 $\mu$M) gave a dose–response curve with an opposite effect to the basal release of glycerol. Results are means ± SE of six different experiments run in duplicate. *$P < 0.05$% (Student's *t*-test for paired data), significant compared to the basal value. (◆) 2 methyl-histamine; (◇) 4-methyl-histamine.

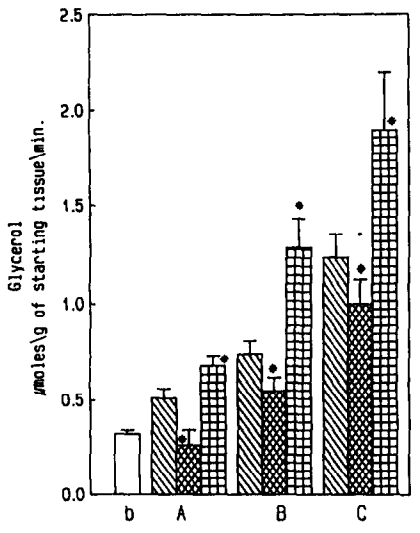


Fig. 4. The effect of 2- and 4-methyl histamine on isoprenaline-induced lipolysis in isolated adipocytes. The effect of 2- and 4-methyl histamine on a pure $\beta$-adrenergic agonist, isoprenaline, was measured in isolated adipocytes. 4-Methyl histamine (0.1 $\mu$M) was added to isoprenaline (5, 10 and 100 nM) stimulated cells and the glycerol release evaluated enzymatically. The maximum effect of isoprenaline shifted from 1 to 0.1 $\mu$M when 4-methyl histamine was present. On the contrary, when 2-methyl histamine (1 $\mu$M) was added to isoprenaline stimulated adipocytes, the amount of glycerol produced by the system was lower than that obtained by isoprenaline alone. The effect is more pronounced at low isoprenaline concentrations and it did not affect maximal isoprenaline stimulation. Results are means ± SE of six different experiments run in duplicate. *$P < 0.05$% (Student's $t$-test for paired data), significant compared to the value obtained with isoprenaline alone. b (□) Basal glycerol release; A (▨) isoprenaline 5 nM, (■) isoprenaline 5 nM + 2-methyl histamine 1 $\mu$M, (▦) isoprenaline 5 nM + 4-methyl histamine 0.1 $\mu$M; B (▨) isoprenaline 10 nM, (■) isoprenaline 10 nM + 2-methyl histamine 1 $\mu$M, (▦) isoprenaline 10 nM + 4-methyl histamine 0.1 $\mu$M; C (▨) isoprenaline 100 nM, (■) isoprenaline 100 nM + 2-methyl histamine 1 $\mu$M, (▦) isoprenaline 100 nM + 4-methyl histamine 0.1 $\mu$M.

Table 2. The effect of histaminergic antagonists, cimetidine and pyrilamine on histamine induced lipolysis in isolated adipocytes

| | +Cimetidine (% of inhibition) | +Pyrilamine (% of increase) |
|---|---|---|
| HI $10^{-5}$ M | 20 | 0 |
| HI $10^{-6}$ M | 96 | 0 |
| HI $10^{-7}$ M | 90 | 65 |
| HI $10^{-8}$ M | 95 | 140 |

Adipocytes were preincubated for 30 min at 37° as described in Materials and Methods in the presence of 1 $\mu$M cimetidine and pyrilamine, then histamine was added and cells were incubated for a further 30 min at the same temperature.

Results are expressed as the percentage of effect compared to the same concentration of histamine without antagonists. Results are means ± SE of five different experiments run in duplicate.

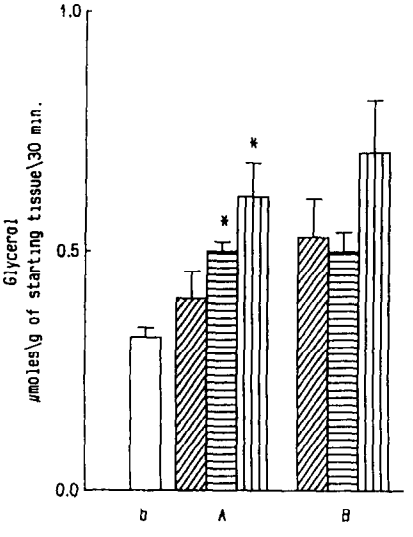


Fig. 5. The effect of SSAO inhibition on histamine-induced lipolysis in isolated adipocytes. Cells were preincubated, with or without 1 $\mu$M pyrilamine, for 30 min at 37° in an atmosphere of 5% $CO_2$ and 95% $O_2$ in the presence of 0.1 mM B24 or semicarbazide. Histamine (10 nM) was then added and lipolysis measured after a further 30 min in the same conditions of temperature and oxygenation. Results are means ± SE of at least five experiments run in duplicate. *$P < 0.05$ (Student's $t$-test for paired data), significant compared to the values obtained with histamine alone. b (□) Basal release of glycerol; A (▨) histamine 10 nM, (▤) histamine 10 nM + B24 0.1 mM, (▥) histamine 10 nM + semicarbazide 0.1 mM; B (▨) histamine 10 nM + pyrilamine 1 $\mu$M; (▤) histamine 10 nM + pyrilamine 1 $\mu$M + B24 0.1 mM, (▥) histamine 10 nM + pyrilamine 1 $\mu$M + semicarbazide 0.1 mM.

noradrenaline concentrations again produces an additive effect on glycerol release as compared to the value obtained with noradrenaline alone (Fig. 2B).

Starting from these observations we extended our measurements using the selective $H_1$ and $H_2$ histaminergic agonists, 2- and 4-methyl histamine. When the effects on glycerol release produced by the selective histamine agonists were compared, we found opposite activities for the 2- and 4-methyl derivatives. As shown in Fig. 3, while 2-methyl histamine inhibited glycerol release, 4-methyl histamine stimulated lipolysis. Both effects were concentration-dependent. In addition, the opposite effect of the two agonists was confirmed in lipolysis induced by the $\beta$ receptor selective agonist, isoprenaline. When 4-methyl histamine (0.1 $\mu$M) was added to isoprenaline, the dose-related curve of the sympathetic agonist shifted to higher values (34 ± 3% of increase), resulting in an additive effect of the two agonists. The opposite happened after the addition of 2-methyl histamine (1 $\mu$M) to

isoprenaline. In this case, an inhibition of glycerol release was observed (45% inhibition at 10 nM and 10% inhibition at 0.1 μM isoprenaline concentration, Fig. 4).

The involvement of an $H_1$ and $H_2$ receptor in histamine activity was further investigated using the selective antagonists, pyrilamine and cimetidine. The lipolytic activity of histamine was increased by the presence of 1 μM pyrilamine and inhibited by 1 μM cimetidine (Table 2).

Adipocyte SSAO was previously demonstrated to be inhibited *in vitro* by semicarbazide and B24 [11, 12] whereas the effect of these substances on intact adipocytes has not previously been verified. The measurement of SSAO inhibition reached in a typical lipolysis experiment was evaluated by adding 0.1 mM semicarbazide to cell samples prepared as usual. Cells were incubated and washed as described in the Materials and Methods. In these conditions 85 ± 3% of enzyme inhibition was reached. This evaluation was possible only when semicarbazide was used (not with B24, which is a reversible inhibitor). With regard to the effect of SSAO inhibition on histamine lipolysis, as shown in Fig. 5 semicarbazide and B24 at 0.1 mM concentration produced a significant increase in the lipolytic activity induced by 10 nM histamine. This effect was no further potentiated by 1 μM pyrilamine. B24 and semicarbazide, at the concentrations used, did not modify the basal release of glycerol.

The histamine concentration in WAT was evaluated as 169 ± 69 ng/mg of proteins.

## DISCUSSION

The active role of histamine in the control of rat adipocyte fat mobilization was investigated by *in vitro* experiments using small pieces of WAT and adipocytes isolated from the same source. In our experimental conditions histamine shows lipolytic activity also in the rat WAT. These results seem to confirm that histamine might be an important endogenous regulator of lipid mobilization of several animal species.

We found that histamine exerts a lipolytic activity in WAT and this is also evident in isolated cells. This effect was found to be less than that of noradrenaline and isoprenaline, in agreement with the well known central role of noradrenergic receptors in the control of WAT lipolysis. Histaminergic glycerol mobilization in adipocytes is probably the result of an interaction of this compound with $H_2$ receptors, which stimulate, and $H_1$ receptors, which inhibit, lipolysis. Both these effects were in fact separate when selective agonists (2- and 4-methyl histamine) and antagonists (pyrilamine and cimetidine) were used. As appeared from the significant increase of lipolysis in the presence of 1 μM pyrilamine, $H_1$ antilipolytic effects predominated at low agonist concentrations (10–100 nM), suggesting different affinity values of histamine for these two receptors. Therefore the effect of histamine on rat adipocyte lipolysis seemed a complex function of its concentration. This double mechanism of histamine regulation might also provide an explanation for the antilipolytic effect of histamine observed in some species.

Although the lipolytic activity of histamine was less than that induced by noradrenergic compounds, we observed that histamine substantially modified the lipolysis induced by submaximally-active noradrenaline and isoprenaline concentrations. The additive effect of histamine on lipolysis induced by noradrenergic agonists might be due to the increase of the intracellular levels of the same second messenger, cAMP, which is known to regulate adipocyte lipolytic activity [26].

We also found that histamine was present in WAT where it could be stored in mast cells surrounding adipocytes or located closely to endothelial cells (Fig. 6). Taken as a whole, these results seem to indicate a possible interaction between adrenergic and histaminergic systems that could be of physiological relevance. Histamine could be a component of stress-induced lipolysis not only in dog and human but also in rat.

DAO is considered to be the main enzymatic pathway responsible for the direct oxidative deamination of histamine in many tissues [27]. In addition, histamine is metabolized by MAO following a preliminary N-methylation by HMT [28]. In our previous papers [11, 12] we were able to assess that in WAT the SSAO enzyme was able to catalyse histamine direct oxidation. Therefore, because of the absence of any measurable DAO in WAT, SSAO activity concurs with MAO in the metabolism of histamine. Similar observations have already been described in other rat tissues [29].

Although the affinity of SSAO towards histamine is about 10-fold lower ($K_m$ = 319.9 ± 21.5 μM) than that measured for the non-physiological benzylamine ($K_m$ = 37.8 ± 4.9 μM), histamine rate of oxidation by SSAO ($V_{max}$ = 5.3 ± 0.2 nmol/mg of protein/min) results very close to that measured for benzylamine ($V_{max}$ = 8.5 ± 0.5 nmol/mg of protein/min). From this point of view, the relative low affinity of SSAO for physiological substrates seems to be verified by other authors [30]. We decided to use semicarbazide, an irreversible but non selective inhibitor of the class EC 1.4.3.6 enzymes, to assess the pharmacological relevance of SSAO inhibition in histamine lipolytic potency. We also confirmed the SSAO involvement in the regulation of histamine lipolysis using a more selective, reversible inhibitor, B24, which at 0.1 mM was found to completely inhibit histamine degradation in adipocyte homogenates.

Our results supported the idea that SSAOs play a role in histamine degradation, in fact the addition of semicarbazide and B24 to intact cells increased the lipolysis, induced by 10 nM histamine, by 53 ± 2.5% and 30 ± 5%, respectively. The difference between the two inhibitors may be explained by the fact that semicarbazide is irreversible whereas B24 is reversible. The measured effects therefore seem rather specific since neither semicarbazide nor B24 affected basal glycerol release. In our opinion these results demonstrate that, in the presence of SSAO inhibition, histamine lipolytic potency can reach almost the same levels as obtained with noradrenaline (Figs 1B and 5). The amplification of the lipolytic effect of 10 nM histamine obtained in the presence

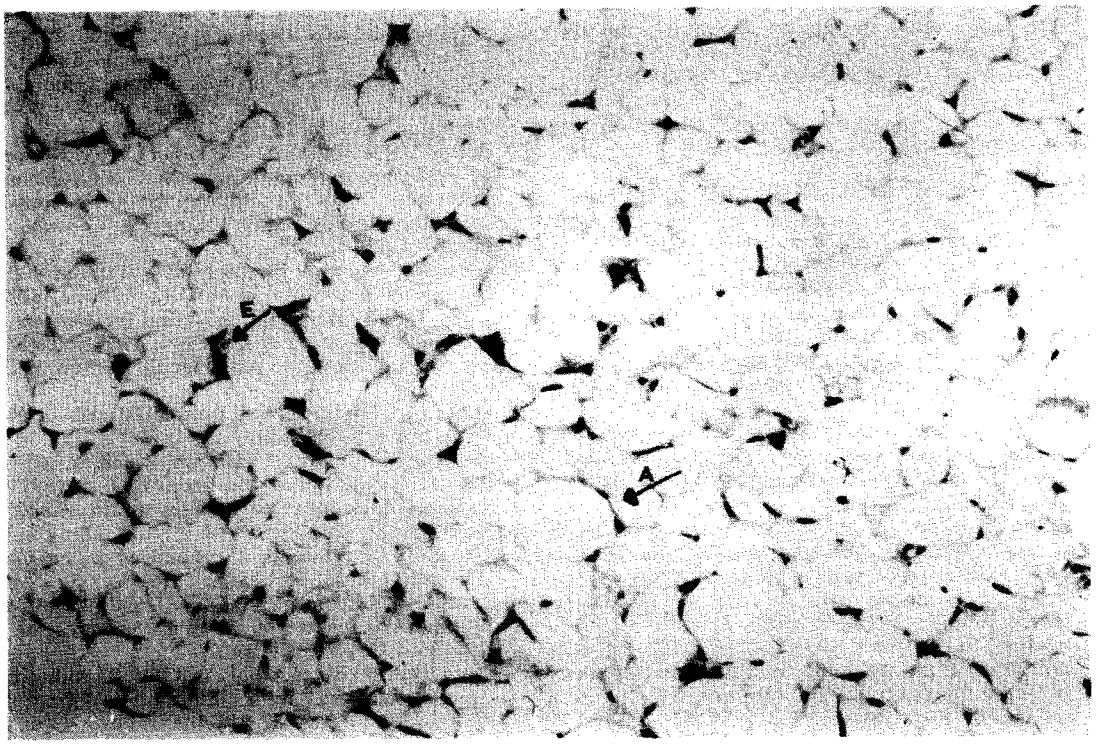


Fig. 6. A photomicrograph (10×) of the epididymal portion of rat WAT. The tissue was fixed in paraformaldehyde (pH 7 in phosphate-buffered saline), sliced (10 μm), embedded in a plastic support and treated with Boyens solution. The staining shows the presence of two populations of mast cells, one of which dispersed among adipocytes, the other one surrounding the endothelial cells. A, a typical adipocyte surrounded by mast cells; E, endothelial cells and mast cells.

of SSAO inhibition is well justified by the membrane-bound nature of the adipocyte SSAO [12] which can actively control amine levels at the receptor sites.

We would like to point out that SSAO is also present inside the cells, where it might regulate intracellular levels of histamine, either taken up or directly synthesized by the cells. Up to now our findings seem to suggest a relationship between SSAO occurrence in adipocyte and cell maturation (a process which allows cells to acquire functional properties, of which lipolysis represents one of the main ones). Moreover, when preadipocyte differentiation is induced in the presence of semicarbazide and B24 the cell transformation is delayed [31]. We concluded that the presence of SSAO in adipocytes is not only a peculiar feature of mature cells but this enzymatic activity might be necessary to sustain adipocyte maturation.

Although the role of histamine in rat lipolysis regulation has yet to be established, we confirm that this amine, in addition to its already known role on cell differentiation, could also regulate WAT physiology. Histamine concentration in WAT might be the result of mast cells degranulation. Therefore, we still have to investigate the possible active participation of adipocytes in histamine synthesis or uptake from the extracellular spaces.

In conclusion, we confirm here the presence of histamine in rat WAT, its involvement in adipocyte lipolysis and the presence of an enzymatic system active in controlling amine function. In fact, as shown by our results, the pharmacological inhibition of SSAO makes histamine lipolytic activity more prominent, probably raising amine concentration in the extracellular spaces. However, to postulate a complete hypothesis on histamine and SSAO function in WAT we still have to investigate the presence in adipocytes of histamine synthesizing enzyme and cellular uptake systems for the amine which might cooperate with SSAO in controlling histamine extracellular turnover. From this point of view SSAO could play a crucial role in controlling the concentration of an amine which can act as a lipolytic or antilipolytic agent depending on its concentrations at receptor sites.

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