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Effects of Histamine on the Metabolism of Isolated Rat Hepatocytes: Roles of H_1- and H_2-Histamine Receptors

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SUMMARY

In isolated rat hepatocytes histamine stimulates in a dose-dependent fashion three of the major metabolic pathways: glycogenolysis (70–80% increase over basal), gluconeogenesis from lactate (50–60%), and ureagenesis (50–60%). It was observed that both H_1 and H_2 receptors mediate the action of histamine and that, in control hepatocytes, the H_1-mediated action predominates over the H_2. The H_1-mediated effect diminished in the absence of extracellular calcium, whereas the H_2-mediated action did not. Interestingly, in hepatocytes from hypothyroid rats, the H_2 action increased and the H_1-mediated effect decreased as compared to those in the controls, with an inversion in efficacy (i.e., $H_1 > H_2$ in the controls and $H_2 > H_1$ in cells from hypothyroid rats). Furthermore, it was observed that pertussis toxin treatment and forskolin both enhance the H_2-mediated effects without altering the H_1-mediated actions of histamine (i.e., $H_1 \cong H_2$). The active phorbol ester, phorbol 12-myristate 13-acetate, did not alter the effect of the autacoid. In summary, the data show that histamine modulates liver metabolism through H_1 and H_2 receptors. The relative importance of the two receptor types in mediating the actions of histamine varies depending on the specific conditions used.

Histamine is one of the so-called local hormones or autacoids. Its role in anaphylaxis and allergy has been known for more than 75 years, and the important role of histamine in the maintenance of homeostasis is increasingly being recognized (see Ref. 1 for a review). The action of histamine is initiated by its interaction with specific receptors located on the external surface of the plasma membrane. Two types of receptors for histamine have been clearly differentiated: the H_1 and the H_2 receptors (2, 3). It has been observed, in a variety of models, that the H_1 receptors are coupled to phosphoinositide turnover and calcium signaling (4–8), whereas the H_2 receptors are coupled to adenylate cyclase in an activatory fashion (9, 10).

Recently, Imoto *et al.* (11) reported the presence of a large number of H_1-histamine receptors in liver membranes. However, there is no information about the action(s) of histamine on liver metabolism. This prompted us to study whether histamine has any action on the major metabolic pathways of this organ, the receptor(s) involved, and their modulation. The results of this study are the subject of the present report.

Materials and Methods

Urease, peroxidase, glucose oxidase, glutamine, ornithine, pyrilamine, histamine, (−)-epinephrine, *dl*-propranolol, PMA, and 6-*n*-propyl-2-thiouracil were obtained from Sigma Chemical Co. Forskolin (7β-acetoxy-8,13 epoxy-1α,6β,9α-trihydroxy-*1abd*-14-en-11-one) was obtained from Calbiochem. Pertussis toxin was purified from vaccine concentrates by the method of Sekura *et al.* (12). Cimetidine, impromidine, and AET were generous gifts from Smith Kline and French. Collagenase was obtained from Cooper Biomedical.

Female Wistar rats (200–250 g) were used. The animals were fed *ad libitum* for the studies of ureagenesis and glycogenolysis and were fasted for 24 hr in those of gluconeogenesis. In some experiments hypothyroid animals were used; hypothyroidism was induced by giving the animals water containing 0.030% 6-*n*-propyl-2-thiouracil for 40–50 days and was assessed by decreased weight gain, dryness of the fur, and decreased levels of triiodothyronine (13). When pertussis toxin was used, it was administered intraperitoneally (50 μg/100 g) to the animals 3 days before the experiment was performed (14).

Hepatocytes were isolated by the method of Berry and Friend (15) and incubated in Krebs-Ringer bicarbonate buffer saturated with O_2/CO_2 (95%:5%), pH 7.4, at 37°. In some experiments no $CaCl_2$ was added to the buffer; the amount of calcium in this buffer was in the range of 10–40 μM (due to contamination of the reagents and water) as determined by atomic absorption spectroscopy; 25 μM EGTA was added to it. To determine the rate of glycogenolysis, cells obtained from fed animals were incubated for 60 min and reactions were stopped by cooling the cell suspensions in ice water. The cell suspensions were centrifuged for 1 min at top speed in a clinical centrifuge and glucose was determined in the supernatant by the glucose oxidase procedure

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ABBREVIATIONS: PMA, phorbol 12-myristate 13-acetate; AET, 2-(2-aminoethyl)-thiazole; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

(16). For the study of gluconeogenesis, the same procedure as described above for glycogenolysis was employed, but cells were obtained from 24-hr fasted rats, and 10 mM lactate was added to the incubation medium; glucose production from endogenous substrates accounted for 10–20% of the total production and was substracted from the values obtained in the presence of lactate. For the study of ureogenesis, the incubation medium was supplemented with 10 mM glutamine and 2 mM ornithine. After 60-min incubations, urea was determined enzymatically in cell supernatants (17).

Results and Discussion

Histamine stimulated in a dose-dependent fashion three of the major metabolic pathways of liver cells: ureagenesis (Fig. 1A), gluconeogenesis (Fig. 1B), and glycogenolysis (Fig. 1C). The EC_{50} for histamine for glycogenolysis and gluconeogenesis (150 ± 80 nM) was similar to the EC_{50} for ureagenesis (270 ± 70 nM). These EC_{50} values are within the range observed in other systems (4–10). The maximal stimulations produced were 40–60% for ureagenesis, 50–60% for gluconeogenesis from lactate, and 80–100% for glycogenolysis. Such stimulations are bigger or within the range of those produced by other hormones such as adrenaline (18) or glucagon (19). Ureagenesis was preferred as metabolic parameter for all of the following studies; similar data were obtained when the other metabolic pathways were studied in preliminary experiments. In Fig. 2, urea production as a function of the time of incubation, in the absence and presence of 10 μM histamine, is presented. It can be observed that urea synthesis was proportional to the time of incubation and that histamine increased the rate of ureagenesis (Fig. 2). A small but consistent stimulation was observed at 10 min (109 ± 0.5% of basal); however, longer incubations were preferred (60 min) because the magnitude of the effect was bigger and more easily detected.

It has been observed that, to produce a sustained effect, the action of some hormones depends on the presence of extracellular calcium whereas that of other hormones does not (20). The extracellular calcium dependency of histamine action was examined. It was observed that, in the absence of calcium plus 25 μM EGTA, the ureagenic action of histamine was markedly reduced (to approximately 30% of its effect in the presence of calcium) but, interestingly, it was not abolished (Fig. 3). In these experiments the EC_{50} values for histamine were 290 ± 75 nM and 390 ± 100 nM in the presence and absence of calcium, respectively.

In order to determine the type of histamine receptor that mediates the actions of the autacoid, the selective antagonists pyrilamine (H_1) and cimetidine (H_2) were used. Surprisingly, it was observed that both antagonists inhibited in a dose-dependent fashion the effect of histamine, but neither blocked it completely (Fig. 4). Pyrilamine was more potent and effective than cimetidine, i.e., pyrilamine blocked 60% of the effect of histamine, whereas cimetidine blocked the effect only 30% (Fig. 4). The possibility that both H_1 and H_2 receptors could mediate the effect of histamine was considered. To test this hypothesis the effects of histamine in the presence of a fixed concentration of one of the antagonists was studied. The effect of 10 μM histamine plus 1 μM cimetidine was blocked by increasing concentrations of pyrilamine (Fig. 4). Similarly, the effect of 10 μM histamine plus 1 μM pyrilamine was antagonized by cimetidine (Fig. 4). The antagonists were without effect on basal ureagenesis at the concentrations tested (not shown). The data indicated two points: first, that both H_1 and H_2 receptors mediate the actions of histamine in liver cells, and, second, that the H_1-mediated action predominates over the H_2-mediated effect.

In order to confirm this observation, the effect of selective H_1 (AET) and H_2 (impromidine) agonists was studied. AET stimulated ureagenesis ≈40% over basal whereas impromidine did so to a much lesser extent (≈20% over basal) (Fig. 5). The effect of the H_1 agonist AET was inhibited by the H_1 antagonist pyrilamine but not by cimetidine (Fig. 6). In contrast, the stimulation of ureagenesis induced by the H_2 agonist impromidine was blocked by cimetidine but not by pyrilamine (Fig. 6). The data illustrate the selectivity of the agents employed and confirm the findings described above, i.e., that both H_1 and H_2 receptors mediate the actions of histamine, that the H_1-mediated effect is predominant, and that the actions are additive (see Figs. 4–6). Interestingly, the effect of the selective H_1 agonist, AET, was markedly reduced in cells incubated in the absence of calcium plus 25 μM EGTA, whereas the action of impromidine (H_2 agonist) was not affected by the absence of the cation (results not shown).

In the following experiments, we tried to determine whether the relative participation of the two histamine receptor types in mediating the action of the autacoid was constant or whether it showed plasticity. Four conditions were explored: the action of the active phorbol ester PMA, hypothyroidism, pertussis toxin treatment, and the effect of forskolin.

The active phorbol ester, PMA, blocks the α_1-adrenergic

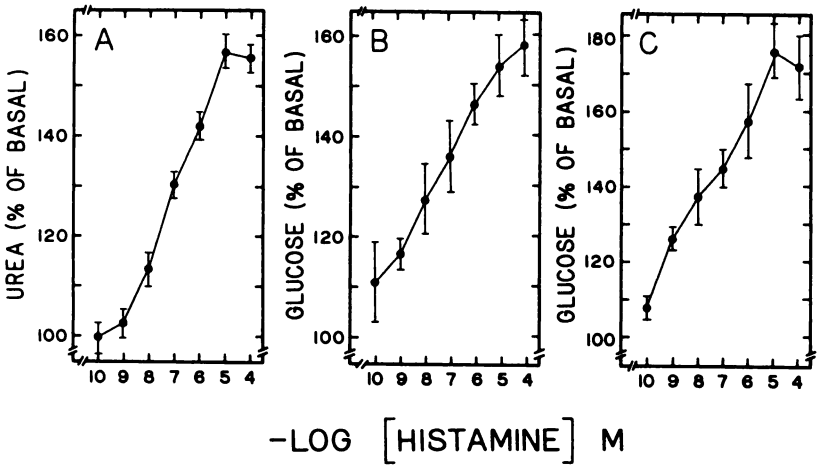

Fig. 1. Effect of histamine on ureagenesis, gluconeogenesis, and glycogenolysis in isolated rat hepatocytes. Hepatocytes were incubated as described under Materials and Methods in the absence or presence of different concentrations of histamine, and ureagenesis (A), gluconeogenesis from lactate (B), and glycogenolysis (C) were determined. Results are expressed as percentage of basal values which were 26 ± 2 nmol/mg of cell wet weight for ureagenesis, 15 ± 1 nmol/mg of cells wet weight for gluconeogenesis from lactate, and 44 ± 2 nmol/mg of cell wet weight for glycogenolysis. Plotted are the means and standard errors (*vertical lines*) of 10–15 determinations using different cell preparations.

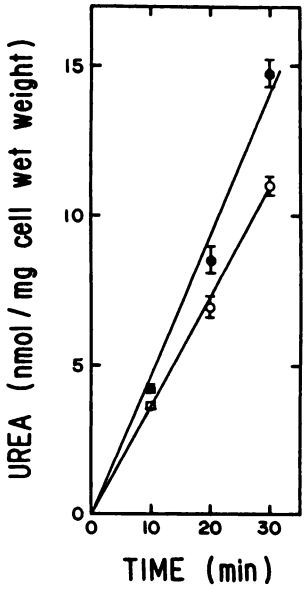

Fig. 2. Time course of the effect of histamine on urea synthesis. Hepatocytes were incubated in the absence (○) or presence (●) of 10^{-5} M histamine. Plotted are the means and standard errors (*vertical lines*) of six determinations using different cell preparations.

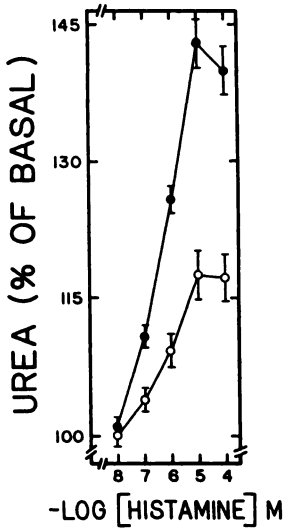

Fig. 3. Effect of extracellular calcium on the stimulation of ureagenesis induced by histamine. Hepatocytes were incubated in regular Krebs-Ringer bicarbonate buffer (1.2 mM $CaCl_2$; ●) or buffer without $CaCl_2$ supplemented with 25 μM EGTA (○) in the absence or presence of different concentrations of histamine. Results are expressed as percentage of basal values which were 28 ± 2 nmol/mg of cell wet weight in regular buffer and 20 ± 2 nmol/mg of cells wet weight in buffer without calcium containing 25 μM EGTA. Plotted are the means and standard errors (*vertical lines*) of four determinations using different cell preparations.

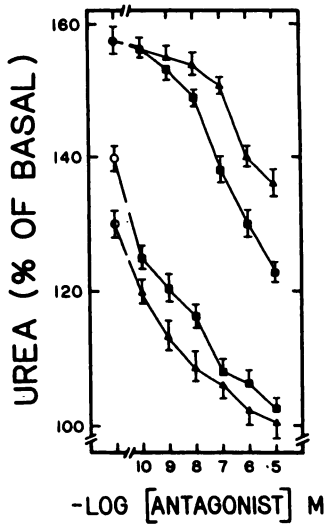

Fig. 4. Effect of pyrilamine (H_1-antagonist) and cimetidine (H_2-antagonist) on the stimulation of ureagenesis induced by histamine. Hepatocytes were incubated in the presence of 10^{-5} M histamine (●), 10^{-5} M histamine + 10^{-6} M cimetidine (○), or 10^{-5} M histamine + 10^{-6} M pyrilamine (◐) alone or with different concentrations of pyrilamine (■) or cimetidine (▲). Results are expressed as percentage of basal ureagenesis which was 29 ± 1 nmol/mg of cells wet weight. Plotted are the means and standard errors (*vertical lines*) of eight determinations using different cell preparations.

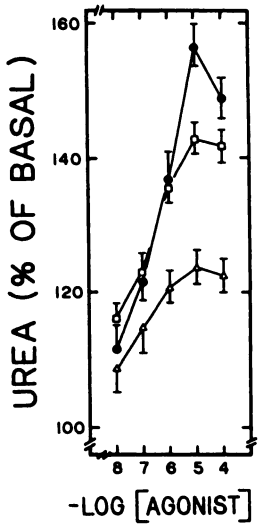

Fig. 5. Effect of histamine, AET, and impromidine on ureagenesis in rat hepatocytes. Hepatocytes were incubated in the absence or presence of different concentrations of histamine (●), AET (□), and impromidine (△).

action in hepatocytes (21–26). We studied the effect of PMA on the stimulation of ureagenesis by histamine and the selective agonists AET and impromidine (Fig. 7). PMA was without effect on the stimulation of ureagenesis by the histamine agonists, whereas the α_1-adrenergic action of adrenaline was clearly blocked (Fig. 7). The data indicate that the responsiveness of liver cells to histamine is not modulated through the activity of protein kinase C. It is important to mention that Orellana *et al.* (27) observed in cultured astrocytoma cells that PMA blocks both carbachol- and histamine-stimulated inositol phosphate formation. These authors (27) suggested that in these cells phorbol esters may act on some component of the transduction rather than the receptors. Interestingly, the action of PMA in liver cells shows more selectivity (Refs. 21–26 and present paper).

It has been observed that in hepatocytes from hypothyroid rats the effects of agents that act through stimulation of adenylate cyclase such as β-adrenergic agonists or glucagon are greatly enhanced (13, 28–30), whereas the actions of those agents that act through calcium signaling are markedly diminished (13, 27, 31, 32). Taking into account these findings, the effect of the histamine agonists was tested in hepatocytes obtained from hypothyroid rats. Interestingly, it was observed

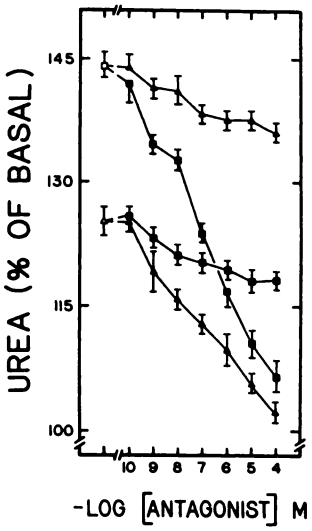

Fig. 6. Effect of the antagonists pyrilamine and cimetidine on the stimulation of ureagenesis induced by AET and impromidine. Hepatocytes were incubated in the presence of 10^{-5} M AET (□) or 10^{-5} M impromidine (△) alone or with different concentrations of pyrilamine (■) or cimetidine (▲). Other indications are as in Fig. 3.

that in these cells the H_2 agonist, impromidine, was more effective than the H_1 agonist, AET (Fig. 8). Again, the effect of histamine seemed to be the addition of both H_1 and H_2 actions (Fig. 8). In other words, the data indicate that in cells from hypothyroid rats the H_1 action is markedly diminished as compared to that in the control cells, whereas the H_2 action is enhanced (Fig. 8).

Pertussis toxin, an exotoxin produced by *Bordetella pertussis*, catalyzes the ADP-ribosylation of the α subunit of the guanine nucleotide-binding regulatory protein involved in inhibition of adenylate cyclase (N_i) (33). It has been observed that such covalent modification of N_i blocks the action of agents that inhibit adenylate cyclase and enhances the action of agents that stimulate this enzyme (reviewed in Refs. 33 and 34). In hepatocytes, it has been shown that treatment with pertussis toxin blocks the action of angiotensin II on adenylate cyclase (35), enhances the accumulation of cyclic AMP produced by β-adrenergic agonist and glucagon (14, 36), and has no effect on the action of agents that act through phosphoinositide turnover and calcium signaling (14, 35, 37, 38). The effect of pertussis toxin on the action of histamine is presented in Fig. 9. It can be observed that in cells from rats treated with the toxin, the H_2-mediated effect is increased whereas the H_1-mediated effect is not altered as compared to the effects observed in the controls (compare Figs. 9 and 5).

Forskolin is a diterpene derivative found in the Indian plant *Coleus forskohli*. It has been elegantly shown by Seamon *et al.* (39) that this agent activates adenylate cyclase both in membranes and intact cells. Interestingly, low concentrations (10–100 nM) of this diterpene, which, by itself does not stimulate cyclic AMP accumulation, magnify the action of agents that stimulate adenylate cyclase such as β-adrenergic agonists, both in fat (40) and liver cells (41). Forskolin also enhanced the H_2-mediated action of histamine in liver cells (Fig. 10). The diterpene was without effect on basal ureagenesis at this concentration (not shown; see Ref. 41).

Imoto *et al.* (11) showed that the number of [^{3}H]pyrilamine-binding sites (putatively H_1-histamine receptors) is very large (around 4700 fmol/mg of membrane protein) and also detected a small number of H_2-histamine receptors in liver membranes. The liver contains many cells in addition to hepatocytes and, therefore, membranes obtained from this organ could contain those from nonparenchymal cells. However, our results are totally consistent with the findings of Imoto *et al.* (11) and indicate that the receptors detected by these authors have functional significance in hepatocytes, especially considering that ureagenesis is a pathway that occurs predominantly in liver cells. Our data also showed consistency with the findings of Imoto *et al.* (11) in indicating the predominance of the H_1- over the H_2-histamine action. In other models the H_1-histamine receptor is coupled to calcium signaling, whereas the H_2-histamine is linked to activation of adenylate cyclase (4–10). The results with hepatocytes from hypothyroid rats could be consistent with the observation that, in these cells, the actions of agent that stimulate adenylate cyclase are enhanced, whereas those involving calcium signaling are decreased (13, 28–32). Similarly, the effects of pertussis toxin and forskolin are consistent with the involvement of adenylate cyclase stimulation in the H_2-mediated action of histamine. However, the coupling

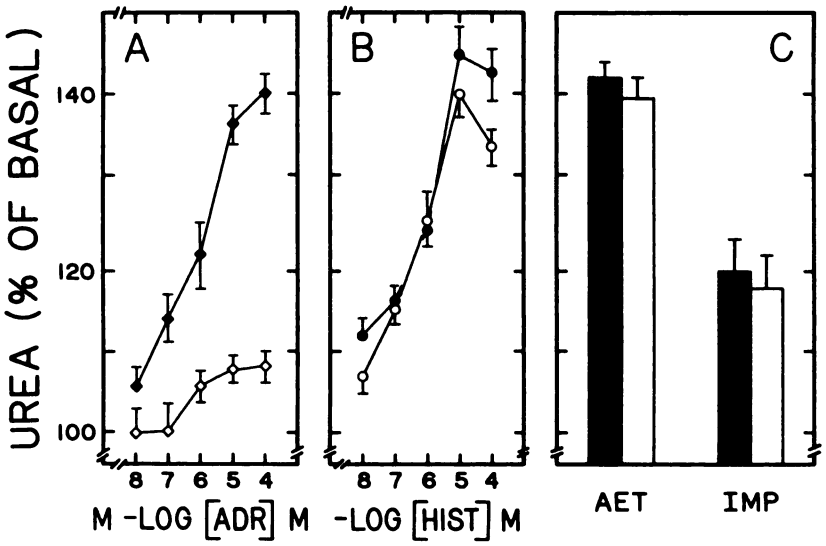

Fig. 7. Differential effect of PMA on the stimulation of ureagenesis by α_1-adrenergic and histamine agonists. Hepatocytes were incubated in the absence [◆ (A), ● (B), ■ (C)] or presence of 10^{-7} M PMA [◇ (A), ○ (B), □ (C)] and with different concentrations of epinephrine + 10^{-5} M propranolol (*ADR*) (A, ◆, ◇) histamine (*HIST*) (B, ●, ○), and 10^{-5} M AET or 10^{-5} M impromidine (*IMP*) (C). Results are expressed as percentage of basal ureagenesis which was 30 ± 2 nmol/mg of cells wet weight. Plotted are the means ± standard errors of six to eight determinations using different cell preparations.

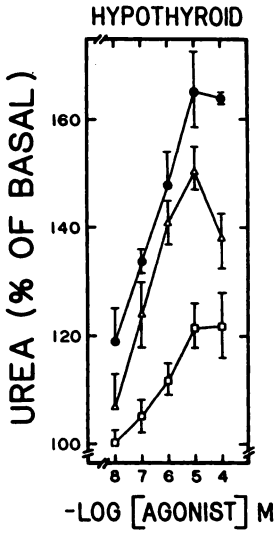

Fig. 8. Effect of hypothyroidism on the stimulation of ureagenesis by histamine agonists. Hepatocytes from hypothyroid rats were incubated in the absence or presence of histamine (●), AET (□), or impromidine (△). Results are expressed as percentage of basal ureagenesis which was 33 ± 2 nmol/mg of cells wet weight. Plotted are the means and standard errors (*vertical lines*) of seven to eight determinations using different cell preparations.

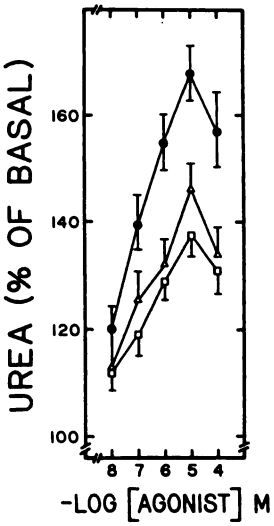

Fig. 9. Effect of pertussis toxin treatment on the stimulation of ureagenesis by histamine agonists. Hepatocytes were incubated in the absence or presence of different concentrations of histamine (●), AET (□), or impromidine (△). Basal ureagenesis was 33 ± 2 nmol/mg cells wet weight. Plotted are the means and standard errors (*vertical lines*) of seven to eight experiments using different cell preparations.

of H_1 and H_2 receptors to their transduction systems (adenylate cyclase and phospholipase C) remains to be directly demonstrated in this cell type. Furthermore, changes at the receptor level (number and/or affinity) may occur during hypothyroidism or pertussis toxin treatment. These aspects are currently being investigated in our laboratory.

In summary, the data clearly indicate, for the first time, the ability of histamine to modulate hepatic metabolism through both H_1- and H_2-histamine receptors. Furthermore, it is shown that the H_1-mediated action predominates in control hepatocytes, whereas the H_2-mediated action predominates in hepatocytes obtained from hypothyroid animals; forskolin and pertussis toxin treatment enhance the H_2-mediated effects. The latter results clearly indicate that there is plasticity in the receptor type that mediates the effects of histamine.

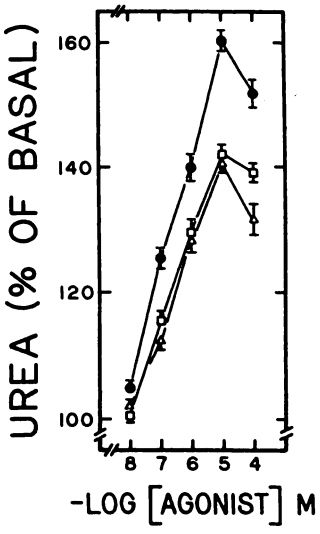

Fig. 10. Effect of forskolin on the stimulation of ureagenesis by histamine agonists. Hepatocytes were incubated in the presence of 10^{-7} M forskolin and with different concentrations of histamine (●), AET (□), or impromidine (△). Basal ureagenesis was 30 ± 1 nmol/mg of cells wet weight. Plotted are the means and standard errors (*vertical lines*) of seven to eight determinations using different cell preparations.

Acknowledgments

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