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R-α-methylhistamine-induced inhibition of gastric acid secretion in pylorus-ligated rats via central histamine H_3 receptors

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1 The effect of central H_3 histamine receptor activation on gastric acid and pepsin production has been investigated in pylorus-ligated rats.

2 Intracerebroventricular injections (i.c.v.) of the selective H_3 agonist, R-α-methylhistamine (0.5–50 nmol per rat) caused a dose-dependent inhibition of gastric acid secretion while intravenous administration (5–500 nmol per rat) was completely ineffective.

3 I.c.v. microinjections of mepyramine, tiotidine and thioperamide (51 nmol per rat), selective antagonists at H_1-, H_2- and H_3-sites respectively, failed to modify the acid secretory response to pylorus ligation.

4 The antisecretory effect of R-α-methylhistamine (5 nmol per rat, i.c.v.) was selectively prevented by the H_3-blocker, thioperamide (51 nmol per rat, i.c.v.), mepyramine and tiotidine pretreatment being completely inactive.

5 Unlike acid secretion, pepsin production was not significantly affected by all the tested compounds.

6 These findings provide the first pharmacological evidence that the activation of central H_3 histamine receptors exerts a negative control in the regulation of gastric acid secretion in conscious pylorus-ligated rats.

Keywords: H_3 histamine receptors; intracerebroventricular; pylorus-ligated rats; gastric acid secretion; pepsin secretion

Introduction

Histamine H_3 receptors were originally described as autoreceptors controlling neuronal histamine release and synthesis in rat brain (Arrang *et al.*, 1983), where their distribution was demonstrated autoradiographically to be highly heterogeneous (Arrang *et al.*, 1987).

Subsequently, pharmacological studies, performed both on central and peripheral tissues with the highly selective H_3 agonist, R-α-methylhistamine, and antagonist, thioperamide, have shown that the third histamine receptor is also localized in non-histaminergic neurones (van der Werf & Timmerman, 1989) and in extraneuronal sites (Ea Kim *et al.*, 1992; Schworer *et al.*, 1992; Cardell & Edvinsson, 1994).

Experimental evidence is accumulating to relate various functional and behavioural effects to central H_3 histamine receptor-mediation. The activation of such cerebral sites seems to provoke a waking effect in cats (Lin *et al.*, 1988; Schwartz *et al.*, 1990), alteration of locomotor activity (Clapham & Kilpatrick 1994), anticonvulsant (Yokoyama *et al.*, 1993) and antinociceptive (Malmberg-Aiello *et al.*, 1994) actions in mice. Modifications of rat intestinal motility (Fargeas *et al.*, 1989) as well as of guinea-pig cardiovascular function (McLeod *et al.*, 1991) have also been described as a consequence of central H_3 stimulation.

Since the work of Hervatin *et al.* (1988), it has been known that peripheral administration of the H_3 selective agonist, R-α-methylhistamine inhibited pentagastrin-, 2-deoxy-D-glucose-, and peptone meal-stimulated gastric acid secretion in conscious cats as well as pentagastrin- and bombesin-induced hypersecretion in the conscious dog. Such inhibitory effects were antagonized by thioperamide. The mechanism of action underlying this phenomenon remains unknown and is still a matter of study even though the presence of H_3 receptors on parasympathetic nerve terminals or on gastric paracrine cells has been suggested (Hervatin *et al.*, 1989; Bado *et al.*, 1991a; Coruzzi *et al.*, 1991; Soldani *et al.*, 1994).

So far, the possible contribution of a central H_3-mediated regulatory influence in affecting gastric acid secretion has not been explored and so cannot be ruled out.

These considerations prompted us to investigate the possible involvement of central H_3 receptors in the control of gastric secretory function. Therefore, we analysed the modification of gastric secretion following central (i.c.v.) or peripheral (i.v.) administration of R-α-methylhistamine in conscious pylorus-ligated rats both in the absence and presence of highly selective H_1, H_2 or H_3 histamine receptor antagonists. The effects evoked by central injection of these antagonists were also investigated.

Preliminary results of this work were communicated to the Tenth World Congresses of Gastroenterology, Los Angeles 1994.

Methods

Female Wistar strain rats weighing 200–250 g were used. In a first series of experiments, the animals were anaesthetized with pentobarbitone (Nembutal, 33 mg kg^{-1}, i.p.) and were placed in a stereotaxic frame. Stainless steel cannulas (23 gauge) were then implanted into the lateral cerebral ventricle of each rat according to the coordinates of Pellegrino's Atlas (Pellegrino *et al.*, 1979): AP–1; L ± 1; DV-4. Each cannula was fixed into the skull with screws and dental cement as a guide for intracerebroventricular microinjections. One week of post-operative recovery elapsed before the experiments were carried out. At the time of the experiments, the rats, fasted for 18 h, were lightly anaesthetized with ether and, after laparotomy, the pylorus was ligated. Care was taken not to occlude or damage major vessels to the stomach. Immediately after closure of the abdominal wound with silk sutures, the vehicle or the test drug was microinjected into the ventricular space over

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a period of 1 min, in a final volume of 10 µl. The micropipette was left in place for an additional 2 min to avoid back diffusion along the cannula.

The H_3-agonist **R**-α-methylhistamine (0.5, 5, 50 nmol per rat) was injected i.c.v. in the absence or presence of the different antagonists: mepyramine, tiotidine, thioperamide (51 nmol per rat). Equimolar doses of the H_1, H_2 and H_3 antagonists were chosen by considering the comparable affinities displayed by these compounds at the respective histamine receptors (Arrang *et al.*, 1983). The antagonists were administered, in a volume of 5 µl, 5 min before the injection of the agonist dissolved in 5 µl saline. Control animals received 10 µl of the vehicle.

In a second series of experiments, **R**-α-methylhistamine (5, 50, 500 nmol per rat) was peripherally (i.v.) injected after pylorus ligation and control animals received the vehicle alone (1 ml kg^{-1}). The animals were killed 2 h after ligation of the pylorus, the abdomen was opened, the stomach removed and the gastric contents were collected and centrifuged (2000 *g*, 5 min). Gastric secretion volume was measured and the acid concentration was determined by titrating 0.1 ml with 0.05 N NaOH to an end point of pH = 7 on an automatic titrator (Radiometer, Copenhagen, Denmark). Gastric acid output was calculated by multiplying the volume by the acid concentration and expressed as microequivalents per 2 h. Peptic activity of the gastric juice samples was measured according to Berstad's method (1970) with bovine haemoglobin used as a substrate. In this method any pepsinogen of the sample was converted to pepsin before the haemoglobin digestion period and, therefore, the measurement of pepsin activity reflected total pepsinogen secretion. The standard curve was made using dilutions of a crystalline preparation of pepsin from gastric porcine mucosa (Merck). Pepsin concentration (µg ml^{-1}) was evaluated and pepsin output was expressed as µg 2 h^{-1}.

Data analysis

The results were expressed as the mean ± s.e.mean. The statistical evaluation was performed with Student's *t* test. *P* values < 0.05 were considered to be significant.

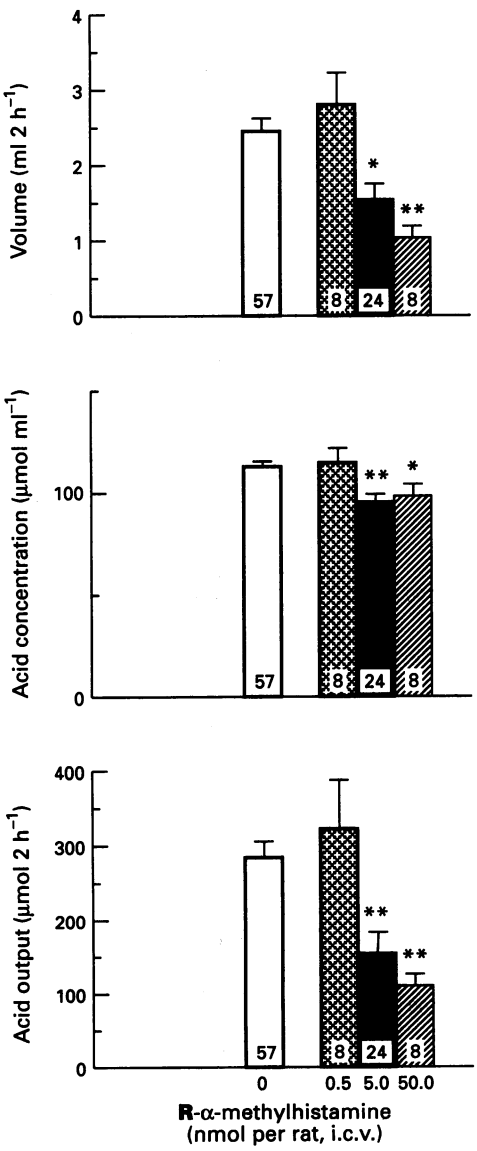

Figure 1 Effect of i.c.v. microinjection of the H_3 agonist, **R**-α-methylhistamine on gastric acid secretion in conscious pylorus-ligated rats. Results are mean ± s.e.mean. Number of rats per group is indicated at the base of each column. $*P < 0.05$, $**P < 0.01$ Student's *t* test as compared with the corresponding vehicle-treated rats.

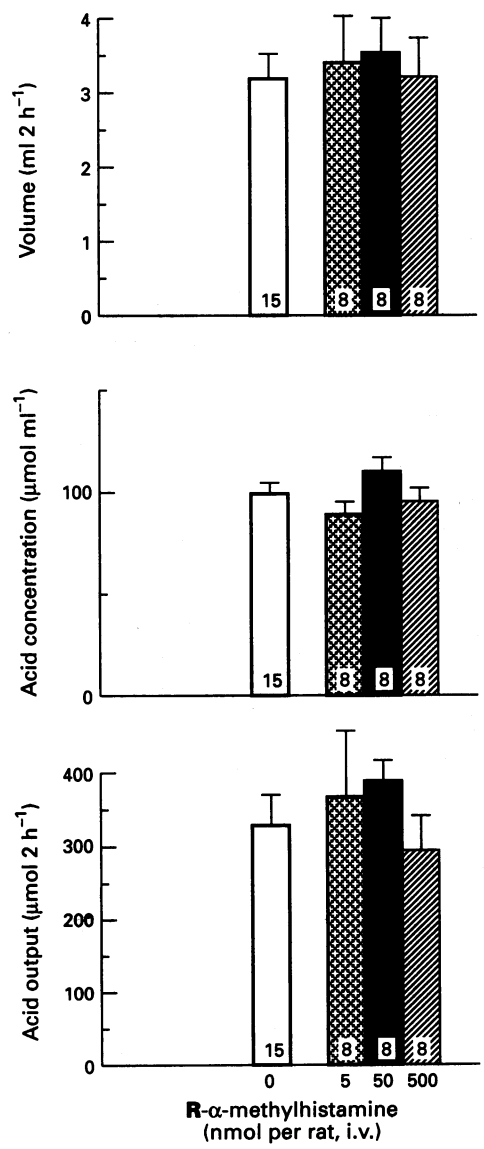

Figure 2 Effect of i.v. injection of the H_3 agonist, **R**-α-methylhistamine, on gastric acid secretion in conscious pylorus-ligated rats. Results are mean ± s.e.mean. Number of rats per group is indicated at the base of each column.

Drugs

R-α-methylhistamine dihydrochloride, tiotidine and thioperamide were synthesized by Prof. P.V. Plazzi *et al.*, Pharmaceutical Department, University of Parma, Parma, Italy.

Mepyramine maleate was obtained from Sigma Chimica, Milano, Italy.

Results

Intracerebroventricular injection of **R**-α-methylhistamine (0.5–50 nmol per rat) dose-dependently inhibited gastric acid secretion induced in conscious rats by pylorus ligation (Figure 1). Total acid output was significantly ($P<0.01$) reduced to 54% and 39% by the administration of 5 and 50 nmol **R**-α-methylhistamine respectively compared with control values. This reduction in acid output was related mainly to the change in volume of secretion and also to a small decrease of the proton concentration.

Conversely, **R**-α-methylhistamine, injected peripherally (5–500 nmol per rat, i.v.), did not significantly affect gastric secretion of conscious pylorus-ligated rats, the secretory parameters (volume, acid concentration and output) of the treated groups resembling the corresponding values of the vehicle-treated animals (Figure 2).

Pretreatment with the selective H_3 blocker, thioperamide (51 nmol per rat, i.c.v.), antagonized the antisecretory effect elicited by 5 nmol per rat **R**-α-methylhistamine whereas the H_1 and H_2 blocking agents, mepyramine (51 nmol per rat, i.c.v.) and tiotidine (51 nmol per rat, i.c.v.) injected at equimolar doses, failed to prevent the inhibitory action of this amine (Figure 3).

On the other hand, mepyramine, tiotidine or thioperamide, injected intraventricularly at the aforementioned doses, in themselves were totally ineffective in modifying acid secretions with respect to control rats (Figure 4).

Neither peripheral administration of the selective H_3 agonist nor treatment with H_1, H_2 and H_3 antagonists significantly influenced gastric pepsin secretion, pepsin concentration and output remaining similar to the corresponding basal values (2023 ± 106 μg ml^{-1} and 4993 ± 368 μg 2 h^{-1}, $n=47$). An apparent reduction of pepsin output to the value of 3295 ± 580 μg 2 h^{-1} was caused by **R**-α-methylhistamine (5 nmol per rat, i.c.v.). However, this thioperamide-sensitive effect seems to be a direct consequence of the changes produced by H_3-receptor brain stimulation on gastric juice volume.

Discussion

The aim of the present study was to ascertain whether central H_3 histamine receptors might be involved in the modulation of rat gastric acid secretion.

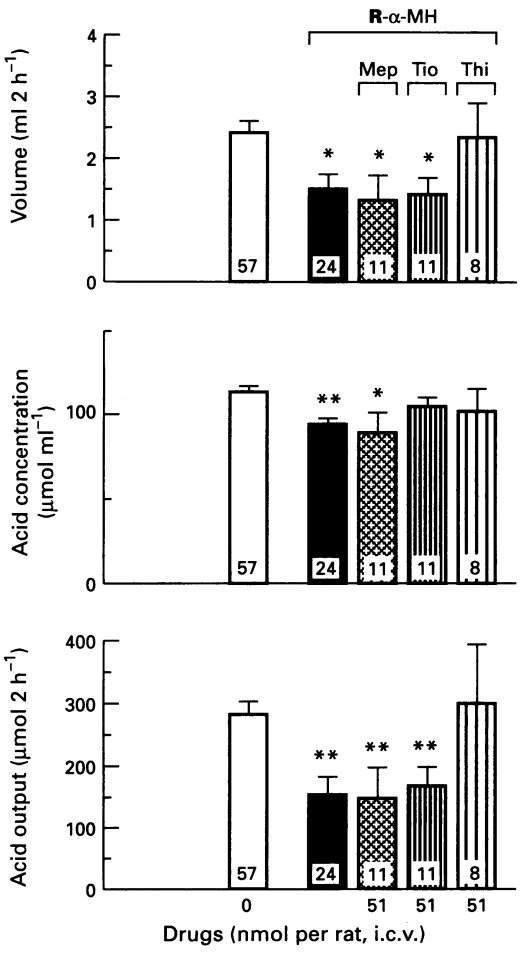

Figure 3 Effect of i.c.v. equimolar microinjections of the selective H_1-, H_2- and H_3-antagonists mepyramine (Mep), tiotidine (Tio) and thioperamide (Thi) on (5 nmol per rat, i.c.v.) **R**-α-methylhistamine (**R**-α-MH)-induced inhibition of gastric acid secretion in conscious pylorus-ligated rats. Results are mean ± s.e.mean. Number of rats per group is indicated at the base of each column. $*P<0.05$, $**P<0.01$ Student's t test as compared with the corresponding vehicle-treated rats.

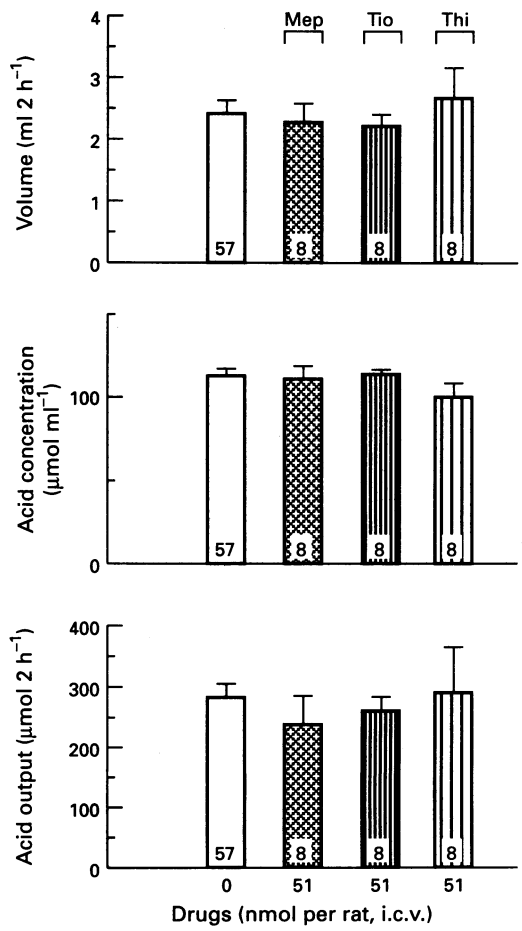

Figure 4 Effect of i.c.v. equimolar microinjections of the selective H_1-, H_2- and H_3-antagonists mepyramine (Mep), tiotidine (Tio) and thioperamide (Thi) on gastric acid secretion in conscious pylorus-ligated rats. Results are mean ± s.e.mean. Number of rats per group is indicated at the base of each column.

Intracerebroventricular injection of the selective H_3 agonist, R-α-methylhistamine (0.5–50 nmol per rat) dose-dependently inhibited the acid hypersecretory response elicited in conscious rats by pylorus ligation. The absence of an antisecretory effect after peripheral administration of this amine, at doses ranging from 5 to 500 nmol per rat, allows us to assume that R-α-methylhistamine, given i.c.v., exerted its inhibitory action directly in the brain and not as a consequence of leakage from the central nervous system. Pharmacokinetic studies recently performed in rats have demonstrated that, after systemic administration, R-α-methylhistamine is rapidly transferred to peripheral tissues and hardly crosses the blood-brain barrier (Yamasaki *et al.*, 1994). Therefore, it is likely that R-α-methylhistamine, at the intravenous doses administered in this study, did not reach the brain in effective concentrations owing to its poor central penetration.

R-α-methylhistamine-elicited inhibition of gastric acid production was selectively prevented by microinjection of the H_3 antagonist, thioperamide, in nanomol doses. This effect of thioperamide was unlikely to be non specific since thioperamide did not modify the gastric secretion of the pylorus-ligated rats not treated with R-α-methylhistamine. The failure of the H_1 and H_2 receptor antagonists to inhibit the H_3-agonist effect provides supportive evidence that the action of R-α-methylhistamine is mediated through central H_3 receptors.

The lack of antisecretory action which we observed in rats injected peripherally with R-α-methylhistamine (about 2.5 μmol kg^{-1}, i.v.) seems to diverge from the results obtained in other animal species. Indeed, R-α-methylhistamine, peripherally injected at low doses in conscious cats (0.2–0.4 μmol kg^{-1} h^{-1}) and dogs (1.2 μmol kg^{-1} h^{-1}), has been proved to inhibit gastric hyperacidity evoked by indirect secretagogues (Bado *et al.*, 1991b; Coruzzi *et al.*, 1991; Soldani *et al.*, 1994). This discrepancy seems to indicate the existence of a different sensitivity between animal species to the stimulation of gastric H_3 receptors. Such different species-related responsiveness to R-α-methylhistamine is consistent with previous findings comparing the cardiovascular responses to peripheral H_3 activation in conscious guinea-pigs, rabbits and rats (McLeod *et al.*, 1994).

Furthermore, numerous studies, recently performed by exploiting different *in vitro* experimental models, seem to strengthen this hypothesis. Data obtained in rabbit gastric mucosa suggested that histamine downregulates its own synthesis acting at H_3-autoreceptors (Hollande *et al.*, 1993) and thioperamide stimulates acid secretion as well as histamine release (Bado *et al.*, 1991b). Conversely, new studies performed on human, rat and mouse isolated gastric mucosa gave rise to contradictory results which described an H_3-mediated opposite inhibitory/stimulatory effect of histamine on somatostatin-secreting gastric cells affecting gastrin as well as histamine secretion (Bado *et al.*, 1992; 1994; Vuyyuru & Schubert, 1993; Vuyyuru *et al.*, 1994). Furthermore, the different methods of R-α-methylhistamine administration used in this study and in the work quoted above could also account for the divergent effects observed on gastric acid secretion.

With regard to pepsin secretion, the data reported here suggest a certain refractoriness of pepsin production to central H_3 receptor mediation.

In summary, our findings led us to conclude that the activation of central H_3 histamine receptors exerts a negative control in the gastric acid secretory processes in conscious pylorus-ligated rats. Several brain sites have been identified as being involved in the control of gastric acid secretion (Taché, 1987; Barocelli *et al.*, 1991) and in some of them, namely lateral hypothalamus and nucleus accumbens, a high density of H_3 receptors has been visualized autoradiographically (Arrang *et al.*, 1987). However, even if it is currently accepted that vagal tone plays a role in the pylorus ligation hypersecretory model (Brodie, 1966) the exact anatomical site as well as the nervous or humoral pathways involved in R-α-methylhistamine inhibitory action require further investigation.

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