The new potent and selective histamine H\textsubscript{2} receptor agonist amthamine as a tool to study gastric secretion

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Summary. The new histamine H\textsubscript{2} receptor agonist amthamine, \([2\text{-amino-5-(2-aminoethyl)-4-methylthiazole}]\), was tested for its activity on gastric acid secretion in different in vivo and in vitro experimental models. Amthamine induced a dose-related increase in acid secretion both in conscious cats with a gastric fistula (ED\textsubscript{50} = 0.069 \text{\mu mol/kg/h}) and in anaesthetized rats with a lumen-perfused stomach (ED\textsubscript{50} = 11.69 \text{\mu mol/kg i.v.}). In this last preparation the efficacy of amthamine was significantly higher than that of histamine and dimaprit. Amthamine was an effective secretagogue also in the rat isolated gastric fundus, behaving as a full agonist (EC\textsubscript{50} = 18.9 \text{\mu mol/l}). In all the experimental models amthamine was more potent than dimaprit (from 3 to 10 fold) and approximately equipotent with histamine, and its effect was competitively antagonized by the histamine H\textsubscript{2} receptor antagonists famotidine or ranitidine. Experiments with H\textsubscript{1} and H\textsubscript{3} receptor antagonists indicated that amthamine is devoid of stimulatory activity at H\textsubscript{1} and H\textsubscript{3} receptors. The present data indicate that amthamine is a full agonist at histamine H\textsubscript{2} receptors and, being more effective and selective than the other compounds of the family, it may represent a good alternative to the other available histamine H\textsubscript{2} receptor agonists for the study of gastric acid secretion.

Key words: Amthamine – Histamine H\textsubscript{2} receptors – Gastric acid secretion – Conscious cat – Rat

Introduction

Both for unravelling the physiological and pharmacological role of receptor systems and for the development of potential drugs interfering with these receptors, the availability of potent and especially selective ligands is very important. For the three types of histamine receptors so far no agonist of either the H\textsubscript{1} or H\textsubscript{2} receptor with combined high potency and selectivity has become available. Recently however, a new histamine H\textsubscript{2} receptor agonist with a pD\textsubscript{2} similar to that of histamine and a high degree of selectivity has been described (Eriks et al. 1991, 1992). This new compound, amthamine, has been developed on the basis of theoretical considerations. A remarkable feature of amthamine is that, instead of an imidazole nucleus, it carries a thiazole moiety. This thiazole nucleus does not allow the existence of two tautomeric species and the activity of the compound is therefore unexpected in the light of the existing ideas about the mechanism of activity of the agonists (Weinstein et al. 1976).

In the present paper we describe the histamine H\textsubscript{2} receptor agonistic effect of this new ligand in several gastric secretion models.

Preliminary results have been presented at the 3rd Joint Meeting of Hungarian, Italian and Polish Pharmacological Societies, Italy, 1992 (Coruzzi et al. 1992b).

Materials and methods

Conscious gastric fistula cat

Female cats weighing approximately 3.5 kg were used. Under pentobarbital anaesthesia (30 mg/kg i.v.) they were equipped with a permanent gastric fistula drained by a gastric cannula (Emas et al. 1967), at least 6 weeks before the study. They were fasted for 18 h before the test, with water ad libitum. Each animal was used once a week. Drugs were administered intravenously by bolus injection or continuous infusion in 0.15 mol/l NaCl through a peristaltic pump (30 ml/h). Gastric juice was collected throughout the whole experiment and divided in 10 min samples. Volume was measured and acid concentration determined by titration to pH 7.0 with NaOH 0.1 mol/l with an automatic titrator (Radiometer, Copenhagen).

The effect of the different secretagogues was tested either by single administrations (one dose for each animal), in order to evaluate the time course of the response, or by cumulative doses progressively increasing every 30 min in order to obtain a complete dose-response curve in the same animal. When an antagonist was used, it was administered by i.v. infusion throughout the whole experiment starting 30 min before the administration of the stimulant.
Acid secretory responses were reported as mean values±SEM in mEq HCl/10 min. The potency ratio of the different secretagogues was assessed by comparing their ED50s, calculated from the individual dose-response curves and expressed as geometric means with 95% confidence limits. The pA2 value of the antagonist was calculated by determining dose ratios in the absence and in the presence of three doses of the antagonist (Arumalakshana and Schild 1959).

Lumen-perfused stomach of the anaesthetized rat

Male Wistar rats (180-200 g) were used. They were fasted for 18 h and were allowed free access to water. After urethane anaesthesia (1.25 g/kg i.p.) the stomach was perfused with saline at 37 °C (60 ml/h) through an oesophageal cannula and the perfusion fluid was collected via a duodenal cannula. Changes in the acid concentration of the perfusate were recorded every 10 min by automatic titration to pH 7 with NaOH 0.01 mol/l (Radiometer, Copenhagen).

Drugs were administered by a rapid intravenous injection or a continuous infusion (6 ml/h). Dose-response curves to agonists were constructed by single administrations in separate animals (only one dose was administered to each animal); for this reason ED50 values for the different agonists were calculated from the mean dose-response curves and were expressed as arithmetic mean with 95% confidence limits. The antagonist was administered by single i.v. injections at the plateau of acid secretion induced by a continuous infusion of the agonist. The inhibitory effect of the antagonist was expressed as percent inhibition against a submaximum dose of the agonist and the ID50 value was calculated from the inhibitory dose-response curve. Acid secretory responses were expressed as mean values±SEM in μEq HCl/kg/min.

Isolated gastric fundus from immature rats

The technique described by Coruzzi et al. (1984) was followed. Fed immature male rats (30-45 g), kept with a lactating female, were used. They were killed by cervical dislocation, the stomach was removed and were allowed access to water. After urethane anaesthesia (1.25 g/kg i.p.) the stomach was perfused with saline at 37 °C (60 ml/h) through an oesophageal cannula and the perfusion fluid was collected via a duodenal cannula. Changes in the acid concentration of the perfusate were recorded every 10 min by automatic titration to pH 7 with NaOH 0.01 mol/l (Radiometer, Copenhagen).

They were killed by cervical dislocation, the stomach was removed and were allowed access to water. After urethane anaesthesia (1.25 g/kg i.p.) the stomach was perfused with saline at 37 °C (60 ml/h) through an oesophageal cannula and the perfusion fluid was collected via a duodenal cannula. Changes in the acid concentration of the perfusate were recorded every 10 min by automatic titration to pH 7 with NaOH 0.01 mol/l (Radiometer, Copenhagen).

Compounds used.
The following drugs were used: histamine dihydrochloride, atropine sulphate and pyrilamine maleate (Sigma Chemical Co., St. Louis, Mo., USA); famotidine base (Sigma-Tau, Pomezia, Italy); ranitidine hydrochloride (Gliax, UK); dimaprit dihydrochloride (RBI, USA); thioperamide (Coockson Chemicals, Ltd., UK). Amthamine was taken from lab stock (Eriks et al. 1992). The chemical structure of amthamine is shown in Fig. 1.

Statistical methods. Acid responses were expressed as means±SEM. ED50s for gastric fistula cats were reported as geometric means with 95% confidence limits. ED50s for anaesthetized rats and EC50s for the isolated rat stomach were expressed as arithmetic means with 95% confidence limits (Tallarida and Murray 1987). Statistical comparisons between two sets of values were made by Student's t-test for unpaired data; comparisons among several groups were made by analysis of variance. In all tests the level of significance was chosen as P<0.05.

Results

Conscious gastric fistula cat

The histamine H2 receptor agonist amthamine administered by rapid i.v. injections (0.015-1 μmol/kg) caused a dose-dependent increase in the volume and acid concentration of the gastric juice, which was rapid in onset and lasted from 30 to 180 min, according to the dose administered (Fig. 2). No significant difference between amthamine and dimaprit was noted as for the time course of the response (data not shown). Cumulative administrations of amthamine resulted in a dose-response curve which was comparable with that of dimaprit as for the maximum response (1.24±0.06 and 1.27±0.11 mEq/10 min for amthamine and dimaprit, respectively) (Fig. 3), but the potency of amthamine was significantly (P<0.05) higher. ED50s values for amthamine and dimaprit are reported in Table 1. The secretory effect of amthamine was competitively antagonized by the histamine H2 receptor antagonist famotidine (Fig. 3). The pA2 value of famotidine against amthamine was 8.0±0.063. The secretory effect of amthamine was not modified by pretreatment with the H3 antagonist thioperamide given at the dose (0.02 μmol/kg/h) that completely antagonized H3-mediated effects (Coruzzi et al. 1991). Also the H1-receptor antagonist pyrilamine (4 mg/kg i.v. followed by 2 mg/kg/h) was unable to modify the maxi-