Characterization of histamine H3 receptors inhibiting 5-HT release from porcine enterochromaffin cells: Further evidence for H3 receptor heterogeneity

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Abstract. The nature of the histamine receptor mediating inhibition of 5-HT release was investigated in strips of the porcine small intestine by investigating the effects of histamine ligands on the overflow of endogenous 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA). The overflow was measured by HPLC, combined with electrochemical detection and represents calcium-sensitive 5-HT release from enterochromaffin cells, as reported previously. The histamine H3 receptor selective agonists (R)-α-methyl-histamine and imetit inhibited the overflow of 5-HT maximally by 50–60%, with EC50 values of 48 and 3.2 nmol/l, respectively. Effects on 5-HT overflow were always accompanied by similar effects on the overflow of 5-HIAA. Thioperamide (100 nmol/l) shifted the concentration response curve of (R)-α-methyl-histamine to the right (pK~ value 8.38). The inhibitory effect of 1 µmol/l (R)-α-methyl-histamine was antagonized in a concentration-dependent manner by thioperamide (IC50: 65 nmol/l) and dimaprit (IC50: 8.6 µmol/l); however, the effect of (R)-α-methyl-histamine was weakly antagonized by burimamide (by 38% at 100 µmol/l) and not significantly affected by other H3 receptor antagonists, such as impromidine, betahistine and phenylbutanoyl-histamine (each up to 100 µmol/l). In conclusion, H3 receptors mediating inhibition of 5-HT release from porcine enterochromaffin cells have a particular pharmacological profile indicating that heterogeneity of H3 receptors may exist. The data suggest that histamine H3 receptors modulating 5-HT release in pig small intestine do not belong to either H3A or H3B receptors as defined in rat tissue.

Key words: Enterochromaffin cells – 5-Hydroxytryptamine release – Histamine H3 receptors – Thioperamide – (R)-α-methyl-histamine – Imetit – Porcine small intestine

Introduction

There is substantial evidence that histamine is an important cellular messenger in the gastrointestinal tract (see Rangachari 1992). Recently, we reported that histamine inhibits the release of 5-HT from enterochromaffin cells of the porcine small intestine (Schwörer et al. 1992). Histamine receptors can be subdivided into three major classes (see Hill 1990), and pharmacological experiments indicated that H3 receptors mediated the effect of histamine on the enterochromaffin cells (Schwörer et al. 1992). However, there is increasing evidence for heterogeneity of H3 receptors. Thus, West et al. (1990) reported that burimamide and thioperamide displaced [3H]-Nα-methyl-histamine from H3 binding sites in rat brain membranes in a biphasic manner, and H3A and H3B binding sites (with high and low affinity for these antagonists) have been defined. In functional experiments, Schlicker et al. (1992) showed that the pharmacological profile of the H3 receptors mediating inhibition of noradrenaline release from mouse brain cortex corresponded well to the H3A sites. Additional support for heterogeneity of H3 receptors has been presented by Clapham and Kilpatrick (1992) showing that betahistine and phenylbutanoyl-histamine were only very weak antagonists at H3 receptors mediating inhibition of [3H]-acetylcholine release in the rat cortex, although these antagonists had been shown to be effective inhibitors of (R)-α-methyl-histamine binding in brain and at the H3 receptors mediating inhibition of [3H]-histamine release (Arrang et al. 1985; Timmerman 1990; Taylor and Kilpatrick 1992).

The aim of the present experiments was to characterize further the H3 receptor involved in the inhibition of 5-HT release from the enterochromaffin cells. Therefore, the potencies of the H3 receptor agonists (R)-α-methyl-histamine and imetit and of several established antagonists (thioperamide, burimamide, impromidine, betahistine and phenylbutanoyl-histamine) were determined and compared with the reported potencies at H3 receptors in other tissues. The H2 receptor agonist dimaprit was also included in the present study because it has been shown
that dimaprit also binds to $H_3$ receptors (West et al. 1990) and behaves as an $H_3$ receptor antagonist with moderate potency (Schliker et al. 1992).

A preliminary account of part of the present results has been given to the German Pharmacological Society (Racké and Schwörer 1992c).

**Methods**

Preparation and incubation of isolated segments of the porcine small intestine. Segments of the small intestine were removed from pigs which underwent pancreatic perfusion as described previously (Schwörer et al. 1987; Racké and Schwörer 1992a). Briefly, pigs fasted overnight (15–25 kg body weight) of either sex were premedicated with azaperone (160 mg i.m.), metamizode (100 mg i.v.) and atropine (0.5 mg i.v.) and artificially respirated. General anaesthesia was maintained by metomidate (200 mg/h i.v.) and piritramide (3.5 mg/h i.v.). A portion of the jejunum (about 10 cm long) was cut along the mesenteric border and strips (1.5–2.5 cm long; 0.5 cm wide) weighing between 50 and 100 mg were prepared. Single strips were suspended under a preload of 10 mN in an organ bath containing 3 ml Krebs solution of the following composition (mmol/l): NaCl 118; KCl 4.7; CaCl_2 2.52; MgSO_4 1.64; NaHCO_3 24.88; KH_2PO_4 1.18; D-glucose 5.6; (+)-ascorbic acid 0.057 and EDTA 0.03. The incubation medium was continuously gassed with 5% CO_2 + 95% O_2 and maintained at 37°C. During the first 20 min the medium was changed every 10 min, thereafter every 5 min. The medium of the first 40 min was discarded, thereafter it was collected in acid-washed glass tubes which contained 50 μl of 57 mmol/l (+)-ascorbic acid, 50 μl of 10 mmol/l EDTA and 65 μl of 1 mol/l perchloric acid (suprapure). Test substances were added to the incubation medium from 55 min onwards; each preparation was exposed to only one concentration of a test drug. In interaction experiments the respective antagonist was present from 20 min before the start of the collection period (i.e. 35 min before the agonist). At the end of the collection period the tissue was blotted and weighted.

Chemical analysis. 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were measured by high pressure liquid chromatography (HPLC) combined with electrochemical detection as described previously (Schwörer et al. 1987). Briefly, the separation of 5-HT and its metabolite was achieved by a reverse phase column (length 250 mm, inner diameter 4.6 mm, prepacked with Shodex ODS-Hypersil, 5 μm) using a mobile phase of 0.1 mol/l phosphate buffer (adjusted to pH 3.0), containing octane sulphonic acid sodium salt (160 mg/l), sodium EDTA (0.3 mmol/l) and methanol (12%, v/v). Quantitation was achieved with electrochemical detectors (Waters 460 or Gynkothek M 20) equipped with glass carbon working electrodes and Ag/AgCl reference electrodes. The potential was set at +0.70 V. Portions of 50–100 μl of the incubation medium were injected directly into the HPLC-column. The limit of detection was between 60 and 120 fmol for 5-HT and between 10 and 25 fmol for 5-HIAA per injection. The amount of 5-HT and 5-HIAA in the incubation media is expressed in units of pmol/g/5 min.

Calculations and statistical analysis. Results are expressed as percentage of the mean overflow observed during the first three collection samples (40–55 min of incubation) of the individual experiments. Mean values of $n$ experiments are given ± SEM. The significance of differences between two mean values was assessed using Student’s t-test. For the comparison of one control with several experimental groups, the significance of differences was evaluated by the modified t-test according to Bonferroni (see Wallenstein et al. 1980). A P value of less than 0.05 was considered significant. Calculation of EC_{50} values was carried out by the use of a computer program (Tallarida and Murray 1988). Antagonism was quantified by calculating pK_B values according to the Eq. (4) given by Furchgott (1972) or by comparison of the potencies (IC_{50} values) of antagonists to inhibit the effect of a single agonist concentration.

**Results**

The mean basal overflow of 5-HT and 5-HIAA from strips of the porcine small intestine incubated in the absence of test substances (determined between 40 and 55 min of incubation) amounted to 251±21 and 743±71 pmol/g/5 min, respectively ($n$ = 174). The time course of the present experiments was similar to that described in previous studies (Racké and Schwörer 1992a, b; Schwörer et al. 1992); as observed in those studies, in the absence of test drugs, the overflow of 5-HT (Fig. 1) and 5-HIAA (not shown) did not change significantly during 40–80 min of incubation (i.e. the period of observation).

Drug-induced changes in the overflow of 5-HT were always accompanied by parallel changes in the overflow of 5-HIAA and will not be documented in detail.

In confirmation of previous observations (Schwörer et al. 1992), (R)-α-methyl-histamine inhibited the overflow of 5-HT in a concentration-dependent manner, maximally by about 60% at 10 μmol/l (Fig. 2). A value of 48 pmol/l was calculated for the concentration causing half maximum effect (EC_{50}). Figure 2 shows also that imetit caused a similar reduction in the overflow of 5-HT, but was about 10 times more potent than (R)-α-methyl-histamine (EC_{50}: 3.2 nmol/l). The concentration response curve of (R)-α-methyl-histamine was shifted to the right in the presence of 100 nmol/l thiopermane (Fig. 2), and a pK_B value of 8.38 was calculated for this antagonist.

The inhibitory effect of 1 μmol/l (R)-α-methyl-histamine on the release of 5-HT was not significantly affected by imipramide, betaistine and phenylbutanoyl-his-

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**Drugs.** Betaistine dihydrochloride (Fluka, Buchs, Switzerland); burimamide, dimaprit dihydrochloride, imetit (2-(4-imidazolyl)ethylsulphoxide dihydrochloride) and imipramide trihydrochloride (Smith Kline Beecham, Betchworth, UK); (R)-α-methyl-histamine oxalate and thiopermane maleate (RBI, Natick, MA); phenylbutanoyl-histamine (Glaxo, Ware, UK).

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**Fig. 1.** Effects of imetit (1 μmol/l, $n$ = 4) or (R)-α-methyl-histamine (R)-α-MeHis, 1 μmol/l, $n$ = 5) on the overflow of 5-HT from strips of the porcine small intestine incubated in vitro. The horizontal bar indicates the period of incubation with the respective test drug. The open circles show control experiments without drug application ($n$ = 11). Ordinate: overflow of 5-HT into the incubation medium expressed as percent of the mean overflow from 40–55 min of incubation in the individual experiments. Given are mean values ± SEM of $n$ experiments. Abcissa: time after onset of incubation.