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# Cardiac and gastric effects of histamine $H_2$ receptor antagonists: no evidence for a correlation between lipophilicity and receptor affinity

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1 A series of histamine $H_2$ receptor antagonists with different lipophilicity were tested in cardiac and gastric assays in order to reveal possible differences in receptor affinity. Lipophilicity of the compounds was expressed as CLOG P (theoretically-determined logarithm of octanol:water partition coefficient) and log $k'$ (logarithm of capacity factor, experimentally-determined by reverse-phase high performance liquid chromatography).

2 Aminopotentidine (APT) and iodoaminopotentidine (I-APT), which are both lipophilic compounds, behaved as insurmountable antagonists of histamine responses in rat isolated gastric fundus ($pK_B = 6.20 \pm 0.16$ and $6.89 \pm 0.19$, respectively) and guinea-pig isolated papillary muscle ($pK_B = 6.34 \pm 0.37$ and $6.81 \pm 0.26$, respectively). They were approximately as effective as ranitidine (RAN) in reducing histamine-induced acid secretion in the anaesthetized rat, $ID_{50}$ values being $0.018 \pm 0.02$, $0.020 \pm 0.03$ and $0.036 \pm 0.01$ $\mu$mol kg$^{-1}$ i.v. for APT, I-APT and RAN, respectively. Both APT and I-APT had a significantly longer duration of action than RAN.

3 The hydrophilic compound, SK&F 92857, was inactive up to 10 $\mu$M in modifying histamine-induced acid secretion in the isolated rat stomach. In the papillary muscle, low concentrations (0.1 - 1 $\mu$M) of this compound produced a competitive antagonism of the histamine responses ($pA_2$ value $= 7.38 \pm 0.11$), while a higher concentration (10 $\mu$M) significantly reduced the maximal response to histamine.

4 RAN competitively antagonized histamine effects with a comparable affinity in cardiac and gastric preparations ($pA_2$ values were $6.42 \pm 0.09$ and $6.78 \pm 0.38$ in heart and stomach, respectively).

5 Results obtained in this study clearly showed that the discrepancies between gastric and cardiac effects observed for some $H_2$ antagonists are not explained solely by differences in lipophilicity of compounds. Moreover, the significant correlation found between CLOG P and log $k'$ parameter, which takes into account, besides their lipophilicity, the ionization of the molecules, suggests that ionization has a similar influence for all the molecules on the partition between the lipophilic and aqueous phase.

**Keywords:** Histamine $H_2$ receptors; heart; stomach; aminopotentidine; iodoaminopotentidine; compound SK&F 92857; lipophilicity

## Introduction

In a previous paper (Coruzzi *et al.*, 1994) it was found that the brain-penetrating histamine $H_2$ receptor antagonist, zolantidine, was unable to modify histamine-induced acid secretion in *in vitro* and *in vivo* preparations from rats, guinea-pigs and cats. In contrast, zolantidine antagonized histamine-induced responses in the guinea-pig atria and papillary muscle, with an affinity comparable to that of ranitidine. The existence of heterogeneity in the $H_2$ receptor population suggested by these findings seems unlikely, when considering previous data with a variety of $H_2$ receptor antagonists, which did not distinguish between $H_2$ receptors in different tissues (for review, see Ganellin & Parsons, 1982; Leurs *et al.*, 1991). The finding that zolantidine blocked histamine responses when tested in gastric homogenates (Parsons, personal communication) strengthens the hypothesis that kinetic factors are responsible for the ineffectiveness of zolantidine in intact tissues. This is in accordance with the original hypothesis of Angus & Black (1979) that very lipophilic compounds had reduced activity on gastric secretion assays relative to their activity on the heart, due to their ready penetration of cell membranes and subsequent loss of antagonist through the gastric mucosal membrane into the gastric acid secretion. In an extensive study (Shankley *et al.*, 1988) it was subsequently found that a significant correlation existed between lipophilicity (log $P_{OCT/H_2O}$) and $pK_B$ underestimation in gastric assays. However, while significantly lower $pA_2$ values were found for muscarinic and $H_2$ receptor antagonists in the isolated whole mouse stomach, no evidence for discrepancies between gastric and cardiac $pA_2$ values was found in rat stomach preparations (Main & Pearce, 1981; Coruzzi *et al.*, 1984; Welsh *et al.*, 1992), thus suggesting species differences or differences due to the use of perfused (mouse) or non-perfused (rat) models. However, the ineffectiveness of zolantidine on rat gastric secretion models both *in vivo* and *in vitro* (Coruzzi *et al.*, 1994) indicates that other factors may be involved. For this reason the study was extended to other histamine $H_2$ receptor antagonists characterized by different degrees of lipophilicity. They include aminopotentidine (APT) and iodoaminopotentidine (I-APT), the activity of which have not been reported in functional gastric secretion assays, which both resemble zolantidine in chemical structure, being piperidinomethylphenoxypropyl derivatives. The compound SK&F 92857, which in a previous study (Shankley *et al.*, 1988) was found to be less effective in gastric tissues than in the heart, although being a relatively hydrophilic $H_2$ antagonist was also included. RAN was used as a reference histamine $H_2$ antagonist because it is equally effective at cardiac and gastric $H_2$ receptors. Lipophilicity of the molecules was expressed as CLOG $P_{OCT/H_2O}$ (theoretically determined partition coefficient in an octanol: water system) and as log $k'$ (capacity factor determined by reverse-phase high performance liquid chromatography), which also takes into account the ionisation of the molecule (Minick *et al.*, 1989; Sun *et al.*, 1994; Yamagami

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& Takao, 1993). Data from the literature concerning zolantidine are reported for comparison.

## Methods

*In vitro* experiments were carried out on the guinea-pig papillary muscle and the gastric fundus from immature rats; *in vivo* experiments were performed on anaesthetized rats with lumen-perfused stomachs.

### *Guinea-pig papillary muscle*

Essentially, a previously described technique was followed (Bertaccini & Coruzzi, 1981). Guinea-pigs of either sex weighing 250–300 g were killed by cervical dislocation and the hearts quickly removed. Left papillary muscles (2 to 3 mm in length and 0.3 to 0.4 mm in diameter) were rapidly dissected free from the ventricle and suspended under 1 g tension in 10 ml organ baths containing Ringer solution (mM, composition: NaCl 154, $NaHCO_3$ 5.9, KCl 5.0, $CaCl_2$ 2.1, glucose 5.6). The solution was kept at 37°C and gassed with 95% $O_2$ and 5% $CO_2$. Two platinum electrodes were used to stimulate the tissues electrically with square wave pulses at 2 Hz frequency, 1 ms duration and 20% above threshold voltage. Changes in force of contraction were recorded by an isometric transducer connected to a pen recorder. During a 60–90 min stabilisation period, tissues were repeatedly exposed to a threshold concentration (0.1 $\mu$M) of histamine at 15 min intervals until a stable response was obtained; after each administration the tissue was washed until the level of contractility had returned to baseline. Once the response to histamine had stabilized, a cumulative concentration-response curve (CRC) to histamine was constructed by increasing doses at 0.5 log unit intervals after each response had reached a plateau (3–4 min); once the maximal effect was obtained, the tissue was repeatedly washed until the pre-drug level of contractility was reached. Two agonist CRCs were constructed in each preparation. Preliminary experiments showed that the second CRC was superimposable, in terms of agonist potency ($EC_{50}$) and maximal response ($E_{max}$), provided that a 60 min interval was allowed between curves and frequent washouts (every 15 min) were made. For antagonist studies, the first CRC to histamine was followed by incubation of antagonist or vehicle for 30 min before a second CRC to histamine was obtained.

In a separate series of experiments, RAN (100 $\mu$M) was co-incubated with APT (10 $\mu$M) before the second CRC to histamine. RAN was incubated 30 min before histamine, whereas APT was added for the last 5 min, since in previous experiments it was shown that the inhibitory effects of this compound did not change following different incubation times (5, 10, 20, 40 or 60 min).

### *Expression of results: determination of antagonist potency*

Values are shown as mean ± s.e.mean. The response to histamine in antagonist-treated preparations was expressed as a percentage of the maximal response to histamine obtained under control conditions. The concentration of agonist required to elicit half maximal response ($EC_{50}$) was calculated from individual log concentration-response curves by linear regression analysis (over the range of 10–90% of the maximal response). For compounds which produced surmountable antagonism (at least three antagonist concentrations) linear regression analysis was used to calculate $pA_2$ values and the slopes of Schild plots (Arunlakshana & Schild, 1959). Concentration-ratios, determined at the $EC_{50}$ level, were derived from control and test curves within each preparation. For compound SK&F 92857 a $pA_2$ value was estimated from single concentrations, which produced surmountable antagonism, using the Gaddum equation: $pA_2 = -\log [B] + \log [CR-1]$, where B is the antagonist concentration and CR is the concentration-ratio at the $EC_{50}$ level. Since both APT and I-APT induced a non-parallel shift to the right of the control CRC to histamine with concentration-dependent depression of the maximal response, the potency of these antagonists was expressed by apparent $pK_B$ values which were calculated by applying the following equation: $K_B = [B]/\text{slope}-1$, in which the slope is that of the double-reciprocal plot of equieffective concentrations of agonist (A) in the absence (1/A) and in the presence (1/A') of the antagonist (B) and [B] represents the antagonist concentration (Kenakin, 1987).

### *Rat gastric fundus*

Fed immature male rats (30–45 g) were used according to the technique described by Coruzzi *et al.* (1984). They were killed by cervical dislocation, the stomach was removed and placed in a perspex tube at 34°C with the mucosa facing into the lumen. The mucosa was bathed in 5 ml oxygenated (100% $O_2$), unbuffered solution containing (mM): NaCl 136, KCl 5.0, $CaCl_2$ 2.4, $MgCl_2$ 2.4, glucose 16.7. The serosal surface was bathed in 30 ml of buffered solution containing (mM): NaCl 110, $NaHCO_3$ 26, KCl 5.0, $CaCl_2$ 2.4, $MgCl_2$ 2.4, glucose 16.7 and gassed with 95% $O_2$ and 5% $CO_2$. The unbuffered mucosal solution was replaced at 15 min intervals with 5 ml of pre-warmed and oxygenated mucosal saline; the acid content of mucosal samples was measured by titration to pH 7.0 with 0.01 M NaOH. CRCs to histamine were constructed by adding, to the serosal solution, single histamine concentrations to each stomach in a randomized order. Each agonist concentration was left in contact with the tissue until the response was fully developed (usually 60 min), then the tissue was washed. After return to baseline, for antagonist studies the stomach was incubated with the antagonist for 60 min before the second histamine administration. Only two concentrations of histamine were given to each stomach. Acid responses are expressed as mean values ± s.e.mean in $\mu$Eq $H^+$ $cm^{-2}$ $h^{-1}$. The affinity estimates of the different antagonists were determined as described for the papillary muscle; concentration-ratios were derived from the mean CRCs obtained in control and in antagonist-treated stomachs.

In a separate series of experiments the reversal of $H_2$ receptor antagonism was investigated by administering to different stomachs approximately equiactive inhibitory concentrations of the antagonists against a submaximal concentration of histamine (30 $\mu$M). The secretory response to histamine was measured before the antagonist, during incubation with antagonist and finally after a washout period. It was previously determined that up to three single administrations of histamine caused reproducible responses, provided a washout period of 60 min was allowed to elapse.

### *Lumen-perfused stomach of the anaesthetized rat*

Male Wistar rats (200–250 g) were used after a 18 h fasting with free access to water. After urethane anaesthesia (1.25 g $kg^{-1}$, i.p.) the stomach was perfused at constant volume (60 ml $h^{-1}$) with saline (0.9% NaCl) at 37°C through an oesophageal cannula and the perfusion fluid was collected via a duodenal cannula. Changes in the acid concentration of the perfusate were recorded at 20 min intervals by automatic titration with 0.01 M NaOH to pH 7 (Radiometer, Copenhagen). The antagonists were administered by single i.v. bolus injections when acid secretion induced by a continuous infusion (6 ml $h^{-1}$) of submaximal doses (20 $\mu$mol $kg^{-1}$ $h^{-1}$) of histamine had reached a plateau. Only one dose of antagonist was used in each experiment. The inhibitory effect of the antagonist was expressed as percentage inhibition of the agonist effect (pre-drug level taken as 100%); the antagonist dose required to inhibit 50% of the agonist response ($ID_{50}$ value) was calculated from the inhibitory dose-response curves. Acid secretory responses are reported as mean values ± s.e.mean in $\mu$Eq $H^+$ $kg^{-1}$ $min^{-1}$.

### *CLOG P determination*

Logarithms of octanol:water partition coefficients for the histamine $H_2$ receptor antagonists under study were theoretically determined (CLOG $P_{OCT/H_2O}$) according to the method described by Hansch & Leo (1979).

### *log k' determination*

This hydrophobic parameter was determined by reverse-phase high performance liquid chromatography (r.p.-h.p.l.c.), according to the methods previously described (Minick *et al.*, 1989). The chromatographic data were obtained using a Water Assoc. (Milford, MA, U.S.A.) Model 600-ms pump, a Model 717 plus autosampler and a Model 484 variable wavelength detector operating at 254 nm. An ultrasphere ODS (150 × 4.6 mm i.d.) Beckman, with 5 mm average particle size, was used. The composition of the mobile phase used was as follows: phosphate buffer 7.5 mM (pH = 7.4) 40% (v/v), $CH_3OH$ 60% (v/v). Data were processed with a PC 486/33i (NEC Technologies Inc.) equipped with software Waters Millenium 2080. Values of *k'* (capacity factor) for $H_2$ antagonists were determined from retention times of the sample compound ($t_r$) and of the unretained reference compound ($t_0$) by means of the equation, $k' = (t_r - t_0)/t_0$.

### *Statistical analysis*

All data in the figures and text are given as mean values ± s.e.mean. $n$ = number of experiments. Statistical significance levels of the differences between groups were determined by one-way analysis of variance or Student's $t$ test for paired or unpaired data where appropriate and a $P$ value of less than 0.05 was considered to be significant. For statistical analysis the computer programme by Tallarida & Murray (1987) was used.

### *Drugs*

Histamine dihydrochloride, forskolin, adrenaline hydrochloride were purchased from Sigma Chemical Co (St. Louis, MO, U.S.A.); ranitidine hydrochloride was obtained from Glaxo (Verona, Italy); aminopotentidine and iodioaminopo-

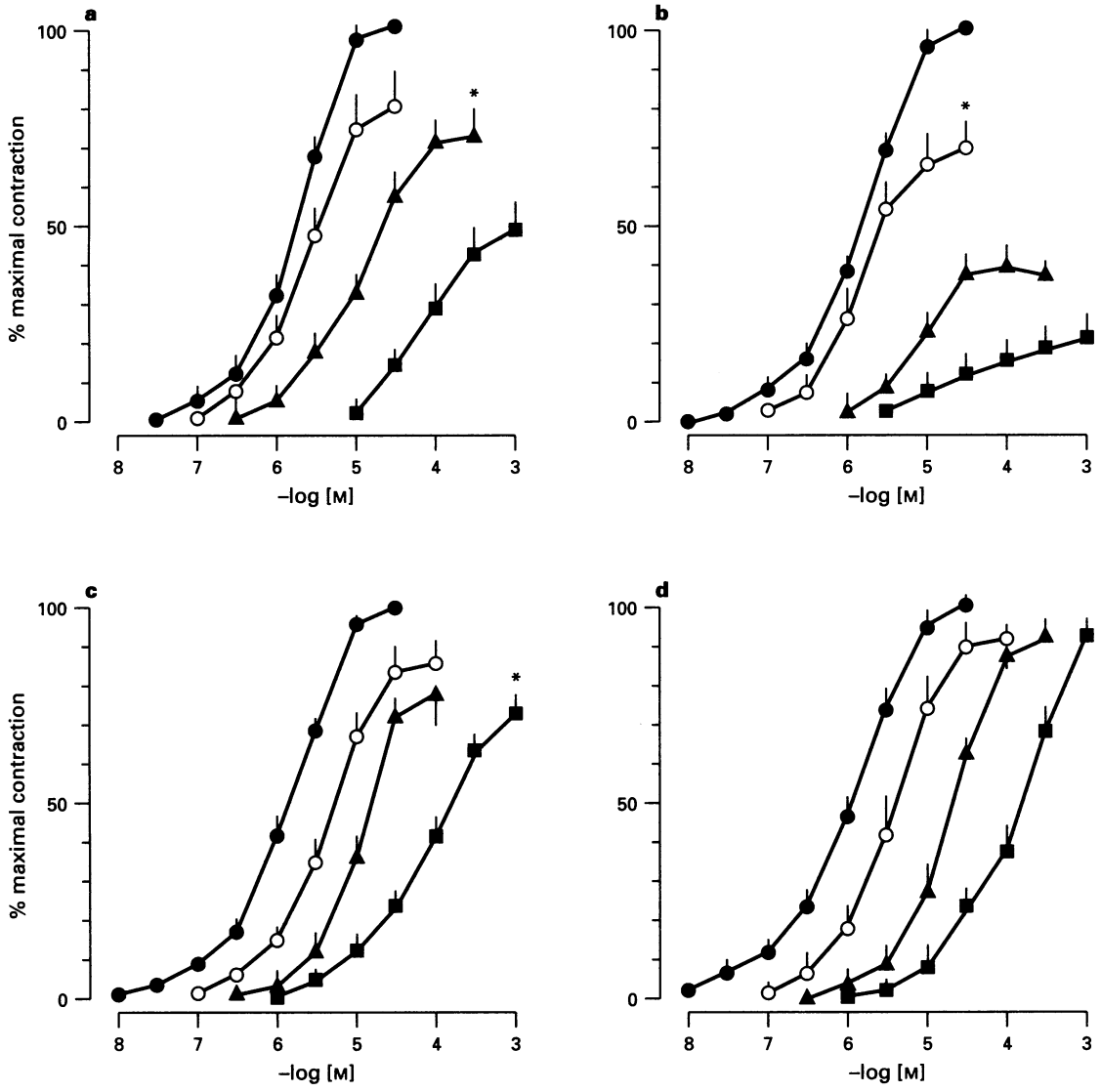


**Figure 1** Guinea-pig isolated papillary muscle: histamine concentration-effect curves obtained in the absence (●) and in the presence of: (a) APT 0.1 (○), 1 (▲) and 10 (■) μM; (b) I-APT 0.1 (○), 1 (▲) and 10 (■) μM; (c) compound SK&F 92857 0.1 (○), 1 (▲) and 10 (■) μM; (d) RAN 1 (○), 10 (▲) and 100 (■) μM. Values are mean ± s.e.mean of 6 measurements. *$P < 0.05$ vs control histamine. For abbreviations, see text.

tentidine were synthesized by Dr Wiro Menge at the department of Medicinal Chemistry of the Leiden-Amsterdam Center for Drug Research; compound SK&F 92857 was a gift from Smith Kline Beecham Pharmaceuticals (UK). Stock solutions of forskolin (10 mM) were prepared in absolute ethanol and stored at 4°C; 1 mM dilutions were made in 50% ethanol and further dilutions in distilled water. APT and I-APT were dissolved in 100% dimethyl sulphoxide (DMSO) and stock solutions (10 mM) were stored at −20°C. The 1 mM dilutions were prepared in 50% DMSO and further dilutions made in distilled water. Separate experiments showed that DMSO had no effect on tissue responsiveness. All the other drugs were prepared in distilled water on the day of the experiments.

## Results

### *Guinea-pig papillary muscle*

In this preparation none of the $H_2$ antagonists tested reduced basal contractility (data not shown). APT (0.1 – 10 μM) and I-APT (0.1 – 10 μM) produced a concentration-dependent antagonism of the contractile response to histamine with a marked depression of the maximal response (Figure 1a and b). Compound SK&F 92857 (0.1 – 1 μM) produced a parallel shift to the right of the concentration-response curve (CRC) to histamine, yielding a $pA_2$ value of 7.38 ± 0.11 ($n = 11$) (Figure 1c). At 10 μM it caused a significant depression of the maximal response (Figure 1c). In contrast, RAN (1 – 100 μM) shifted the histamine CRC to the right in a parallel fashion, while leaving the maximal contraction unchanged (Figure 1d). Table 1 shows the affinity estimates for the above compounds in addition to the data previously obtained (Coruzzi *et al.*, 1994) with zolantidine.

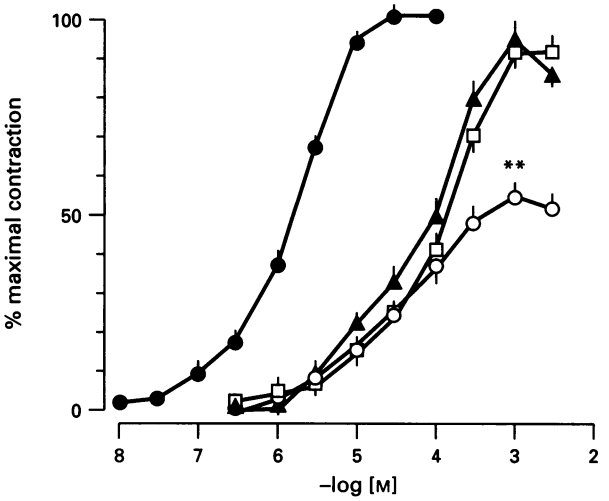


**Figure 2** Guinea-pig isolated papillary muscle: effects of APT 10 μM (○), ranitidine (RAN) 100 μM (□) and the combination (▲) of APT 10 μM and RAN 100 μM on the concentration-effect curves to histamine (control, ●). The tissues were treated first with RAN for 25 min, followed by APT in the last 5 min. Values are mean ± s.e.mean of 6 measurements. $^{**}P < 0.01$ vs control histamine. For abbreviations, see text.

**Table 1** Activity of a series of histamine $H_2$ receptor antagonists with different lipophilicity (CLOG $P_{OCT/H_2O}$) on cardiac and gastric tissues

| *Compound* | *Guinea-pig papillary muscle* | *Rat gastric fundus* | *Lumen-perfused stomach* | *CLOG P* |
|---|---|---|---|---|
| **Ranitidine** | 6.42 ± 0.09[a] (0.89 ± 0.04) | 6.78 ± 0.38[a] (0.99 ± 0.04) | 0.036 ± 0.01[b] | 0.27 |
| **Zolantidine*** | 6.78 ± 0.27[a] (0.94 ± 0.03) | Inactive up to 100 μM | Inactive up to 30 μmol kg$^{-1}$ | 5.58 |
| **Aminopotentidine** | 6.34 ± 0.37[c] | 6.20 ± 0.16[c] | 0.018 ± 0.02[b] | 2.96 |
| **Iodoaminopotentidine** | 6.81 ± 0.26[c] | 6.89 ± 0.19[c] | 0.020 ± 0.03[b] | 4.33 |
| **SK&F 92857** | 7.38 ± 0.11[d] | Inactive up to 10 μM | NT | 1.02 |

[a][i.e. apparent $pK_B$] $pA_2$ calculated with the Schild regression; in parenthesis Schild plot slopes (not significantly different from unity) [b]$ID_{50}$ value in μmol kg$^{-1}$, i.v.; [c]calculated according to Kenakin (1987); [d]$pA_2$ value was determined from only two antagonist concentrations, since higher concentrations depressed the maximal response to histamine. NT = not tested. *data from Coruzzi *et al.* (1994).

APT and I-APT, both administered at a concentration (10 μM) which significantly reduced the maximal effect of histamine, did not modify the response to adrenaline (data not shown). To investigate further the nature of the insurmountable antagonism of APT, receptor protection experiments were performed by co-incubating APT 10 μM with RAN 100 μM. Since in preliminary experiments it was observed that the degree of antagonism by APT 10 μM did not change when using either 5 or 20 or 40 min incubation time, RAN 100 μM was incubated 30 min before histamine, whereas APT 10 μM was added for the last 5 min. In the presence of APT 10 μM the maximal contractile response to histamine was restored to 94% of the control by 100 μM RAN (Figure 2).

### *Rat gastric fundus*

All the compounds tested did not significantly alter basal acid secretion in the range of concentrations 0.1–10 μM (data not shown). APT (0.1–1 μM) and I-APT (0.1–1 μM) produced concentration-related, non-parallel shifts to the right of the CRCs to histamine and decreased the maximum response by 80–85% at the highest concentrations tested (Figure 3a and b). Compound SK&F 92857 (1–10 μM) failed to affect the acid response to histamine (Figure 3c). In contrast, RAN (0.3–3 μM) produced the expected parallel, rightward shift of the CRC to histamine, with no depression of the maximal secretory response (Figure 3d). Quantitative data concerning the affinity estimates of the different compounds tested are shown in Table 1.

APT (1 μM), I-APT (1 μM) and RAN (3 μM) did not modify the acid response to the intracellular secretagogue forskolin (Figure 4).

Reversibility experiments showed that washout of the tissues induced a recovery of the response to histamine (30 μM) after 1 h in the case of ranitidine and APT, whereas there was no sign of recovery in the case of I-APT (Figure 5).

### *Lumen-perfused stomach of the anaesthetized rat*

Histamine (20 μmol $kg^{-1}$ $h^{-1}$) caused an increase in acid secretion which was maximal after 45–60 min and was stable up to 4–5 h (Figure 6). Both APT (0.003–0.3 μmol $kg^{-1}$, i.v.) and I-APT (0.03–0.1 μmol $kg^{-1}$, i.v.) produced a dose-dependent inhibition of the acid response to histamine, with a potency comparable to that of RAN (Table 1). In some experiments, the duration of action of maximal doses of APT and I-APT was studied in comparison with RAN. In the case of RAN, a complete recovery from inhibition was observed after 4–5 h. In contrast, in the case of APT and I-APT, only 5–10% recovery was evident after 6 h (Figure 6). Compound SK&F 92857 was not tested in this assay, because of the small amount of material available.

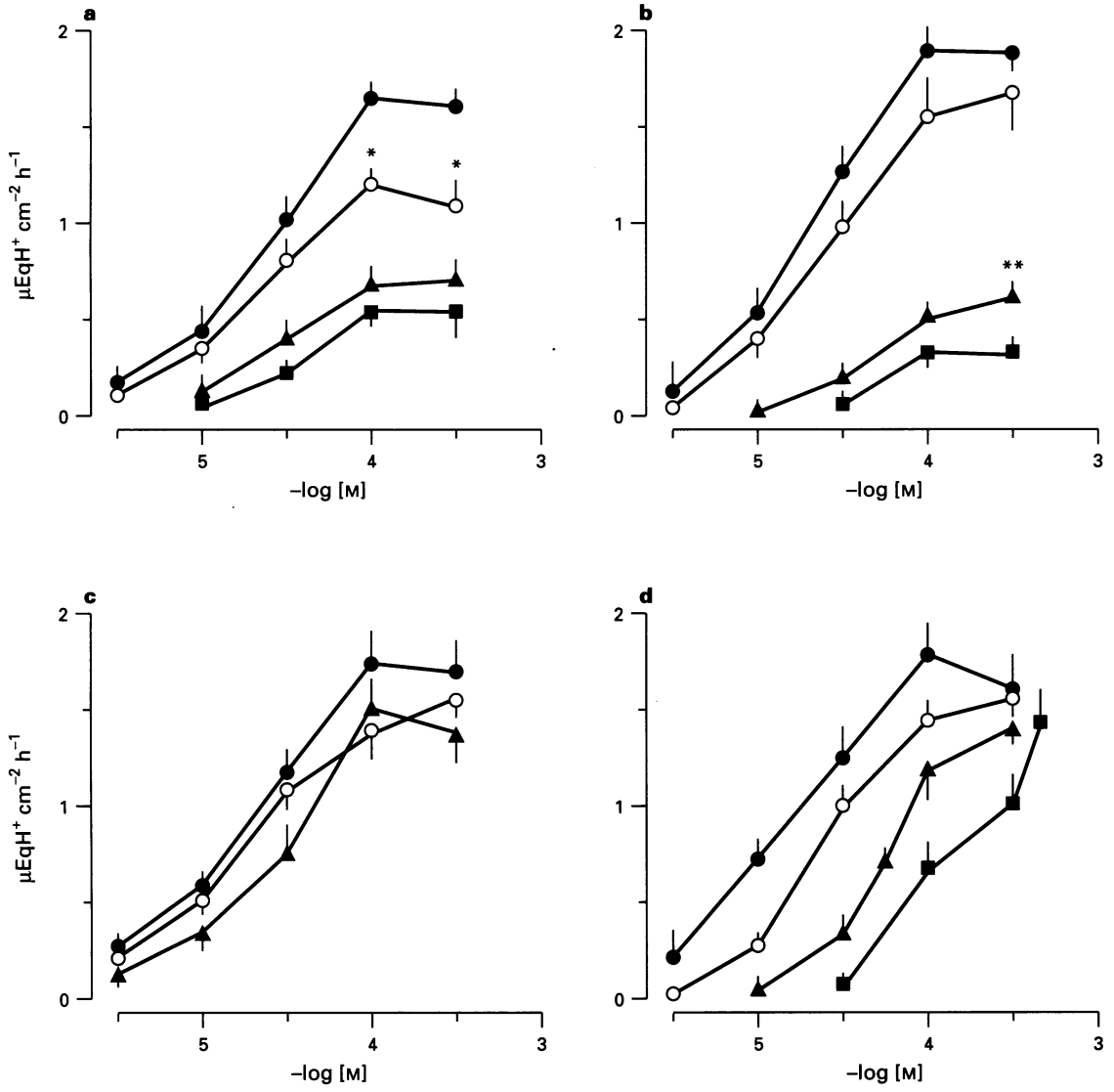


**Figure 3** Isolated gastric fundus of the immature rat: histamine concentration-effect curves obtained in the absence (●) and in the presence of: (a) APT 0.1 (○), 0.3 (▲) and 1 (■) μM; (b) I-APT 0.1 (○), 0.3 (▲) and 1 (■) μM; (c) compound SK&F 92857 1 (○) and 10 (▲) μM; (d) RAN 0.3 (○), 1 (▲) and 3 (■) μM. Values are mean ± s.e.mean of 6–8 measurements. $*P<0.05$ and $**P<0.01$ vs control histamine. For abbreviations, see text.

*Lipophilicity studies*

Calculated LOG $P_{OCT/H_2O}$ values for the various $H_2$ antagonists are presented in Table 1; zolantidine is by far the most lipophilic drug followed by I-ATP and APT. Compounds SK&F 92857 and RAN are moderately hydrophilic drugs. Log $k'$ values for the compounds under study are presented in Figure 7. A significant correlation ($r=0.99$) was found between this parameter and the CLOG P value.

## Discussion

We have examined the cardiac and gastric effects of a series of histamine $H_2$ receptor antagonists characterized by different degrees of lipophilicity, in order to find out whether the physicochemical properties of the antagonist could explain a lower affinity in the gastric assay. Results obtained in the present study showed that the lipophilic $H_2$ receptor antagonists, APT and I-APT, are able to antagonize the response to histamine in both gastric and cardiac tissues, whereas the hydrophilic antagonist, compound SK&F 92587 antagonized histamine responses in cardiac but not in gastric preparations. The inhibitory activity of APT and I-APT on gastric secretion models was rather unexpected, considering that they are lipophilic compounds, with partition coefficients (octanol:water) similar to that of zolantidine, an antagonist which was unable to inhibit histamine responses in the stomach (Coruzzi *et al.*, 1994). Discrepancies between gastric and cardiac effects have been described for some histamine $H_2$ receptor agonists and antagonists by different groups (Black *et al.*, 1985; Shankley *et al.*, 1988; Coruzzi *et al.*, 1995; Buschauer *et al.*, 1995). 'Cardioselective' histamine $H_2$ receptor agonists have been proposed, due to their lower activity on gastric secretion relative to the heart and these

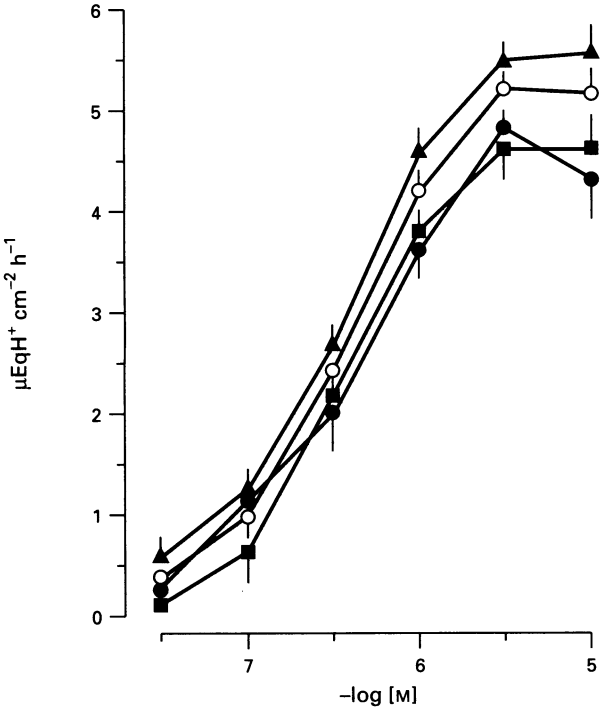


**Figure 4** Isolated gastric fundus of the immature rat: concentration-effect curves to forskolin in the absence (●) and in the presence of RAN 3 μM (▲), APT 1 μM (○) and I-APT 1 μM (■). Values are mean±s.e.mean of 5 measurements. For abbreviations, see text.

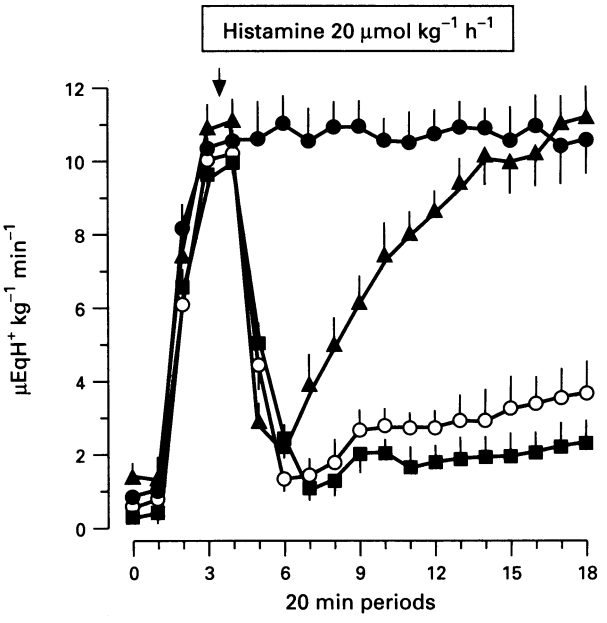


**Figure 6** Lumen-perfused stomach of the anaesthetized rat: time course of the inhibitory effects of RAN 1 (▲), APT 0.3 (○), and I-APT 0.1 (■) μmol kg$^{-1}$, i.v. on the acid response to continuous i.v. infusion of histamine (●). The $H_2$ antagonists were administered at the arrow. Values are mean±s.e.mean of 6 measurements. For abbreviations, see text.

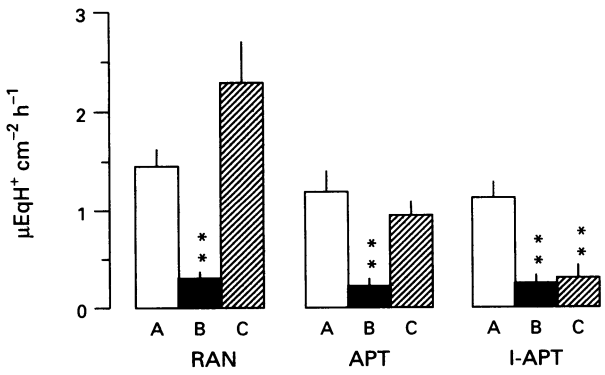


**Figure 5** Isolated gastric fundus of the immature rat: reversibility experiments showing the effect of washout on the action of ranitidine (RAN), APT and I-APT. Columns represent the response to histamine in the absence (A) or in the presence (B) of the $H_2$ antagonists and (C) after washout of the tissue (see text for details). Mean values±s.e.mean. $^{**}P<0.01$ vs the corresponding control response (A). For abbreviations, see text.

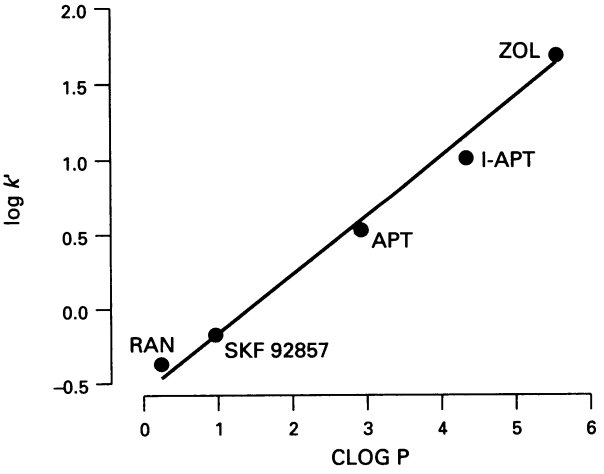


**Figure 7** Correlation between CLOG $P_{OCT/H_2O}$ and log $k'$ for a series of $H_2$ antagonists. See text for abbreviations of the compounds.

compounds could have a possible therapeutic value for the management of congestive heart failure (Buschauer, 1989). The tissue selectivity of these substances was attributed mainly to pharmacokinetic factors rather than to heterogeneity between gastric and cardiac histamine $H_2$ receptors. In fact, most of the functional studies employing isolated gastric glands or oxyntic cells rather than intact tissues, did not reveal $pK_B$ or $pA_2$ estimates for histamine $H_2$ antagonists different from those found in the guinea-pig atrium. Zolantidine inhibited histamine-stimulated adenylate cyclase in guinea-pig gastric mucosal homogenates ($pK_i = 7.2$, Parsons, personal communication), indicating that this compound has a relatively high affinity for the gastric mucosal $H_2$ receptor. Moreover, preliminary binding experiments performed with guinea-pig gastric and cardiac membranes showed that zolantidine was able to displace [$^{125}$I]-APT from $H_2$ receptors with a comparable affinity in heart and stomach assays (Coruzzi, unpublished). Taken together, these findings suggest that the lack of effect of zolantidine in intact tissues is due to kinetic factors rather than heterogeneity of gastric histamine $H_2$ receptors. In a very extensive study (Shankley *et al.*, 1988), performed in the isolated mouse whole stomach, a good correlation was found between log $P_{OCT/H_2O}$ (as a measure of the lipophilicity of the molecule) and the underestimation of $pK_B$ values of lipophilic $H_2$ antagonists. However, the activity of APT and I-APT in gastric secretion models and the lack of antisecretory effect of compound SK&F 92857, which is a more hydrophilic substance, does not reconcile with the hypothesis that the lipophilicity is the main factor in determining the discrepancies between gastric and cardiac effects of $H_2$ antagonists. These data are in accordance with previous data (Shankley *et al.*, 1988) obtained with the $H_2$ antagonist, oxmetidine. In fact, this compound did not distinguish between cardiac and gastric effects, although having a log $P_{OCT/H_2O} = 2.12$. It is possible, however, that the partition coefficient octanol:water, although being one of the most common parameters used to explain brain penetration of neutral organic compounds, is not the most appropriate parameter for revealing possible differences in activities at gastric and cardiac $H_2$ receptors. Therefore an effort was made to explain such differences by calculating a hydrophobic parameter (log $k'$) on r.p.-h.p.l.c. utilizing a mobile phase containing a 7.5 mM phosphate buffer (pH = 7.4) and $CH_3OH$. In contrast to log P, this parameter takes into account the ionisation of the molecule as do other parameters, such log $D_{OCT/7.4}$ or $\Delta$ log $P_{OCT-ALK}$, which have been used to describe the ability of ionisable drugs to cross the blood-brain barrier (Young *et al.*, 1988; Ter Laak *et al.*, 1994). The significant correlation found in our study between CLOG $P_{OCT/H_2O}$ and log $k'$ indicates that ionization has a similar influence for all molecules on their partition between liphophilic and aqueous phase, and, as a consequence, it does not explain the discrepancies observed between gastric and cardiac effects.

### *Insurmountable antagonism by APT and I-APT*

These compounds produced a rightward displacement of the CRC to histamine with a reduction in the maximal response in both cardiac and gastric assays. Our data do not entirely agree with previous functional studies carried out in the guinea-pig isolated atrium, from which $pA_2$ values of 7.22 and 7.56 were calculated for APT and I-APT, respectively, with Schild plot slopes which were not significantly different from unity (Hirschfeld *et al.*, 1992). In the guinea-pig papillary muscle and rat gastric fundus APT and I-APT exhibited a lower potency than in the atrium and, in addition, produced insurmountable antagonism. It was confirmed, however, that I-APT was slightly more potent than APT. Since there is no evidence that histamine $H_2$ receptors in the atrium and papillary muscle are different, it is possible that kinetic factors related to the high lipophilicity of these compounds are responsible for these differences. In the case of $H_2$ receptor agonists, some discrepancies were obtained between atrium muscle chronotropic and papillary muscle inotropic responses. Those compounds with more lipophilic structural components expressed significantly lower $pD_2$ values in the papillary muscle (Buschauer, 1989). Radioligand binding studies in guinea-pig cerebral membranes revealed that APT and I-APT have high affinity for $H_2$ receptors ($pK_i = 8.01$ and 9.15, respectively), being significantly more potent in binding assays than in functional experiments (guinea-pig atrium); this presumably reflects an easier access of these compounds to the $H_2$ receptors in membrane preparations rather than an $H_2$ receptor heterogeneity (Hirschfeld *et al.*, 1992).

Several different mechanisms can account for the insurmountable antagonism produced by APT and I-APT in heart and stomach: non-specific effects involving sites other than $H_2$ receptors, allosteric interaction, irreversible or slowly reversible antagonism. The possibility of non-specific effects was investigated in experiments in which stimuli other than histamine were used. However, neither APT nor I-APT modified the acid secretion induced by forskolin (which acts at intracellular sites beyond the $H_2$ receptor) and the inotropic response to adrenaline in the papillary muscle, indicating that both compounds are selective for $H_2$ receptors at concentrations that depressed the maximal response to histamine. Indeed, other studies have previously shown that these compounds are highly selective for $H_2$ receptors and [$^{125}$I]-APT has been considered the most valuable probe for labelling $H_2$ receptors in different tissues (Ruat *et al.*, 1990; Hirschfeld *et al.*, 1992). Receptor-protection experiments in the present study showed that the co-administration of the surmountable antagonist ranitidine was capable of restoring the maximal response to histamine after APT, suggesting that APT and ranitidine interact with a common binding site. Similar results were obtained in preliminary experiments with I-APT but not with zolantidine; the depression of the maximal histamine response produced by zolantidine was maintained in receptor-protection experiments (unpublished data). This clearly differentiates the insurmountable antagonism caused by high concentrations of zolantidine, which is reported to be due to an interaction with non-histamine sites (Calcutt *et al.*, 1988; Coruzzi *et al.*, 1994).

An alternative explanation for the insurmountable antagonism caused by APT and I-APT might be that the incubation time of 30 min was not sufficient for equilibrium to be reached. However, this is unlikely since the inhibitory potency of these compounds was similar after 5 or 60 min incubation times, indicating that the equilibrium is rapidly reached, at least in the papillary muscle. This is not consistent with the possibility that APT and I-APT act as irreversible antagonists at $H_2$ receptors, since irreversible antagonists exhibit a time-dependent antagonism (Kenakin, 1987). However, the resistance to washout in isolated preparations and the long duration of action observed in anaesthetized animals indicate that both APT and, to a greater extent, I-APT may slowly dissociate from the receptor. In accordance with this finding, a significantly longer time was required for full development of the histamine responses in the presence of these drugs. A slow dissociation from receptors has been proposed to explain the insurmountable antagonism induced by angiotensin non-peptide antagonists at $AT_1$ receptors (Robertson *et al.*, 1992), whereas an allosteric interaction might explain the insurmountable antagonism by methysergide at vascular 5-HT receptors (Kaumann & Frenken, 1985). Moreover, conformational changes in the receptor induced by the insurmountable antagonist could reduce the efficiency of stimulus-response coupling, according to the allosteric model proposed by de Chaffoy de Courcelles *et al.* (1986). All these models implicate an accessory binding site and a change in the state of the receptor as a consequence of insurmountable antagonist interaction. However, the recently proposed two-state receptor model (Robertson *et al.*, 1994), can explain the insurmountable antagonism at angiotensin $AT_1$ receptors without postulating extra binding sites. From our experiments,

however it is difficult to establish which is the best model to explain the insurmountable antagonism induced by APT and I-APT.

The more pronounced rightward shift of the CRC to histamine observed in the papillary muscle in comparison with rat gastric fundus could be related to a larger receptor reserve for histamine $H_2$ receptors in the heart, so that APT and I-APT, although reducing agonist-receptor occupancy will not produce a depression of the maximal response. A different number of spare receptors could also explain why APT and I-APT did not produce insurmountable antagonism in the right atrium (Hirschfeld *et al.*, 1992); in this tissue, in fact, a receptor reserve of approximately 97% at maximal histamine responses has been calculated (Kramer *et al.*, 1987).

Finally, functional studies in intact tissues have to consider a variety of factors which can influence the estimation of agonist and/or antagonist affinity: uptake or metabolism of the agonist, uptake of the antagonist, intracellular effects, difficulty in reaching the receptor, equilibration of the antagonist, etc. Thus, before postulating heterogeneity of histamine $H_2$ receptors, kinetic properties of $H_2$ antagonists under study have to be carefully investigated, since they could be responsible for the anomalous data obtained in functional experiments (insurmountable antagonism, differences in affinity estimates, etc). On the other hand, it seems interesting that the physicochemical properties of the antagonist could address the action to specific tissues: in particular, the lack of gastric antisecretory effects could be of therapeutic value for any $H_2$ antagonist which might be used for the treatment of non-gastrointestinal diseases, like schizophrenia or other psychiatric disorders (Deutsch *et al.*, 1993).

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