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In vivo characterization of histamine H_1- and H_2-receptors in the rat stomach microcirculation

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1 Using a video microscope system, an *in vivo* microcirculation preparation was designed to characterize histamine receptors in the rat stomach submucosal arterioles (diameter 50 μm).

2 Each *in vivo* stomach microcirculation preparation received topically applied multiple concentrations of either the selective histamine H_1- orH_2- receptor agonist and antagonist and the respective responses (changes of the arteriolar diameter) were used to construct the concentration-response curves.

3 Results showed that both 2-thiazolylethylamine and impromidine, the respective selective H_1- and H_2-receptor agonists, produced concentration-dependent vasodilator responses in the *in vivo* stomach submucosal arterioles. These vasodilator responses were competitively and selectively blocked by the respective H_1-receptor antagonist mepyramine and the H_2-receptor antagonist cimetidine.

4 Data from each *in vivo* preparation were examined separately to yield a Schild plot and a Hill plot, from which the *in vivo* estimates of the pA_2 value, the slope of the Schild plot, and the Hill coefficient were obtained.

5 The estimated pA_2 values for mepyramine (9.60 ± 0.033, mean ± s.e. mean) and cimetidine (5.98 ± 0.037) conformed to similar values found in other tissues, showing that the rat stomach microvascular H_1- and H_2-receptors are of the same nature as similar receptors elsewhere.

6 Both the Hill and the Schild plots yielded regressions with unity slopes, indicating that the agonist-receptor and the antagonist-receptor reactions followed a simple one-to-one stoichiometry.

7 The findings in the present study are discussed and compared with those from other *in vitro* tissue preparations; it appears that the present *in vivo* technique is a satisfactory system for characterizing receptors in the vascular smooth muscle of the microcirculation.

Introduction

The pharmacological action of histamine has been shown to be mediated by two distinct classes of receptors, namely a mepyramine-sensitive histamine H_1-receptor system (Ash & Schild, 1966) present in the smooth muscle in the gut and bronchi, and a burimamide-sensitive histamine H_2-receptor system (Black, Durant, Duncan, Ganellin & Parsons, 1972) found in the acid secreting gastric parietal cell, atria and the rat uterus. In the circulatory system, there is evidence showing that the vasodilator effects of histamine involved both H_1- and H_2-receptors in producing systemic hypotension (Black *et al.*, 1972) and in dilating arteries in canine skeletal muscle (Powell & Brody, 1976) and intestine (Pawlik, Tague, Tepperman, Miller & Jacobson., 1977), cat and rat mesentery (Guth & Smith, 1978*a*), rabbit ear (Galeno, Knuepfer & Brody, 1979), cat stomach (Harvey, Owen & Shaw, 1980) and rat brain (Gross, Harper & Teasdale, 1981).

In the rat stomach microcirculation, Guth & Smith (1978*b*) and Guth, Moler & Smith (1980) have demonstrated the dual involvement of H_1- and H_2-receptors in the histamine-induced arteriolar vasodilatation but they could not characterize these two receptors in their study because it was difficult to demonstrate H_2-receptor mechanism unless H_1-receptors were blocked. The present investigation is a further evaluation of the vasodilator response of arterioles in the rat stomach microcirculation to the selective stimulation of histamine receptors. The aim of the present study is two fold: first, it is designed to show the effects of two selective histamine receptor

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agonists, i.e., the H_1-receptor agonist 2-thiazolylethylamine dihydrochloride (Durant, Ganellin & Parsons, 1975) and the H_2-receptor agonist impromidine trihydrochloride (Durant, Duncan, Ganellin, Parsons, Blakemore & Rasmussen, 1978) in the arterioles of the submucosal microcirculation of the rat stomach. Hitherto, these two selective histamine receptor agonists have not been tested *in vivo* in any kind of vascular smooth muscle preparation. The second aim is to demonstrate that these vascular histamine H_1- and H_2-receptors are homogeneous with similar histamine receptors in other tissues.

Methods

Animal preparation

Before the start of the experiment, male Sprague-Dawley rats (100–120g) were deprived of solid Purina chow for 48 h but allowed free access to 10% (w/v) glucose solution. Pilot studies had determined that feeding with glucose solution was better for the subsequent *in vivo* video microscopy than merely giving tap water *ad libitum*. The rat was anaesthetized with sodium pentobarbitone (40 mg/kg, i.m.) and allowed to breathe spontaneously. A midline upper abdominal incision was made to expose the stomach which was exteriorised. The stomach was next slit opened along its greater curvature, avoiding as far as possible major anastomosing blood vessels (Koo, 1982). In this manner, the stomach wall was converted into a flat sheet so that the mucosal surface was exposed and could be directly visualised. The rat in the right lateral position was then transferred to a specially designed microscope stage while the right half of the intact full-thickness stomach wall was mounted by pins on a raised circular Perspex viewing platform, with the mucosal surface uppermost. A shallow groove around the circular viewing platform had been previously filled with silicone rubber to provide a substrate for pins. Rectal temperature of the rat was maintained near 37°C by means of a heating pad.

The *in vivo* stomach preparation, once exposed, was continuously suffused with Krebs solution which contained the following (in mM): NaCl 118, KCl 4.7, $MgSO_4.7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, CaCl 2.5 and glucose 11.6. It was equilibrated with a gas mixture of 95% O_2 and 5% CO_2 in order to give a pH 7.4 at 37°C. The Krebs solution was delivered to the *in vivo* stomach preparation by a peristaltic pump (Watson-Marlow, Model 501) via a glass tube (i.d. 3 mm, length 300 mm) wrapped with a heating coil. A built-in thermistor at the tip of the glass tube detected the temperature of the suffusing solution and fed back to an electronic circuit which controlled the current passing through the coil to stabilize the temperature. Connected to a syringe infusion pump (Sage Instruments, Model 355), a piece of polyethylene tubing (size PP50) was inserted inside the glass tube but terminated about 20 mm from the tip of the glass tube. When the peristaltic pump delivered the suffusing Krebs solution via the glass tube at a rate of 1.8 ml/min and the syringe infusion pump delivered the drug solution via the polyethylene tubing at 0.2 ml/min, the overall rate of suffusion was 2.0 ml/min. Since the end of the polyethylene tubing was some distance from the tip of the glass tube, there was sufficient space for mixing of the drug with Krebs solution before the equilibrated solution was applied to the *in vivo* stomach preparation. The difference of infusion rates also dictated a 10 fold dilution of the drug. On account of the dead space in the suffusing system, it took 15–20 s for the diluted and mixed drug solution to reach the *in vivo* stomach preparation. A mock test was performed for every preparation with Evans Blue solution infused via the polyethylene tubing to ensure that good mixing had occured in the glass tube and that the Evans Blue solution was evenly distributed to the mucosal surface of the stomach.

The *in vivo* stomach preparation was observed by a video microscope system as has been described (Koo & Liang, 1977; 1979). Briefly, the preparation was transilluminated by a halogen-tungsten lamp and observed through a Leitz UM32x/0.30 long-working-distance objective lens. The image was optically coupled to a video camera through a microscope eyepiece (2.5 ×) and observed on the screen of a video monitor. In the present study, only the arterioles in the submucosal microcirculation of the non-secretory part of the stomach (the rumen) were observed. The rumen in the rat adjoined the oesophagus and had mainly squamous epithelium which appeared relatively transparent under *in vivo* transillumination.

The present opitcal-video system had a linear magnification of 500 ×. Prior calibration with a stage micrometer scale, allowed the diameter of an arteriole in the stomach submucosal microcirculation to be measured directly from the screen of the video monitor by a pair of sliding calipers. Pilot studies determined that the accuracy of the diameter measurement was ±0.5 mm on the video screen, or an equivalent of ± 1 μm referred to the microcirculation.

Histamine receptor stimulation and blockade

The histamine H_1- and H_2-receptors on the vascular smooth muscle of the arterioles in the rat stomach submucosal microcirculation were stimulated by topically applying cumulative concentrations of 2-thiazolylethylamine and impromidine respectively.

Essentially, the respective drug was freshly prepared before the start of the experiment by either dissolving or diluting in Krebs solution. The drug solution was then infused via the polyethylene tubing for 2 min, diluted 10 times while mixing with the suffusing Krebs solution in the glass tube and had a contact time of 100–105s with the vascular smooth muscle of the arteriole in the *in vivo* preparation. Pilot studies determined that such duration of contact time was sufficient to produce a maximal vasodilator response, and that a longer duration might cause reabsorption of the drugs to produce systemic effects in addition to local responses. The assumption made in the present experimental procedure was that the elicited responses were from a drug concentration the magnitude of which was equal to that in the suffusing solution. The time cycle required to complete each cumulative concentration-response curve varied between 25 to 35 min. The diameter of the arterioles was measured before and after the application of drugs.

Local blockade of histamine receptors at the microvascular site was performed by suffusing the *in vivo* stomach preparation with Krebs solution containing the desired concentration or either the selective H_1-receptor antagonist mepyramine maleate (May & Baker) or the selective H_2-receptor antagonist cimetidine (Smith, Kline & French) respectively. After 30 min of contact with the antagonist, the agonist in cumulative concentrations was topically applied as before. Usually, experiments with at least four different concentrations of each antagonist were performed.

Data analysis

For each *in vivo* stomach preparation, a family of cumulative concentration-response curves for each histamine receptor agonist was constructed by plotting the absolute changes of the diameter of an arteriole, with and without the respective selective receptor antagonism, versus the logarithm of the concentrations of the topically applied agonist, in a manner similar to that described by Van Rossum (1963) for *in vitro* systems. The effective concentrations of the agonist which produced the half-maximal response (the EC_{50}values) were determined from the curves and results were expressed as the geometric means with their 95% confidence intervals (Fleming, Westfall, De La Lande & Jelett, 1972). These EC_{50}values, in the absence and presence of the respective selective antagonists, were also used to calculate the concentration-ratios of the agonist for the different antagonist concentrations. A regression of log (agonist concentration-ratio − 1) on log (antagonist concentration) was plotted to yield a pA_2value (Schild, 1947; Arunlakshana & Schild, 1959) for each of the *in vivo* stomach preparations. The final estimate of pA_2 was expressed as the mean (± s.e.mean) of the individual estimates (Ott, Weiner, Cheng & Woodward, 1981).

The degree of saturation at equilibrium of the H_1- or H_2-receptors on the vascular smooth muscle of the arterioles was analysed using the Hill plot (Hill, 1910). Briefly, the data from the concentration-response curves were transformed to yield a Hill plot which was a linear regression of $\log(E_A/E_{max} - E_A)$ on log (agonist concentration) where E_{max} and E_A were responses (i.e., changes of arteriole diameter) at maximal stimulation and at a particular concentration of the agonist respectively. The Hill coefficient was the slope of the regression line.

Results

Results of the microcirculatory studies in the rat stomach submucosa showed that both histamine H_1- and H_2-receptor agonists caused arteriolar dilatation in all *in vivo* preparations and that the vasodilator responses were concentration-dependent (Figure 1). The size of the arterioles studied was similar in all rats, averaging $49.8 \pm 0.69\,\mu m$ (mean±s.e.mean, $n = 18$). The maximal increase of the diameter of the arterioles induced by the histamine receptor agonists was $10.4 \pm 0.15\,\mu m$, or an equivalent increase of 20.9 (20.1–21.8)% (mean, with 95% confidence intervals) above the control diameter. 2-Thiazolylethylamine dilated the arteriole over the approximate concentration range 3 nM to 1 μM while impromidine had its effective vasodilator concentration range from 10 nM to 3 μM (Figure 1). Table 1 summarizes the EC_{50} values for both 2-thiazolylethylamine and impromidine on the vascular smooth muscle of the arterioles in the rat stomach submucosal microcirculation. 2-Thiazolylethylamine was about 2.5 fold more potent than impromidine in eliciting the arteriolar dilatation response.

Experiments such as that illustrated in Figure 1a also showed that the H_1-receptor antagonist mepyramine produced concentration-related dextral displacements of the 2-thiazolylethylamine concentration-response curves without affecting significantly their slope or maximum. The threshold antagonistic concentration was 1 nM while the maximal response was never affected by the antagonist. Similar results were obtained for the cimetidine antagonism of impromidine stimulation of the H_2-receptors (Figure 1b). The threshold antagonistic concentrations were between 1 and 3 μM. When 2-thiazolylethylamine was applied to the preparation pretreated with cimetidine (concentration range from 1 μM to 100 μM), no shifting of the

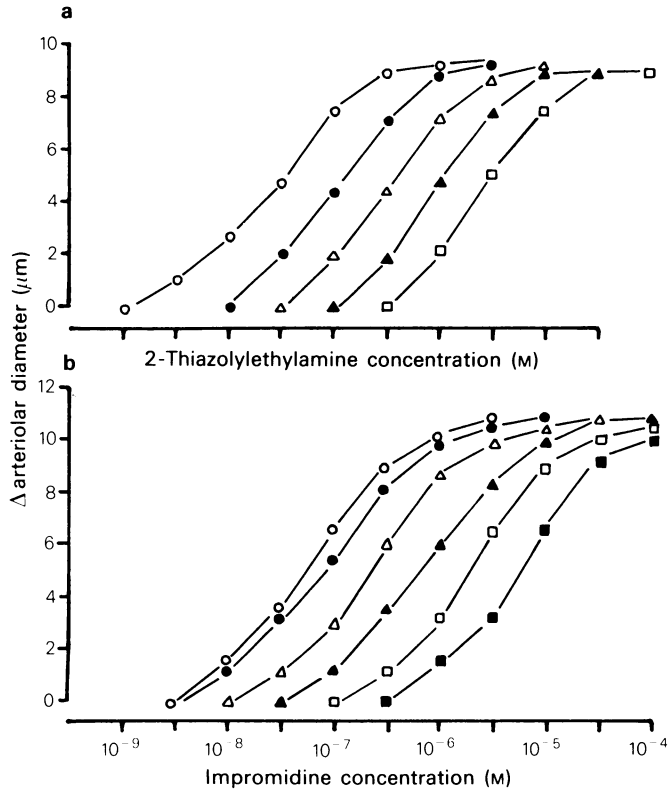

Figure 1 (a) Representative concentration-response curves of 2-thiazolylethylamine from an arteriole (diameter 49 μm) in the submucosal microcirculation of the rat stomach without the selective H_1-receptor antagonist (○) and after 30-min equilibration with the H_1-antagonist mepyramine 1 nM (●), 3 nM (△), 10 nM (▲) and 30 nM (□). The control diameter of the arterioles in the group ($n = 7$) was 50.5 ± 1.09 μm (mean ± s.e.mean). (b) Similar representative concentration-response curves in an arteriole (diameter 47 μm) showing stimulation of the selective H_2-receptor agonist impromidine and the blockade by the selective H_2-receptor antagonist cimetidine. Concentrations for cimetidine were 0 μM (○), 1 μM (●), 3 μM (△), 10 μM (▲), 30 μM (□) and 100 μM (■). The control diameter of arterioles for this group ($n = 11$) was 49.3 ± 0.95 μm. (At high concentrations of cimetidine, the impromidine concentration-response curves did not quite reach the maximum response compared to the response before cimetidine blockade, and this was attributed to the fact that 10^{-4} M impromidine was the highest concentration available in this study).

concentration-response curve was evident, thus confirming the lack of cimetidine effect on the H_1-receptor system. Similarly, mepyramine in the concentration range from 1 nM to 30 nM did not block the impromidine-induced vasodilator effect on the H_2-receptor system.

The respective pA_2 values for the 2-thiazolylethylamine-mepyramine pair and the impromidine-cimetidine pair are summarized in Table 2 and graphically represented in Figure 2. The slopes of the Schild plots were all close to, and did not deviate significantly from unity.

Table 1 Effective concentrations of histamine H_1- and H_2-receptor agonists which produced the half maximal response (the EC_{50} values), and the calculated Hill coefficient (slope of the Hill plot) for 2-thiazolylethylamine (selective H_1-receptor agonist) and impromidine (selective H_2-receptor agonist)

Agonist	*Number of preparations*	*EC_{50} ($\times 10^{-8}$ M)*	*Hill coefficient*
2-Thiazolylethylamine	6	2.37 (2.01–2.80)†	1.01 ± 0.019*
Impromidine	6	6.08 (5.81–6.35)†	0.98 ± 0.013*

† Values are geometric means (with 95% confidence intervals) and * values are means ± s.e.mean.

Table 2 pA_2 values for mepyramine (selective H_1-receptor antagonist) and cimetidine (selective H_2-receptor antagonist) reported in various tissues and in the present study

Species and tissue	*Agonist-antagonist pair*	*pA_2*	*Slope of Schild plot*	*Reference*
Guinea-pig, ileum	Histamine-mepyramine	9.46 ± 0.22**	—	Schild (1947)
Guinea-pig, ileum	Histamine-mepyramine	9.36	—	Marshall (1955)
Guinea-pig, ileum	Histamine-mepyramine	9.3	—	Arunlakshana & Schild (1959)
Guinea-pig, ileum	Pyridylethylamine-mepyramine	9.2	—	Arunlakshana & Schild (1959)
Guinea-pig, lung	Histamine-mepyramine	9.4	—	Arunlakshana & Schild (1959)
Guinea-pig, trachea	Histamine-mepyramine	9.394 ± 0.077**	—	Ison, Franks & Soh (1973)
Cat, cerebral arteries	Histamine-mepyramine	9.07	0.71	Edvinsson & Owman (1975)
Rat, stomach arterioles	2-Thiazolylethylamine-mepyramine	9.60 ± 0.033*	1.02 ± 0.013*	Koo (Present study)
Guinea-pig, atrium	Histamine-cimetidine	6.10 (6.04–6.17)†	0.81 (0.62–1.00)†	Brimblecombe *et al.* (1975)
Rat, uterus	Histamine-cimetidine	6.09 (5.92–6.27)†	0.96 (0.82–1.10)†	Brimblecombe *et al.* (1975)
Guinea-pig, atrium	Impromidine-cimetidine	6.53 (6.09–6.98)†	0.81 ± 0.21*	Durant *et al.* (1978)
Dog, parietal cell	Histamine-cimetidine	6.0	0.92 (0.81–1.03)†	Soll (1980)
Guinea-pig, papillary muscle	Histamine-cimetidine	6.41 ± 0.09*	—	Bertaccini & Coruzzi (1981)
Guinea-pig, papillary muscle	Impromidine-cimetidine	6.71 ± 0.19*	—	Bertaccini & Coruzzi (1981)
Guinea-pig, trachea	Dimaprit-cimetidine	6.10 ± 0 09*	0.97	Tomioka & Yamada (1982)
Rat, stomach arterioles	Impromidine-cimetidine	5.98 ± 0.037*	1.05 ± 0.026*	Koo (Present study)

*Mean ± s.e.mean, **mean ± s.d., †mean (with 95% confidence intervals).

Table 1 also summarises the results of the Hill plots from which slope of unity was obtained for all histamine H_1- and H_2-receptor stimulations in every *in vivo* stomach preparations. Figure 3 shows a representative set of Hill plots of both histamine H_1- and H_2-receptors stimulation and blockade in two *in vivo* stomach preparations.

Further experiments were performed to exclude the possibility of desensitizing the histamine receptors in the extended period during which the cumulative concentration versus response curves were obtained. For instance, in the H_2-receptor system, after a control impromidine concentration-response curve and a further curve with cimetidine pretreatment were obtained, the procedure was repeated using only impromidine to obtain a third curve. Results as exemplified in Figure 4 showed that these three curves (i.e., one before and one after cimetidine treatment, and one after two applications of multiple concentrations of impromidine) had similar slopes and similar maxima. Without cimetidine, there was no shifting of the curve. A similar result was also obtained for the H_1-receptor system.

In a separate study (Koo, unpublished data) comparing the effects of histamine, acetylcholine and pentagastrin on the stomach submucosal arterioles, it was found that pentagastrin produced a greater maximal response than histamine or acetylcholine, although acetylcholine was the most potent vasodilator among these three drugs. However, in the present study, pilot experiments showed that both 2-thiazolylethylamine and impromidine produced similar maximal responses in the same arteriole, and a single application of a high concentration of either of the two agonists did not produce a greater-than-maximal response.

Discussion

The experimental results obtained in the present study on the rat stomach confirmed previous findings by other investigators on the stomachs of cats and rats (Guth *et al.*, 1980, Harvey *et al.*, 1980) that vasodilator responses were mediated by both histamine H_1- and H_2-receptors. However, the present study showed that it was not necessary to block the H_1-receptor system in order to elicit the H_2-receptor-mediated responses. The 2-thiazolylethylamine- and the impromidine-induced vasodilatations were also in agreement with studies on the rat gastric submucosal microcirculation (Guth *et al.*, 1980) and in the femoral and the mesenteric vasculatures of the cat (Owen, Harvey & Gristwood, 1979) respectively.

In the present study, the agonists concentration-response curves were assumed to follow an expression similar to the one proposed by Hill (1910), viz.,

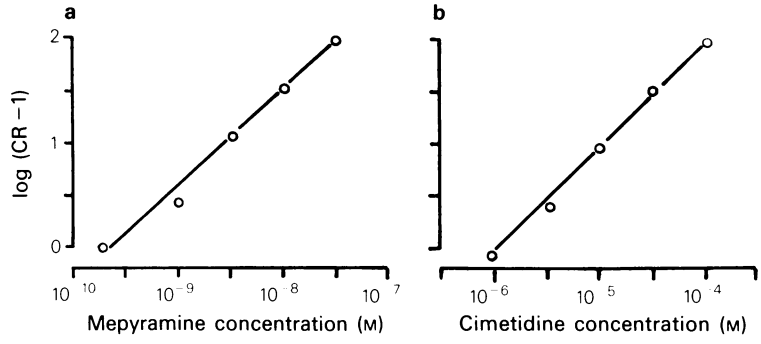

Figure 2 (a) Representative Schild plot showing the antagonism of 2-thiazolylethylamine by mepyramine on the vasodilator response of an arteriole in the submucosal microcirculation of the rat stomach. Antagonism was expressed by 2-thiazolylethylamine concentration-ratio (CR) required for equal responses with and without mepyramine blockade. Each concentration-ratio (CR) was calculated from the ratio of the EC_{50} values (effective concentrations producing the half-maximal response) estimated from each of a separate pair of concentration-response curves shown in Figure 1(a). The pA_2 value of mepyramine in this *in vivo* preparation is 9.55 with the Schild slope of 1.02. (b) Similar representative Schild plot for the antagonism of impromidine by cimetidine. The pA_2 value of cimetidine in this *in vivo* preparations is 6.08 with the Schild slope of 0.97.

$E_A/E_{max} = 1/(1 + K_A/A^n)$ where E_{max} and E_A were maximal response and response at a particular agonist concentration A, respectively. K_A was the dissociation constant of the agonist-receptor complex, and n was the Hill coefficient. If the agonist-receptor reaction followed a one-to-one stoichiometric relationship, the value of n should equal unity. Indeed, results from the present study confirmed this simple stoichiometric relation for the selective H_1- and H_2-agonist with their respective receptors (Table 1).

The present results demonstrated that each concentration of mepyramine gave a parallel displacement of the 2-thiazolylethylamine concentration-response curve, suggesting that mepyramine was a competitive antagonist for 2-thiazolylethylamine in the H_1-receptor system in the vascular smooth muscle of the arterioles in the sub-mucosal microcirculation of the rat stomach. Additional evidence supporting this contention was obtained from the Schild plot. For simple competitive antagonism on a one-to-one basis, the regression should be linear and have a slope of unity (Arunlakshana & Schild, 1959). In the present study, a slope of 1.02 ± 0.013 (mean±s.e.mean) was obtained (Table 2), and there was no significant deviation of the slope from unity. Further evidence was obtained from the Hill plots (Figure 3) which gave slopes not significantly different from unity.

Similarly, the present study showed a simple competitive antagonism between cimetidine and impromidine for the H_2-receptor system in the vascular smooth muscle of the stomach arteriole. Briefly, cimetidine displaced the impromidine concentration-response curves in a parallel manner, the Schild plot gave a slope of 1.05 ± 0.026 (mean±s.e.mean, Table 2) which was not significantly different from unity. The Hill plots such as those shown in Figure 3 also had slopes not deviating from unity.

For the mepyramine antagonism on the H_1-receptor system, a pA_2 value of 9.60 with narrow limits (s.e.mean = 0.033, $n = 6$) was obtained in the present study (Table 2), and this pA_2 value agreed closely with the values reported by other investigators in several other tissue preparations. This similarity indicated that the H_1-receptors on the vascular smooth muscle of the arterioles on the submucosal microcirculation in the rat stomach were homogeneous with the H_1-receptors in other tissues of several other animal species.

In studying the cimetidine antagonism on the H_2-receptor system, a pA_2 value of 5.98 with limits of ± 0.037 (s.e.mean, $n = 6$) was obtained in the vascular smooth muscle of the present *in vivo* stomach preparation (Table 2). Again, this pA_2 value was in close agreement with reported pA_2 values. Therefore, the H_2-receptors on the vascular smooth muscle should be of the same nature as the H_2-receptors elsewhere.

For a number of years, there was a difficult problem in attaining a near equilibrium state in a tissue preparation under *in vivo* experimental conditions, and also in obtaining the assessment of antagonist concentration at the receptor sites for *in vivo* situations. Indeed, very few estimates of pA_2 values for mepyramine and cimetidine were carried out under *in vivo* conditions. For studies of the receptor types on the vascular smooth muscle of arterioles in the microvasculature, conventional *in vitro* organ bath procedures were not feasible, because the calibre of the arterioles were microscopic. These microscopic arterioles are of great importance not only in the

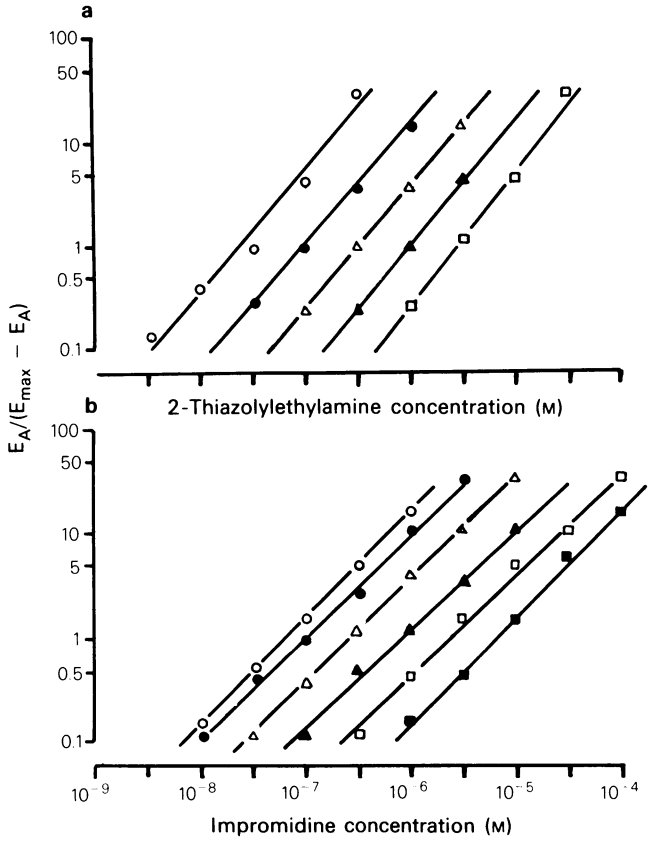

Figure 3 Representative Hill plots showing linear regressions of log (E_A/ (E_{max} – E_A)) on log (agonist concentration). (a) Selective H_1-receptor stimulation of vascular smooth muscle in arteriole by 2-thiazolylethylamine, in the absence of selective H_1-receptor blockade (○) and in the presence of mepyramine 1 nM (●), 3 nM (△), 10 nM (▲) and 30 nM (□). The respective Hill coefficients for this *in vivo* preparation are 0.97 (○), 1.03 (●), 1.06 (△), 1.20(▲) and 1.14 (□). (b) Similar selective H_2-receptor stimulation and blockade by impromidine and cimetidine, respectively. Concentrations of cimetidine are 0 μM (○), 1 μM (●), 3 μM (△), 10 μM (▲), 30 μM (□) and 100 μM (■). The respective Hill coefficients for this *in vivo* preparation are 1.00 (○), 0.97 (●), 0.99 (△), 0.95 (▲), 0.97 (□) and 1.04 (■).

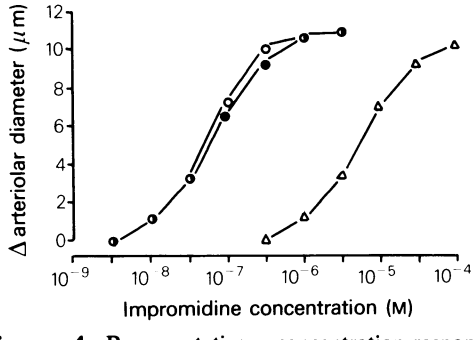

Figure 4 Representative concentration-response curves of impromidine from a stomach submucosal arteriole, with (△) or without (○, ●) cimetidine (100 μM) blockade. open circles (○) represent data before cimetidine blockade while closed circles (●) are results obtained after two cumulative applications of various concentrations of impromidine.

control of the total peripheral resistance of the systemic circulation, but also in the control of perfusion of blood in the downstream capillary bed. The present *in vivo* experimentation allowed the observation of local microcirculatory effects (i.e., changes of the arteriole diameter) in response to local stimulation by a receptor agonist. Moreover, the continuous suffusing system on the exposed tissue preparation enabled the local blockade of the receptor system by a selective antagonist. The relatively short time cycle to generate a concentration-response curve was also an advantage because several concentration-response curves with different blockers could be obtained in a single *in vivo* preparation. The techniques used to estimate the pA_2 values of the antagonists in the present *in vivo* procedures conformed to the description by Arunlakshana & Schild (1959) in which the agonist concentration was varied in the presence of a constant concentration of the antagon-

ist, so that the Schild regression was obtained over a range of several antagonist concentrations in a single *in vivo* preparation. Furthermore, in the present study, the unit slope in all Schild regression not only demonstrated that the antagonism was competitive, but also showed that the *in vivo* experimental procedures were carried out under equilibrium conditions (Kenakin, 1982). Presumably in these small arterioles there is little barrier for diffusion of the suffused drugs in reaching the receptor sites in the vascular smooth muscle. Thus the experimental procedures described in the present study proved satisfactory for characterizing the histamine receptors on the vascular smooth muscle under *in vivo* conditions.

Although the method of measuring the arteriolar diameter as reported in the present study has a high degree of accuracy, it does not provide information on the time-related changes during the entire phase of vasodilatation. Usually, during the 100 s period of receptor stimulation, only one or two diameter measurements could be made accurately, and it was difficult to determine the time-related responses from these few measurements. Therefore, in the present study, only the maximally dilated diameter at a given agonist concentration after reaching equilibrium was measured.

In conclusion, the present experiments provide *in vivo* evidence that both histamine H_1- and H_2-receptors are present in the vascular smooth muscle of the arterioles in the submucosal microcirculation of the rat stomach, and that the vascular histamine H_1- and H_2-receptors are of the same nature as similar receptors in other tissues.

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