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Pharmacological characterization of histamine receptors in the human temporal artery

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1 The subtypes of histamine-receptors which mediate dilatation of small human temporal arteries have been characterized *in vitro* using 'selective' agonists and antagonists.
2 Dilatory responses were studied after preconstriction with prostaglandin $F_{2\alpha}$ since contraction was not seen at histamine concentrations up to 10^{-4} M. Histamine caused a concentration-related relaxation of cerebral vessels with an IC_{50} value of $2.8 \pm 0.6 \times 10^{-7}$ M.
3 Cimetidine caused a parallel shift to the right of the histamine concentration-response curve whereas mepyramine was without observable effect. This suggests the presence of histamine H_2-receptors only. However, combined treatment with mepyramine and cimetidine caused a more marked displacement of the concentration-response curve to the right. Schild analysis indicated that in situations of near complete blockade of the histamine H_1-receptor subtypes, simple competitive antagonism at H_2-receptors can be revealed with a pA_2 value of 6.58 for cimetidine. The apparent pA_2 value for mepyramine was 8.58.
4 The 'selective' H_1-receptor agonists pyridylethylamine, 2-methylhistamine and thiazolylethylamine, and the H_2-receptor agonists dimaprit, impromidine and 4-methylhistamine all mimicked the histamine response, but all except impromidine were less potent than histamine. The order of potency was impromidine > thiazolylamine > 4-Me-histamine > 2-Me-histamine > dimaprit > pyridylethylamine > tele-Me-histamine.
5 These results indicate that the histamine-induced dilatation in small human temporal arteries is mediated by both H_1- and H_2-receptors and that the latter subtype of histamine receptors predominates.

Keywords histamine receptors human temporal arteries H_1-receptors H_2-receptors vasodilatation

Introduction

The multiple function of histamine as a chemical messenger in the mediation of both physiological and various pathological vascular events, such as migraneous neuralgia or vascular headache, has been indicated in previous studies (Diamond *et al.*, 1976; Hardebo *et al.*, 1980; Gross, 1982). In man, histamine produces a headache often associated with increased pulsation in the temporal artery, and a rise in blood, and occasionally urine, histamine occurs in patients during an attack of cluster headache (Anthony & Lance, 1971, Medina *et al.*, 1979; Sjaastad & Sjaastad, 1970).

The reactions to histamine in the external carotid vascular bed have been studied in animals both *in vitro* and *in situ*. In the rabbit and cat histamine has been found to be a vasoconstrictor agent (Furukawa, 1954; Parsons &

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Owen, 1973, Ercan & Turker, 1975, Edvinsson & Owman, 1975). This response seems to be a reflection of a balance between a histamine H_1-receptor mediated vasoconstriction and a histamine H_2-receptor mediated vasodilatation (Glover *et al.*, 1973; Edvinsson & Owman, 1975). In the monkey histamine caused a histamine H_2-receptor mediated vasodilatation (Duckworth *et al.*, 1976) and in the dog it caused an H_1- and H_2-receptor mediated vasodilatation (Saxena, 1975). However, it is not known if these results are applicable to human extracranial arteries, since there might be differences in the type of receptor stimulated and in the relative amount of receptor subtypes in different vessels.

Very few data are available on the effects of histamine on human extracranial vessels. Glover *et al.* (1973) reported that histamine causes vasodilatation of human temporal arteries *in vitro* and although this study provides quantitative evidence of dilator H_2-receptors, the data do not allow calculation of pA_2 values. Hardebo *et al.* (1980) reported that human temporal arteries dilate upon administration of histamine via an action probably through H_2-receptors, but did not test for the presence of H_1-receptors.

Effective tools are now available which may assist in clarifying the role of histamine in the external carotid vascular bed by the use of selective histamine H_1- and H_2-receptor agonists and antagonists (Owen, 1977; Owen *et al.*, 1979). The present study was performed in order to characterize in detail the histamine receptors mediating the dilator effects in human temporal arteries.

Methods

Material was obtained from eight patients (four males and four females aged 34–63 years) who were undergoing neurosurgical operations. None of the patients had received an H_1-receptor antagonist as a pre-medication and they were not suffering from cluster headache or migraine. Small branches of the superficial temporal artery were immediately dissected out and placed in ice-cold Krebs-Ringer solution and kept at 4° C during transportation to the laboratory (about 2 h). Vessels with a diameter of 0.5–1.0 mm were cut in ring segments (approximately 2–4 mm in length) and mounted between two L-shaped metal prongs in temperature-controlled (37° C) 2.5 ml tissue baths. The baths contained an aerated (5% CO_2 in O_2) buffer solution with the following composition (mM): NaCl 119; KCl 4.6; $CaCl_2$ 1.5; $MgCl_2$ 1.2; NaH_2PO_4 1.2; $NaHCO_3$ 15 and glucose 11; pH was kept at 7.4.

The metal prongs were attached to Grass FT-03C transducers and a Grass polygraph for measurement and registration of circular vasomotor effects following exposure to various agents (Högestätt *et al.*, 1983). After mounting the vessels were subjected to a passive load of 4 mN and allowed to stabilize at this tension for 90 min. Concentration-response data were derived by cumulative addition of histamine or other agonists to the tissue bath. Full concentration-response curves were run for all agents tested. Dilatory effects were studied after the arteries had been precontracted by addition of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in a concentration of 3×10^{-6} M which yielded a stable level of contraction which lasted long enough to allow for analysis of relaxant responses (i.e. up to 30 min). Antagonists were added to the tissue baths 20 min before the responses to histamine were tested. The values for relaxation are expressed as percentage of the level of contraction due to 3×10^{-6} M $PGF_{2\alpha}$. The responses were characterized in terms of I_{max} (maximum effects of the drugs) and IC_{50} (concentration eliciting half maximum response) values. Vessel reactivity was tested with a modified buffer solution containing 124 mM potassium which was achieved by an equimolar substitution of NaCl for KCl. At the end of each experiment a potassium-induced contraction was used as an indicator of the reactivity of the vessel. When appropriate mean values ± s.e. mean are given. Out of the *n* values (the number of vessel segments) cited in Figures 1–5 there are never more than two vessel segments from the same patient.

Quantitation of antagonism

Concentration-response curves to histamine were obtained in the absence or presence of various concentrations of either cimetidine or mepyramine, or both. The IC_{50} values of histamine were calculated as well as the concentration ratios (IC_{50} after antagonist/IC_{50} before antagonist). The displacement to the right of the concentration-response curves were utilized in an analysis according to the Gaddum-Schild equation: log (concentration ratio – 1) = logB–logK_B, where B is the concentration of the antagonist and K_B is the apparent dissociation constant for the receptor antagonist complex. Data are presented as a Schild plot where the intercept of the curve with the abscissa represents the mean pA_2 value.

Drugs

Histamine dihydrochloride (Sigma Chemical Co, St Louis, MO, U.S.A.), mepyramine maleate (May and Baker Ltd, Dagenham, U.K.), cimetidine, 2-pyridylethylamine (PEA), 2-methylhistamine, 2-thiazolylethylamine (TEA), tele-methylhistamine, impromidine, dimaprit and 4-methylhistamine (kind gifts from the Smith, Kline & French Laboratories, Welwyn Garden City, U.K.). The drugs were dissolved in 0.9% w/v NaCl solution (saline). All concentrations are expressed as the final molar concentration in the bath.

Results

Dilatory responses were studied in arterial segments in which a preconstriction was achieved with 3×10^{-6} M $PGF_{2\alpha}$. The application of $PGF_{2\alpha}$ resulted in a strong and stable constriction. In these preconstricted vessel segments, histamine invariably produced concentration-related relaxations with 81 ± 4% inhibition of the $PGF_{2\alpha}$ induced level of preconstriction. The IC_{50} for histamine was $2.8 \pm 0.6 \times 10^{-7}$ M. The cumulative application of histamine induced no constriction in the majority of vessels, but in a few there was a weak constriction at concentrations of and above 10^{-4} M (same result in vessels without preconstriction by $PGF_{2\alpha}$). Application of the 124 mM potassium containing buffer invariably caused a strong and stable constriction which rapidly disappeared on washout.

Antagonist experiments

The histamine-induced response in $PGF_{2\alpha}$ precontracted vessel segments was shifted in parallel to the right by cimetidine in concentrations of 1×10^{-6} and 1×10^{-5} M (Figure 1). Administration of mepyramine up to a concentration of 1×10^{-6} M did not significantly alter the histamine induced concentration-response curve (Figure 2).

Combined administration of cimetidine and mepyramine caused a more marked displacement of the histamine-induced concentration-response curve to the right than that achieved by histamine H_2-receptor blockade alone. In the presence of mepyramine 1×10^{-6} M, cimetidine in concentrations between 1×10^{-7} M and 1×10^{-5} M caused a parallel displacement to the right of the histamine-induced response without any reduction in the maximum response (Figure 3a). Similarly, administration of mepyramine in the presence of cimetidine 1×10^{-5} M now caused parallel shifts to the right at mepyramine concentrations between 1×10^{-8} M and 1×10^{-6} M (Figure 3b).

A Schild analysis using the concentration-ratios for cimetidine in the presence of mepyramine 1×10^{-6} M yielded a line with a slope of 1.12 suggesting a simple competitive antagonism at a histamine H_2-receptor site. The resulting pA_2 value for cimetidine was 6.58. When using the concentration-ratios for cimetidine alone, Schild analysis yielded a line with a slope of 0.54 and a pA_2 value of 6.72. A Schild analysis for mepyramine in the presence of cimetidine $1 \times$

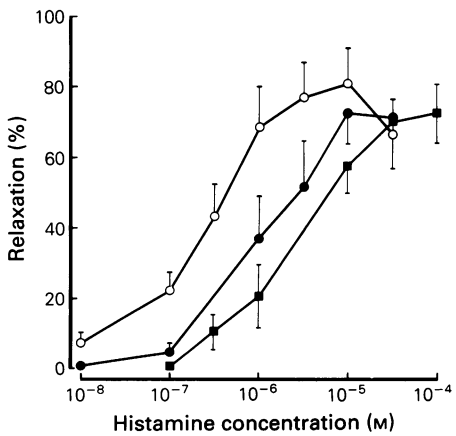

Figure 1 Relaxant effect of increasing concentrations of histamine on small human temporal arteries precontracted with $PGF_{2\alpha}$. Studies were performed without cimetidine (○) and in the presence of cimetidine in concentrations of 1×10^{-6} M (●) and 1×10^{-5} M (■). Mean values are shown, $n = 10$; vertical lines indicate s.e. mean.

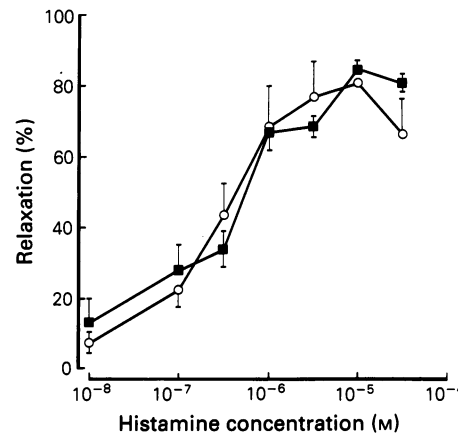

Figure 2 Relaxant effect of increasing concentrations of histamine on small human temporal arteries precontracted with $PGF_{2\alpha}$. Responses to histamine prior to treatment with antagonist are indicated by (○). The concentration-response curves were unaltered by mepyramine 1×10^{-6} M (■). Mean values are shown, $n = 10$; vertical lines indicate s.e. mean.

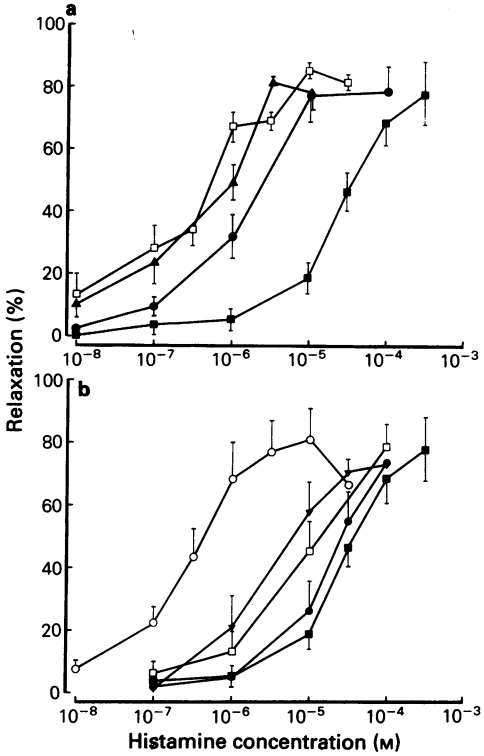

Figure 3 Relaxant effect of increasing concentrations of histamine on small human temporal arteries precontracted with $PGF_{2\alpha}$. (a) Responses to histamine in the presence of mepyramine 1×10^{-6} M are indicated by (□). Cimetidine 1×10^{-7} M (▲), 1×10^{-6} M (●) and 1×10^{-5} M (■) all in the presence of mepyramine 1×10^{-6} M. (b) Responses to histamine are indicated by (○) and responses to histamine in the presence of cimetidine 1×10^{-5} M are indicated by (▼). Mepyramine 1×10^{-8} M (□), 1×10>0–7 M (●) and 1×10^{-6} M (■), all in the presence of cimetidine 1×10^{-5} M. In (a) and (b) mean values are shown, $n = 10$; vertical lines indicate s.e. mean.

10^{-5} M yielded a line with a slope of 0.37 and an apparent pA_2 value of 8.58 (Figure 4).

Agonist experiments

The relatively selective H_2-receptor agonists impromidine, dimaprit and 4-Me-histamine and the relatively selective H_1-receptor agonists TEA, 2-Me-histamine, PEA and tele-Me-histamine all relaxed $PGF_{2\alpha}$ precontracted arterial segments in a concentration-dependent manner (Figures 5a and b). The maximum effects were approximately equal to that of histamine varying between 81–109%. The potency of all agonists except impromidine was lower than

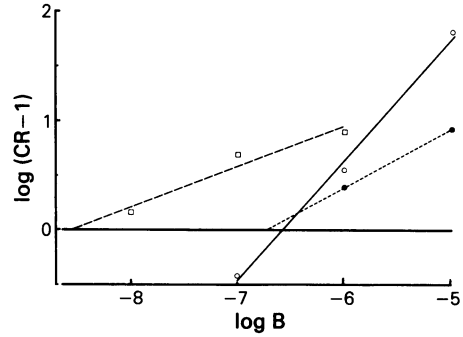

Figure 4 Schild plots for: cimetidine in the presence of mepyramine 1×10^{-6} M (○) ($pA_2 = 6.58$, $y = 7.33 + 1.12x$ and $r = 0.99$); cimetidine without simultaneous H_1-receptor blockade (●) ($pA_2 = 6.72$ and $y = 3.63 + 0.54x$) and mepyramine in the presence of cimetidine 1×10^{-5} M (□) ($pA_2 = 8.58$, $y = 3.17 + 0.37x$ and $r = 0.97$). B is the antagonist concentration and CR is the concentration ratio.

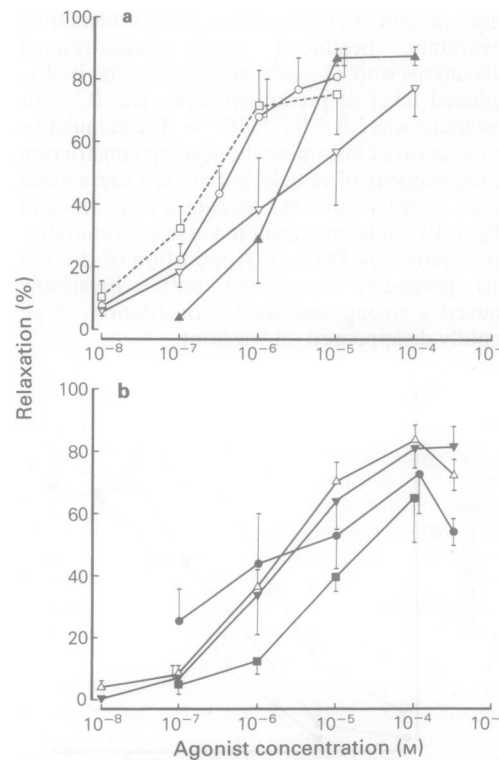

Figure 5 Relaxant effect of increasing concentrations of (a) histamine (○) and the histamine agonists 2-Me-histamine (▽) ($n = 5$), PEA (▲) ($n = 4$) and impromidine (□) ($n = 5$) and (b) of the histamine agonists TEA (●) ($n = 5$), dimaprit (▼) ($n = 6$), 4-Me-histamine (△) ($n = 5$) and tele-Me-histamine (■) ($n = 4$) in small human temporal arteries precontracted with $PGF_{2\alpha}$. Mean values are shown with vertical lines indicating s.e. mean.

Table 1 Comparison of the selectivity of histamine H_1- and H_2-receptor agonists relative to that of histamine, $n = 4$–6

	Potency of histamine activity			
Agonists	*Human temporal artery*	*Human cerebral artery**	*G.p. ileum (H_1)***	*G.p. atrium (H_2)***
Histamine	100	100	100	100
Impromidine	200	14.7	$< 10^{-3}$	4810
4-Me-histamine	19.0	1.9	0.2	43
Dimaprit	16.1	16.5	$< 10^{-4}$	71
TEA	69.4	20.1	26.0	2.2
2-Me-histamine	18.3	8.6	16.5	4.4
PEA	15.7	5.1	5.6	2.5
Tele-Me-hist	5.0	0.2	0.4	< 0.1

* Adapted from Ottosson *et al.* (1988). ** Adapted from Ganellin (1982). TEA = 2-thiazolylamine, PEA = 2-pyridylethylamine and 2-Me-histamine = 2-methylhistamine.

that of histamine itself; the most potent impromidine was followed by TEA, 4-Me-histamine, 2-Me-histamine, dimaprit, PEA and tele-Me-histamine (Table 1).

The impromidine-induced response was shifted in parallel to the right by a cimetidine concentration of 1×10^{-5} M, the concentration-ratio being almost 10 ($n = 3$). A mepyramine concentration of 1×10^{-6} M was without effect. Similarly, the 4-Me-histamine induced response was shifted in parallel to the right by cimetidine 1×10^{-5} M with a concentration ratio of just over 5 ($n = 3$), whereas mepyramine 1×10^{-6} M was without effect.

Discussion

The administration of histamine to human temporal arteries precontracted with $PGF_{2\alpha}$ results in dilatation of the vessels. In most other species investigated, there is a predominance of H_2-receptor participation in the vasodilator responses to histamine and a H_1-receptor mediated vasoconstriction in the external carotid vascular bed (Edvinsson & Owman, 1975; Saxena, 1975; Duckworth *et al.*, 1976; Parsons & Owen, 1973; Ercan & Turker, 1975). However, in human temporal arteries only vasodilatation was seen, and this response seemed mainly to be mediated via H_2-receptors. Thus, H_2-receptor blockade with cimetidine caused a parallel displacement to the right of the histamine concentration-response curve, whereas H_1-receptor blockade with mepyramine was without effect. Combined treatment with both H_1- and H_2-receptor antagonists resulted in further displacement to the right of the histamine-induced concentration-response curve. This indicates that the H_2-receptor predominates in mediating dilatation in this vascular region. With increasing H_2-receptor blockade the H_1-receptor mediated dilatation can be unmasked as shown by the additional displacement to the right of the histamine-induced concentration-response curve by mepyramine in the presence of cimetidine. This might also explain why no simple competitive antagonism at one receptor type by Schild analysis was seen with H_2-receptor blockade alone (Figure 4), because here the dilatory response was influenced by the effect of histamine on the H_1-receptor population. On the other hand, in the presence of effective H_1-receptor blockade (mepyramine 1×10^{-6} M) the experiments revealed a simple competitive antagonism at the H_2-receptor site. However, H_1-receptor blockade in the presence of effective H_2-receptor blockade (cimetidine 1×10^{-5} M) yielded a line with a slope significantly different from 1.0, indicating that there might be some other factor influencing the competitive antagonism at the H_1-receptor site, or merely reflecting the presence of a low concentration of H_1-receptors.

For cimetidine, pA_2 values of 6.1–7.0 have been reported in different test preparations and for mepyramine, pA_2 values of 8.0–9.3 in different test preparations (c.f. Ottosson *et al.*, 1988). Thus, the pA_2 values found in the present study (cimetidine 6.58 and mepyramine 8.58) are well in accordance with those found in histamine receptor subtype characterizations carried out previously.

The displacement to the right of the histamine concentration-response curve by H_2-receptor blockade, the lack of effect of H_1-receptor blockade alone and the pronounced displacement to the right obtained with combined H_1- and H_2-receptor blockade has been observed in previous experimental models; examination of

systemic blood pressure in the cat and dog (Black *et al.*, 1975), and in peripheral resistance vessels of the hind-limb, mesentery and stomach of the cat (Flynn & Owen, 1975). Exceptions do exist, e.g. such as pial arteries of the cat, where Wahl & Kuschinsky (1979) obtained no additional displacement to the right upon combined H_1- and H_2-receptor blockade.

The present result is an example of a drug-receptor interaction where a single agonist (histamine) interacts with two independent receptor subtypes (H_1 and H_2) to produce a common physiological effect (dilatation). Ariens *et al.* (1959) have suggested that the slope of the concentration-response curve is under such circumstances primarily determined by the interaction of the agonist (histamine) with the receptor to which it binds with greater affinity (in this case H_2). The interaction with the other receptor (H_1) would be concealed until unmasked by a competitive antagonist (cimetidine) for the interaction of the agonist with the first receptor. According to this, the receptor having the lower dissociation constant determines the shape of the concentration-response curve, which is well in accordance with our findings for the H_2-receptor. However, the dominance of a given receptor will not only depend on the relative dissociation constants of histamine for H_1- and H_2-receptors but may also depend on the spare receptor reserve for each response.

Further support for the involvement of both H_1- and H_2-receptors in the vasodilator response of human temporal arteries to histamine was obtained by the experiments with the relatively selective receptor agonists. All agonists, both H_1- and H_2-receptor agonists, mimicked the histamine-induced response and all had an I_{max} close to that of histamine. The potency of the relatively selective H_1- and H_2-receptor agonists are in temporal arteries comparable to values obtained in human cerebral arteries, but with a somewhat higher potency for the H_2-receptor agonists in the temporal arteries (Table 1). For comparison the standardized models for demonstrating agonist activity at H_1-receptors (stimulation of the contraction of guinea pig ileum) and H_2-receptors (the rate of atrial beating in the guinea pig heart) have been included in Table 1.

The presence of H_2-receptors in human temporal arteries is further indicated by the displacement to the right of the impromidine and 4-Me-histamine concentration-response curves by cimetidine.

In conclusion, the effects of relatively selective H_1- and H_2-receptor agonists and antagonists revealed that histamine induced dilatation of human temporal arteries is mediated by both H_1- and H_2-receptors, but the H_2-receptor mediated relaxation is predominant. As histamine is thought to be involved in the vasodilatation of cranial vessels in attacks of certain types of headache (see Hardebo *et al.*, 1980), the clinical importance of our findings would be that a combined histamine H_1- and H_2-receptor blockade is necessary to counteract the effect of histamine on the human temporal artery. Such medication has been tried for attacks of cluster headache (Russel, 1979), but as pointed out by this author the doses of the antagonists may have been insufficient to produce a clinical effect.

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