Br. J. Pharmac. (1979), **66**, 517–520

CHARACTERIZATION OF VASCULAR HISTAMINE RECEPTORS IN THE RAT

JAMES R. POWELL & L. BLAIR SHAMEL

Department of Pharmacology, School of Medicine, Emory University, Atlanta, Georgia 30322, U.S.A.

1 In the rat the decrease in blood pressure caused by histamine involves activation of both H_1- and H_2-receptors. Since arterial pressure measurements alone do not permit the separation of responses into cardiac and vascular components, the following experiments were undertaken to study vascular histamine receptors.

2 Vascular responses were studied in the autoperfused hindquarters of anaesthetized rats. Intra-arterial histamine caused vasodilatation which was only partially attenuated by treatment with mepyramine, an H_1-receptor antagonist. Treatment with metiamide, the H_2-receptor antagonist, did not affect vasodilatation caused by histamine but did attenuate vasodilatation which persisted after mepyramine.

3 Intra-arterial 4-methylhistamine, an H_2-receptor agonist, caused vasodilatation which was reduced by metiamide. The H_1-receptor agonist, 2-(2-pyridyl)ethylamine also caused vasodilatation which was blocked by mepyramine.

4 It is concluded that in the rat, histamine causes vasodilatation mediated by both H_1- and H_2-receptors.

Introduction

The depressor effect of histamine has been shown to involve both H_1- and H_2-histamine receptors in the dog, cat, rat, and guinea-pig (Black, Duncan, Durant, Ganellin & Parsons, 1972; Parsons & Owen, 1973; Brimblecombe, Owen & Parsons, 1974; Black, Owen & Parsons, 1975; Powell & Brody, 1976). In the dog (Powell & Brody, 1976) and cat (Flynn & Owen, 1974) the fall in blood pressure in response to histamine appears to be caused largely by vasodilatation due to activation of both H_1- and H_2-histamine receptors. The systemic arterial pressure measurements in rats used by Brimblecome *et al.* (1974) did not permit the separation of the depressor response to histamine into cardiac and vascular components. The following experiments were undertaken to determine the vascular actions of histamine receptor stimulation in the rat.

Methods

Male Sprague–Dawley rats (Hiram Davies Farms, Stockbridge, GA 30281) weighing 270 to 580 g were pretreated with atropine sulphate (0.1 mg/kg, i.p.) to decrease bronchial secretions and anaesthetized with sodium pentobarbitone (50 mg/kg, i.p.). The trachea was cannulated with a 5 cm length of polyethylene tubing (PE 205). Blood pressure was measured from the left common carotid artery by means of a short length of polyethylene tubing (PE 50) and a Statham P23De pressure transducer. Intravenous drug injections were made into the cannulated left external jugular vein. The hindquarters of the rats were perfused with blood according to the methods of Brody, Schaffer & Dixon (1963). The aorta was approached through a midline abdominal incision. The ileolumbar arteries and veins were ligated bilaterally. After administration of heparin (1000 u/kg, i.v.) the aorta was cannulated cranially and caudally with polyethylene tubing (PE 90) connected to Silastic tubing (0.8 mm i.d. × 4.1 mm o.d.) which passed through a rotary-type perfusion pump (Cole-Parmer Model 7013 pumping head and Model 7545 variable speed motor). The total extracorporeal volume was 0.4 ml. The flow rate to the hindquarters was adjusted initially so that the perfusion pressure measured with a Statham P23AC pressure transducer between the pumping head and the hindquarters approximated systemic arterial pressure. The flow rate remained constant for the rest of the experiment so that changes in perfusion pressure reflected changes in vascular resistance of the hindquarters. The flow rates in 10 animals averaged 6.8 $\pm$ 0.4 ml/min (mean $\pm$ s.e.mean). Intra-arterial drug injections were made directly into

0007-1188/79/080517-04 $01.00

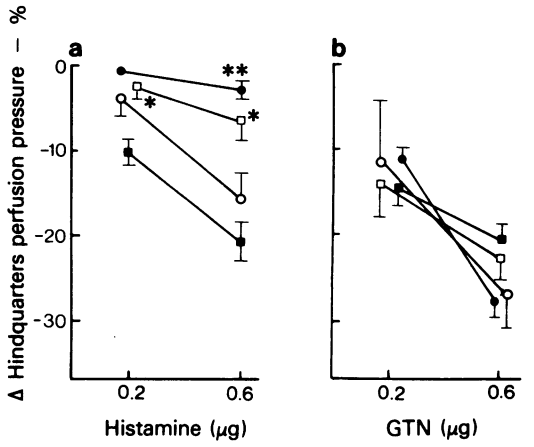

Figure 1 Summary of the effects of mepyramine (14.2 mg/kg, i.v.) metiamide (30, mg/kg, i.v.) and the combination of both antihistamines on the vasodepressor response of the rat to histamine (a) and glyceryl trinitrate (GTN) (b). In one set of animals, histamine and GTN were injected intra-arterially into the perfused hindquarters of anaesthetized rats before and after blockade of H_1-receptors with mepyramine, then again after subsequent H_2-receptor blockade with metiamide. In another set of animals the order of histamine receptor blockade was reversed. (■) Control, $n = 10$; (□) after mepyramine, $n = 5$; (○) after metiamide, $n = 5$; (●) after mepyramine and metiamide, $n = 10$. Points and vertical lines are means ± s.e. * Changes in perfusion pressure significantly different (* $P < 0.05$) from control after treatment with a single antihistamine. ** Values after both antihistamines significantly different (** $P < 0.05$) from treatment with a single antihistamine.

the perfusion tubing near the distal cannulation. Systemic arterial and hindquarters perfusion pressures were recorded on a Grass Model 5 polygraph. Rectal temperature was maintained near 37°C by means of a heating pad. Data were analyzed by *t* tests for dependent and independent data as described by Dixon & Massey (1969).

Drugs

Metiamide, 4-methylhistamine dihydrochloride, and 2-(2-pyridyl)ethylamine dihydrochloride were obtained as generous gifts from Smith, Kline and French, Ltd. Other drugs used were mepyramine maleate, histamine dihydrochloride, atropine sulphate, heparin sodium (all from Sigma Chemical Co., St. Louis, MO.), and glyceryl trinitrate (Parke-Davis and Co., Detroit, MI.). Drugs were dissolved in distilled water, except metiamide which was dissolved in 1 N HCl, neutralized with 0.1 N NaOH and brought to volume with distilled water. Drug doses are expressed as the base.

Results

As seen in Figure 1, intra-arterial histamine caused vasodilatation as reflected by decreases in perfusion pressure. The vasodilatation caused by histamine was not significantly influenced by metiamide (30 mg/kg, i.v.), but was reduced by mepyramine (14.2 mg/kg, i.v.). The combination of both antihistamines caused greater attenuation of the responses to histamine than did mepyramine given alone which was statistically significant for the 0.6 μg dose. As illustrated in Figure 1b, glyceryl trinitrate (GTN) also caused vasodilatation. This vasodilatation was not affected significantly by mepyramine or metiamide given alone or in combination.

Figure 2 shows that 4-methylhistamine (4-MH) the H_2-receptor agonist (Black *et al.*, 1972) caused vasodilatation which was attenuated by metiamide but not by mepyramine. The combination of both antihistamines caused an attenuation of the responses to 4-MH which was greater than the attenuation caused by metiamide alone. Figure 2b indicates that 2-(2-pyridyl)ethylamine (PEA), the H_1-receptor agonist (Durant, Ganellin & Parsons, 1975) caused vasodilatation that was attenuated by mepyramine but not by metiamide, whether given alone or after mepyramine.

As shown in Table 1, mepyramine caused a reduction in systemic arterial pressure when given alone or in combination with metiamide. Metiamide had no effect on arterial pressure and neither antihistamine altered hindquarters perfusion pressure.

Discussion

The results of other studies (Brimblecombe *et al.*, 1974; Szelenyi & Theimer, 1977) indicate that the fall in blood pressure in the rat following intravenous histamine is due to activation of both H_1- and H_2-histamine receptors. The present study indicates that at least a portion of the depressor effect of histamine is due to vasodilatation resulting from activation of vascular H_1- and H_2-receptors by histamine.

Since mepyramine has been defined as the prototype H_1-receptor antagonist (Ash & Schild, 1966), that portion of the vascular effects of histamine blocked by mepyramine represents activation of H_1-receptors. It might be argued that in this study the failure of mepyramine to cause a complete attenuation of the vascular effects of histamine indicates that the dose of mepyramine used in the experiments (14.2 mg/kg) caused an incomplete block of

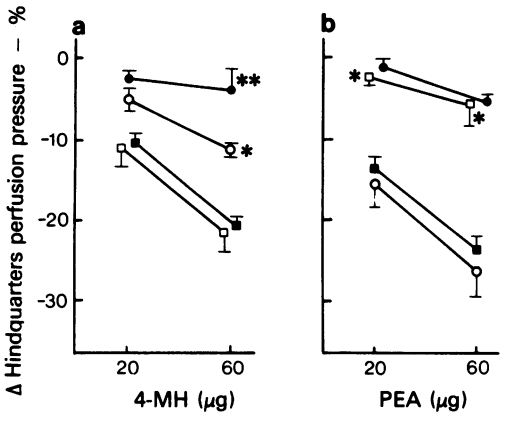

Figure 2 Summary of the effects of mepyramine (14.2 mg/kg, i.v.) metiamide (30 mg/kg, i.v.) and the combination of both antihistamines on the vasodepressor response of the rat to 4-methylhistamine (4-MH) (a) and 2-(2-pyridyl) ethylamine (PEA) (b). In one set of animals 4-MH and PEA were injected intraarterially into the perfused hindquarters of anaesthetized rats before and after blockade of H_1-receptors with mepyramine, then again after subsequent H_2-receptor blockade with metiamide. In another set of animals the order of histamine receptor blockade was reversed. (■) Control, $n = 10$; (□) after mepyramine, $n = 5$; (○) after metiamide, $n = 5$; (●) after mepyramine and metiamide, $n = 10$. Points and vertical lines are means ± s.e. * Changes in perfusion pressure significantly different (* $P < 0.05$) from control after treatment with a single antihistamine. ** Response after blockade of both H_1- and H_2-receptors significantly different (** $P < 0.05$) from response after treatment with a single antihistamine.

H_1-receptors. This does not appear to be the case since in preliminary experiments doubling or even tripling this dose did not cause further attenuation of the response to histamine, suggesting that the dose of mepyramine used caused a maximal blockade of H_1-receptors. The dose of metiamide used (30 mg/kg) also appeared to be maximal for blockade of H_2-receptors since tripling the dose of metiamide did not cause any further attenuation of responses to the doses of histamine used.

Mepyramine given alone or after metiamide caused a long-lasting reduction in mean arterial pressure. This lowering of blood pressure raises the possibility that the attenuating effects of mepyramine on vasodilatation were not related to a blockade of histamine receptors but instead occurred as a result of the low arterial pressure. Two factors argue against this possibility. First, mepyramine did not significantly affect hindquarters perfusion pressure whether given alone or after metiamide. This would indicate that mepyramine had minimal direct effects on the hindquarters circulation. Second, if the lowering of arterial pressure by mepyramine reduced the ability of the hindquarters vessels to dilate, it would be expected that vasodilator responses to any agent would be reduced. Such was not the case since vasodilatation caused by GTN was not significantly affected by mepyramine treatment. It may be concluded, therefore, that the reduction in vasodilatation in response to histamine was due to blockade of vascular H_1-receptors.

Histamine receptor agonists produced vascular effects similar to those produced by histamine. 4-MH, which has been reported to be a selective H_2-receptor agonist (Black *et al.*, 1972), produced vasodilatation which was blocked by metiamide given alone but not by mepyramine. The attenuation of vasodilatation due to 4-MH was greater after combined antihistamine treatment than after metiamide alone suggesting that the specificity of 4-MH for H_2-receptors is only relative. These data support the conclusion of Owen (1975) in regard to the specificity of 4-MH toward cardiovascular H_2-receptors in the cat. The responses of the hindquarters to PEA, a selective H_1-receptor agonist (Durant *et al.*, 1975) were

Table 1 Effects of intravenous mepyramine and metiamide, alone or in combination, on systemic arterial and hindquarters perfusion pressure in the rat

Drug treatment	n	*Mean arterial pressure (mm Hg, mean ± s.e.)*	*Hindquarters perfusion pressure (mmHg, mean ± s.e.)*
None	10	132 ± 5	123 ± 3
Mepyramine (14.2 mg/kg)	5	100 ± 9*	119 ± 10
Metiamide (30 mg/kg)	5	120 ± 6	138 ± 16
Mepyramine + metiamide	10	83 ± 9*	130 ± 7

* Different from control (no drug treatment), $P < 0.05$.

attenuated by mepyramine only. This would indicate that PEA has greater specificity towards H_1-receptors than does 4-MH towards H_2-receptors.

In previous studies in which only arterial pressure measurements were used (Brimblecombe *et al.*, 1974; Szelenyi & Theimer, 1977) it was not possible to separate the depressor effect of histamine into cardiac and vascular components. Since arterial pressure is largely the product of cardiac output and peripheral vascular resistance, it follows that a fall in arterial pressure could be due to a decreased cardiac output, a decreased vascular resistance, or a combination of both mechanisms. This study provides new evidence that in the rat, histamine causes vasodilatation by activation of both H_1- and H_2-histamine receptors. The rat is therefore a suitable model for studying the interaction of histamine and related drugs with both types of vascular histamine receptor.

L.B.S. was a pre-doctoral trainee supported by USPHS Grant TMI GM-00179. A portion of this work was also supported by the Pharmaceutical Manufacturers Association Foundation. The authors wish to thank Smith, Kline and French, Ltd. for the supplies of histamine receptor agonists and metiamide.

References

ASH, A.S.F. & SCHILD, H.O. (1966). Receptors mediating some actions of histamine. *Br. J. Pharmac. Chemother.*, **27**, 427–439.

BLACK, J.W., DUNCAN W.A.M., DURANT, C.J., GANELLIN, C.R. & PARSONS, M.E. (1972). Definition and antagonism of histamine H_2-receptors. *Nature*, **236**, 385–390.

BLACK, J.W., OWEN, D.A.A. & PARSONS, M.E. (1975). An analysis of the depressor responses to histamine in the cat and dog: involvement of both H_1- and H_2-receptors. *Br. J. Pharmac.*, **54**, 319–324.

BRIMBLECOMBE, R.W., OWEN, D.A.A. & PARSONS, M.E. (1974). The cardiovascular effects of histamine in laboratory animals. *Agents and Actions*, **4**, 191–192.

BRODY, M.J., SCHAFFER, R. A. & DIXON, R.L. (1963). A method for the study of perpheral vascular responses in the rat. *J. appl. Physiol.*, **18**, 645–647.

DIXON, W.J. & MASSEY, F.J. (1969). *Introduction to Statistical Analysis.* pp. 119–123. New York: McGraw Hill.

DURANT, G. J., GANELLIN, C.R. & PARSONS, M.E. (1975). Chemical differentiation of histamine H_1- and H_2-receptor agonists. *J. med. Chem.*, **18**, 905–906.

FLYNN, S.B. & OWEN, D.A.A. (1974). Vascular histamine receptors in the cat. *Br. J. Pharmac.*, **52**, 122P.

OWEN, D.A.A. (1975). The effects of histamine and some histamine-like agonists on blood pressure in the cat. *Br. J. Pharmac.*, **55**, 173–179.

PARSONS, M.E. & OWEN, D.A.A. (1973). Receptors involved in the cardiovascular response to histamine. In *International Symposium on H_2-Receptor Antagonists.* ed. Wood, C.J. & Simkins, M.A. pp. 127–136. London: Smith, Kline and French.

POWELL, J. R. & BRODY, M. J. (1976). Identification and specific blockade of two receptors for histamine in the cardiovascular system. *J. Pharmac. exp. Ther.*, **186**, 1–14.

SZELENYI, I. & THEIMER, K. (1977). Involvement of both H_1- and H_2-receptors in the depressor responses of blood pressure to histamine in the rat. *Arzneim. Forsch.*, **27**, 809–811.

(Received July 18, 1978.
Revised January 10, 1979.)