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Histamine H_3 agonist decreases arterial blood pressure in the guinea-pig

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Abstract—Intravenous injection of an H_3-agonist, (*R*)-α-methylhistamine, dose-dependently caused a transient fall in mean arterial pressure of guinea-pigs. This pressor response was not reduced by combined mepyramine/cimetidine (up to 1 mg kg^{-1}), atropine or propranolol, but was attenuated by either a selective H_3-antagonist, thioperamide, or a competitive inhibitor of nitric oxide synthesis, N^G-monomethyl L-arginine. The reduction by the inhibitor of nitric oxide synthesis was reversed by L- but not D-arginine. Histamine activated the H_3-sites since its depressor response (obtained with mepyramine and cimetidine) was similar to that of (*R*)-α-methylhistamine. Our data indicate that H_3-sites could exist in the cardiovascular system of guinea-pigs and that their stimulation might be mediated through the L-arginine/nitric oxide pathway.

Two main types of histamine receptors, the H_1 and H_2 receptors, participate in the cardiovascular regulation of mammals. In blood vessels in-vitro, a novel H_3 receptor has been recognized on both perivascular nerve endings of guinea-pig mesenteric artery (Ishikawa & Sperelakis 1987) and endothelial wall of rabbit middle cerebral artery (Ea Kim et al 1992). The H_3 receptor is pharmacologically distinct from the H_1 and H_2 receptors and it controls both histamine synthesis and release in the nerve terminals (Arrang et al 1987). The H_3-receptor-mediated relaxation may involve neuronal control of blood vessels (Ishikawa & Sperelakis 1987); however, it may also involve the endothelial release of nitric oxide, since in the rabbit middle cerebral artery, it is partially reduced by inhibitors of nitric oxide synthesis derived from L-arginine (Ea Kim et al 1992). Up to now, there has been no indication of whether this third class of histamine receptor contributed to the depressor response to histamine in-vivo. The purpose of the present investigation was to demonstrate that (*R*)-α-methylhistamine, a selective and potent H_3 agonist, decreased blood pressure in anaesthetized guinea-pigs and that this pharmacological effect is distinct from that observed in H_1- and H_2-receptor activation.

Materials and methods

Male Dunkin-Hartley guinea-pigs, 350–500 g, were anaesthetized with urethane (1·5 g kg^{-1}, i.p.) and kept warm (37 ± 0·5°C) with an overhead lamp. The left carotid artery was cannulated with PE50 tubing for blood pressure measurement. Systolic, diastolic or mean arterial pressure (MAP) was monitored via Gould Statham tranducers on a Gould polygraph BS-272. Heart rate was obtained from the arterial pulse wave and recorded on the polygraph output.

The preparation was allowed to equilibrate for at least 20 min before drug administration via the pre-cannulated right jugular vein. Baselines of MAP and heart rate were 51 ± 2 mmHg (n = 45) and 247 ± 6 beats min^{-1} (n = 45), respectively. Difference in systolic and diastolic arterial pressure values was approximately 2 mmHg. There was an inherent oscillation of 4·5 mmHg associated with respiration, but the average trace was stable up to 1 h, after which the preparation was discarded. After blood pressure and heart rate stabilization, 0·1 mL physiological saline was injected into each preparation as vehicle control. The following drugs were used: (*R*)-α-methylhistamine dihydrochloride (methylhistamine), thioperamide (kindly donated by Dr J. C. Schwartz, France); histamine dihydrochloride, L- and D-arginine hydrochloride, atropine sulphate, dimethylsulphoxide (Sigma Chemical Co, USA); 2-thiazolylethylamine dihydrochloride (2-TEA), dimaprit dihydrochloride, cimetidine (SmithKline Beecham, UK); mepyramine maleate (Sandoz, France); propranolol hydrochloride (ICI Pharma, France); N^G-monomethyl L-arginine acetate (L-NMMA) (Research Biochemical Inc., USA); urethane (Cooper, France); saline (0·9% sodium chloride) (OSI, France). Thioperamide was dissolved in dimethylsulphoxide (5 mg mL^{-1}). Stock solutions of all drugs were prepared daily and diluted with saline. The dosage of drugs was chosen according to our own preliminary experiments. Each drug administration was in 0·1 mL except for thioperamide (0·2 mL) and spaced 10 min apart except for the antagonist or inhibitor which were injected 1 min before the agonist.

Results are expressed as mean ± s.e.m. Statistical significance of the difference between mean values was evaluated by Student's *t*-test and $P < 0.05$ indicated a significant difference.

Results

Methylhistamine (0·1–15 μg kg^{-1}) induced a transient dose-dependent decrease in MAP of urethane-anaesthetized guinea-pigs with an ED50 value of 1·9 ± 0·2 μg kg^{-1} (n = 7) (Fig. 1). The maximum fall in MAP which occurred at a dose of around 7 μg kg^{-1} was about 15 mmHg. This effect was accompanied by a non-significant bradycardia (Fig. 1). Histamine and dimaprit, an

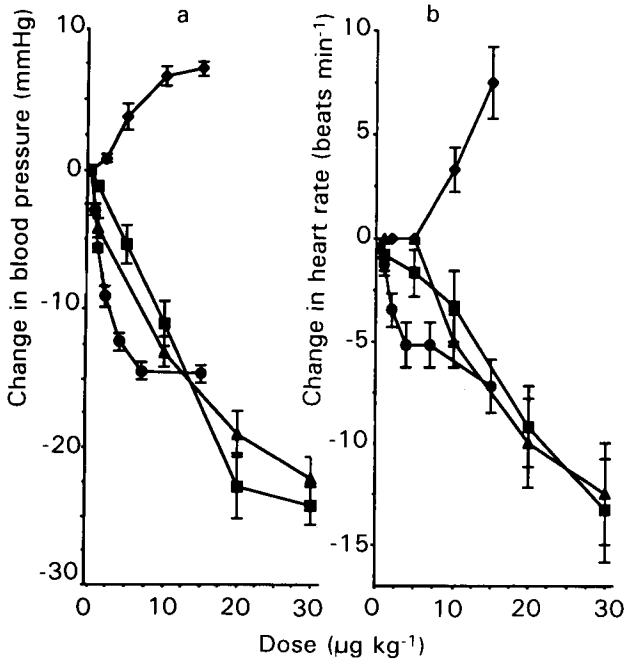

FIG. 1. Dose-response curves for the effects of methylhistamine (●), dimaprit (▲), 2-TEA (◆) and histamine (■) on (a) mean arterial pressure (MAP) and (b) heart rate in urethane-anaesthetized guinea-pigs. Baselines of MAP and heart rate were 51 ± 2 mmHg (n = 45) and 247 ± 6 beats min^{-1} (n = 45), respectively.

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Table 1. Effect of various drugs on the change in mean arterial pressure (MAP) induced by methylhistamine or histamine in anaesthetized guinea-pigs.

Treatment	Dose (mg kg^{-1})	Change in MAP (mmHg)
Methylhistamine (7 μg kg^{-1})		
Control		−14·5±1·0
Mepyramine/cimetidine	0·5	−14·0±1·0
	1·0	−13·8±0·8
Thioperamide	0·1	−5·0±0·5**
	0·5	−1·1±0·4**
	1·0	+1·8±0·8**
L-NMMA	1	−4·8±0·6**
	5	−3·3±0·7**
	10	−2·0±0·3**
L-NMMA with L-arginine	1	−8·6±0·5*
	5	−7·5±0·8*
	10	−7·0±0·3*
Histamine (10 μg kg^{-1})		
Control		−24·0±2·0
Mepyramine/cimetidine	1	−13·0±2·7*

* $P<0·01$, ** $P<0·001$ compared with the corresponding control.

H_2 agonist (1–30 μg kg^{-1}) caused a sustained dose-dependent fall in MAP with ED50 values of 11·6±0·7 and 9·3±0·5 μg kg^{-1}, respectively (n=6) (Fig. 1). The maximum reductions were 24·1±1·3 and 22·3±1·4 mmHg, respectively (n=6). These effects were accompanied by a transient significant bradycardia (Fig. 1); the slopes of regression lines obtained from dose-response curves in MAP for histamine (−1·1±0·1, n=6) and dimaprit (−1·0±0·1, n=6) were significantly different from those obtained for methylhistamine (−2·6±0·2, n=7) ($P<0·001$ and $P<0·001$, respectively). Unlike methylhistamine, the dose-response curve for 2-TEA, an H_1 agonist (1–15 μg kg^{-1}), indicated a small dose-dependent increase in MAP (ED50 5·6±0·6 μg kg^{-1} and maximum increase 7·1±0·4 mmHg (n=6)) with a non-significant tachycardia (Fig. 1).

In the presence of simultaneous injection of mepyramine, an H_1 antagonist and cimetidine, an H_2 antagonist (0·5 or 1 mg kg^{-1}), the fall in MAP induced by 7 μg kg^{-1} methylhistamine was not significantly changed (Table 1). Under the same conditions, both the responses to histamine (30 μg kg^{-1}) were partially reduced to a level which was significantly different from baseline (changes in MAP: histamine alone 24±2 mmHg, n=7; histamine+combined H_1/H_2 antagonists 13±2 mmHg, n=7). The same doses of mepyramine or cimetidine have no significant effect on the baseline blood pressure or heart rate. In addition, the pressor response to methylhistamine was not significantly attenuated by atropine (0·2 mg kg^{-1}) or propranolol (0·2 mg kg^{-1}) (not shown, n=6). The H_3 antagonist, thioperamide (0·1, 0·5 and 1 mg kg^{-1}) dose-dependently restored MAP to control levels or even slightly increased it (Table 1), but did not significantly affect the baseline blood pressure or heart rate. Table 1 also shows that the competitive inhibitor of nitric oxide synthesis, L-NMMA (1, 5 or 10 mg kg^{-1}), partially reduced the methylhistamine response but slightly increased the baseline blood pressure and heart rate; higher doses of L-NMMA (>10 mg kg^{-1}) did not further reduce the methylhistamine response (not shown, n=6). The reduction in MAP decrease was significantly reversed in the presence of 1, 5 or 10 mg kg^{-1} L- but not D-arginine. The same doses of L- or D-arginine have no direct effect on MAP of guinea-pigs (data not shown, n=6).

Discussion

The data provide evidence of pressor responses to several histamine agonists in urethane-anaesthetized guinea-pigs in-vivo.

Firstly, methylhistamine induced a transient fall in MAP accompanied by a non-significant bradycardia. The finding that a combined injection by a blockade dose of H_1 and H_2 antagonists failed to prevent the methylhistamine response is compatible with the hypothesis that the latter was not due to activation of H_1 or H_2 receptors. This is supported by the present finding that the dose-response curve for methylhistamine is significantly different from that obtained from the H_1 or H_2 agonist. Moreover, when the H_1 or H_2 receptor was blocked by the same dose of mepyramine or cimetidine, 2-TEA or dimaprit did not affect the baselines of MAP or heart rate of guinea-pigs (personal communication). Furthermore, the response to methylhistamine was not affected by β-adrenoceptor or muscarinic antagonists. Since the H_3 agonist-induced vasodepression was dose-dependently reduced or even slightly reversed by the selective H_3 antagonist, thioperamide, the overall action of this agonist may be assumed to be mediated via an H_3-receptor site. This result is reminiscent of that reported by Ishikawa & Sperelakis (1987) and of a previous report (Ea Kim et al 1992) for histamine in nerve terminals of the guinea-pig mesenteric artery and in the endothelial wall of rabbit middle cerebral artery, respectively.

Secondly, histamine evoked a dose-dependent sustained decrease in MAP of the guinea-pig which was accompanied by a bradycardia. This result is compatible with that of some investigators (Black et al 1975; Harvey & Owen 1980) for histamine. Those authors suggested that the time-course of depressor response to histamine in numerous species could be assumed to be due to H_1-mediated immediate fall and H_2-mediated sustained fall in MAP. However, in our experiment in the guinea-pig, 2-TEA, an H_1 agonist, increased rather than decreased MAP, and when both the H_1 and H_2 components of histamine response were blocked by a mixture of mepyramine/cimetidine, both the fall in MAP and the bradycardia were partially, but not completely, reduced and the remaining response was similar to that observed with methylhistamine alone. This result confirms the hypothesis that exogenous histamine could also activate H_3-receptor sites, inducing hypotension and that this third class of receptors might exist in cardiovascular tissue of guinea-pigs.

Thirdly, although we do not provide direct evidence that methylhistamine releases dilator substances from endothelium or nerve terminals of the cardiovascular system, one line of evidence suggests that the hypotension in the guinea-pig to the H_3 agonist may be mediated through the release of nitric oxide or nitric oxide-like compounds, since L-NMMA, which has been shown to inhibit competitively and in an enantiomerically specific manner endothelial or neuronal nitric oxide synthesis from L-arginine (Knowles et al 1990), antagonized in a dose-dependent fashion the pressor effect of the amine. In addition, the inhibition by L-NMMA was reversed by L- but not D-arginine as previously described (Palmer et al 1988; Moore et al 1990). Furthermore L- or D-arginine had no direct effect on MAP of the guinea-pig and this is in agreement with the findings of Rees et al (1990) in the rat. This result suggests that cardiovascular tissues of the guinea-pig possess a biochemical pathway converting L-arginine to nitric oxide which might participate in hypotension to methylhistamine. Van de Voorde & Leusen (1982) identified an endothelium H_1 receptor in rat aorta, which when stimulated caused relaxation through endothelium-derived relaxing factor (nitric oxide), since this effect was diminished under hypoxia, in the presence of 5, 8, 11, 14 eicosatetraynoic acid (a non-specific inhibitor of cyclo-oxygenase and lipoxygenase), quinacrine (a phospholipase A_2-inhibitor) or hydroquinone (an oxygen radical scavenger). On the other hand,

Toda et al (1982) showed that the H_1 receptor-mediated relaxation of canine mesenteric arteries could be mediated through PGI_2. More recently (Ea Kim et al 1992), we demonstrated that the H_3-receptor-mediated relaxation could involve the endothelial release of both nitric oxide and PGI_2, since it was reduced by tranylcypromine (a PGI_2-synthesis inhibitor) and L-NMMA or L-NAME inhibitors of nitric oxide synthesis. The present data suggest that the methylhistamine-induced fall in MAP of guinea-pigs might be mediated via the L-arginine nitric oxide pathway. The pressor effect induced by the agonists tested was not a urethane-dependent mechanism since similar reponses were obtained with these agonists in our own preliminary experiment in pentobarbitone-anaesthetized guinea-pigs. Further studies are needed to explain the molecular mechanism responsible for activation of H_3-receptor sites at this level.

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References

Arrang, J. M., Garbarg, M., Lancelot, J. C., Lecomte, J. M., Pollard, H., Robba, M., Schunack, W., Schwartz, J. C. (1987) Highly potent and selective ligands for histamine H_3-receptors. Nature 327: 117–123

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. Pharmacol. 54: 319–324

Ea Kim, L., Javellaud, J., Oudart, N. (1992) Endothelium dependent relaxation of rabbit middle cerebral artery to a histamine H_3-agonist is reduced by inhibitors of nitric oxide and prostacyclin synthesis. Br. J. Pharmacol. 105: 103–106

Harvey, C. A., Owen, D. A. A. (1980) Time-course of depressor responses to histamine. Br. J. Pharmacol. 68: 187P

Ishikawa, S., Sperelakis, N. (1987) A novel class (H_3) of histamine receptors on perivascular nerve terminals. Nature 327: 158–160

Knowles, R. G., Palacios, M., Palmer, R. M. J., Moncada, S. (1990) Kinetic characteristics of nitric oxide synthase from rat brain. Biochem. J. 269: 207–210

Moore, P. K., Al-Swayeh, O. A., Chong, N. W. S., Evans, R. A., Gibson, A. (1990) L-N^G nitroarginine (L-NOARG) a novel L-arginine reversible inhibitor of endothelium-dependent vasodilatation in vitro. Br. J. Pharmacol. 99: 408–412

Palmer, R. M. J., Rees, D. D., Ashton, D. J., Moncada, S. (1988) L-Arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. Biochem. Biophys. Res. Commun. 153: 1251–1256

Rees, D. D., Palmer, R. M. J., Schulz, R., Hodson, H. F., Moncada, S. (1990) Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br. J. Pharmacol. 101: 746–752

Toda, N., Konishi, M., Miyazaki, M. (1982) Involvement of endogenous prostaglandin I_2 in the vascular action of histamine in dogs. J. Pharmacol. Exp. Ther. 223: 257–262

Van de Voorde, J., Leusen, I. (1982) Vascular endothelium and the relaxation effect of histamine on aorta of the rat. Arch. Int. Pharmacodyn. 256: 329–330

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Partitioning and thermodynamics of dipyridamole in the *n*-octanol/buffer and liposome systems

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Abstract—The thermodynamics of partitioning (K) of dipyridamole has been determined in *n*-octanol/buffer and liposome-buffer systems at pH 7·4. Dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) were used to prepare multilamellar liposomes. Partitioning of dipyridamole did not depend on the amount of *n*-octanol employed, however, partitioning was dependent upon the quantity of DMPC employed to prepare liposomes. Plots of log K vs $\frac{1}{T}$ were linear in the *n*-octanol and liposome systems. Partitioning was generally greater in liposomes than in the *n*-octanol/buffer system. Among liposomes, the partitioning was greater in DMPC liposomes at all temperatures. The values of enthalpy (ΔH) and entropy (ΔS) were positive in both the *n*-octanol and liposome systems. These values were lower in DMPC liposomes and were comparable in the *n*-octanol and DPPC liposomes. Thus, the interaction of dipyridamole depends on the rigidity of lipid bilayers and liposomes constitute a more selective partitioning system than the *n*-octanol/buffer system.

Dipyridamole is a widely used coronary vasodilator and antithrombic drug (FitzGerald 1987). In the past few years, attention has also been focused on its ability to potentiate the cytotoxic effects of various antitumour drugs (Grem & Fischer 1989). Recently, a further possible use of dipyridamole emerged in the treatment of human immunodeficiency virus type-1 (HIV-1) infections. Dipyridamole potentiates the antiviral effects of 3′-azido-3′-deoxythymidine (AZT) and 2′,3′-dideoxycytidine (ddC) in HIV-infected human monocyte/macrophages in-vitro (Szebeni et al 1989).

The distribution of drugs in membranes was first quantitatively described by Collander (1951) to be related to their partition coefficients in bulk oil/water systems. Among the many oils which have been investigated, semipolar solvents yield better correlations with the partitioning of solutes in model and biological membranes than non-polar solvents (Leo et al 1971; Diamond & Katz 1974). In particular, *n*-octanol has been a useful reference system for extrathermodynamic studies on a variety of systems (Hansch & Dunn 1972). Although the water-saturated *n*-octanol/water system presumably possesses structural characteristics as a result of the formation of water/*n*-octanol clusters (Smith et al 1975), it has been suggested that the bulk oil lacks sufficient structural similarities to biological membranes to account for the role of steric influences of drug molecular structure on membrane partitioning, transport, drug-receptor interactions and, hence, biological activity (Rogers & Wong 1980). The present study compares the partitioning behaviour of dipyridamole in *n*-octanol/buffer and buffered liposome systems using a thermodynamic approach and, from this, evaluates the interaction of dipyridamole with lipid bilayer systems in comparison with the *n*-octanol/buffer system.

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