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EFFECTS OF METHYLATED DERIVATIVES OF HISTAMINE ON THE ISOLATED GUINEA PIG AORTA

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The effects of histamine and various methylated derivatives of histamine ie (R) α-methylhistamine, Nα, Nα-dimethylhistamine, 1-methylhistamine and 3-methylhistamine on the vascular system was investigated using isolated quinea pig aorta. Histamine and Nα, Na-dimethylhistamine, (0.1-100 μM) both caused a concentration-dependent contraction of the aorta, which was decreased with cimetidine (100 mM) and abolished by pyrilamine (1 μM). (R) α-methylhistamine produced a concentration-dependent relaxation in aortic rings pre-contracted with KCl (50 mM) which was potentiated in the combined presence of pyrilamine (1mM) and cimetidine (100 μM), but was abolished when impromidine (10 μM) was also added to these two antagonists, suggesting that this response was mediated by H_3 receptors. Both 1-methylhistamine and 3-methylhistamine (0.1-100 μM) failed to produce either contraction of the aorta or relaxation in pre-contracted preparations. The results suggest that in this preparation histamine derivatives with methylation of the nitrogen atom as opposed to the terminal carbon atom at the end of the histamine side chain does not produce significant H_3 receptor activation, further methylation of the histamine ring in the 1 - or 3 - positions leads to a marked reduction of the vasoactive effects of the molecules.

Key words: histamine receptors, methylhistamines, aorta (quinea pig)

INTRODUCTION

The functions of histamine in homeostasis, particularly in local homeostasis, is a subject of increasing interest. However, the possible functional roles of histamine metabolites has been less extensively studied. Different species of animals may respond quite differently and unpredictably to histamine derivatives. The main catabolic route of histamine in mammals involves methylation of the ring and side chain nitrogen and thus at least four natural methylated

histamines can be produced (Navert et al., 1985). In addition several methylated derivatives of histamine have been synthesised in studies of the structural-activity relationships of the novel H_3 receptor, where methylation of the side chain and the integrity of the imidazole ring seem to be important for receptor recognition. Thus, in vascular response to histamine three types of histamine receptors H_1 (Ash and Schild, 1966), H_2 (Black et al., 1972) and H_3 (Ea-Kim and Oudart, 1988) can participate. Recent articles provide evidence for the presence of a novel H_3 receptor on histaminergic nerve endings in rat brain tissues (Arrang et al., 1987), perivascular autonomic nerve terminals of the quinea pig mesenteric artery (Ischikawa and Sperelakis, 1987) and presynaptic cholinergic neurons in human airways (Ichinose and Barnes, 1989). Nα - methylhistamine and Nα, Nα - dimethylhistamine are both potent H_3-receptor agonists but show little selectivity between the three classes of histamine receptor (Arrang et al., 1983), while (R) α - methylhistamine is a potent agonist of histamine H_3 - receptors (Van de Werf and Timmerman, 1989). In an earlier study, we showed that one methylated product of histamine, Nα - methylhistamine, had effects on all three types of histamine receptors in the isolated quinea pig heart (Anđelković et al., 1990).

The present study was undertaken to investigate the effects of other methylated products of histamine, ie (R) α - methylhistamine, Nα, Nα - dimethylhistamine, 1-methylhistamine and 3-methylhistamine on the vascular system using isolated quinea pig aorta and to compare their effects with that of histamine.

MATERIALS AND METHODS

The preparations and experimental protocol

Guinea pigs of either sex weighing between 200-350 g were stunned and the heart together with a length of aorta removed and placed in Krebs-bicarbonate solution at $+4^0$ C and gassed with 95% O_2 and 5% CO_2. The specimens of proximal descending aorta were cut into rings (6 mm) with great care to avoid stretching and damaging the endothelial cells. The presence of endothelium was confirmed histologically because it was shown that acetylcholine did not cause relaxation in pre-contracted aorta of the quinea pig, although microscopic examination proved the presence of endothelium (Van de Voorde and Leusen, 1984). An isolated ring of aorta was placed in an organ bath (10 ml) containing Tyrode's solution at $+37^0$ C and gassed with 95% 0_2 and 5% C^0_2. The Tyrode solution used was composed of (mM): NaCl 136.9, KCl 2.69, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42 and glucose 5.55. An initial tension of 1 g was applied (on the base of a length-tension curve using 50 mM of KCl) and 1h allowed for the preparation to adapt. Changes in the tension of the smooth muscle indicating vasoconstriction or dilation were measured using an isometric tension transducer and physiological recorder (IPM, Tuzla, Yugoslavia). The viability of the preparation was tested using the constriction response to the chosen concentration of KCl (50 mM) after a cumulative concentration-response curve to KCl (10-100 mM) had been constructed. Histamine (0.1-100 μM) and

Nα, Nα-dimethylhistamine (o.1-100 μM) were administered under resting conditions in a cumulative manner. (R) α -methylhistamine (o.1-100 μM) was administered after KCl-induced (50 mM) pre-contraction. At the end of the (R) α methylhistamine-induced response papaverine (100 μM) was added to cause complete relaxation. 1-methylhistamine and 3-methylhistamine, (0.1-100 μM) were administered under resting conditions and in preparations after KCl-induced (50 mM) pre-contraction. Pyrilamine (1 μM), cimetidine (100 μM) or impromidine (10 μM) were incubated alone or in combination 30 min before the agonists were added.

Analysis of data

In the case of histamine and Nα, Nα -dimethylhistamine responses, the contraction obtained as the response to 100 μM of agonist was taken as 100% and other responses to lower concentrations of certain agonists compared to these. (R) a -methylhistamine-induced responses are expressed as a percentage of the maximum KCl-induced constriction. The concentration required to produce 50% of the maximum response (EC_{50}), the maximum response (Em) and the slope of linear regression lines were calculated from concentration-response curves plotted for each agonist alone and in the presence of different antagonists.

Statistics

All values are expressed as mean value +/- standard error mean (X +/- S.E.M.). The slopes of linear regression lines and correlation coefficients were evaluated. Em were compared using Student's t-test. P values smaller than 0.05 were considered to be significant.

Drugs

The following drugs were used: histamine dihydrocloride (Sigma), papaverine hydrocloride (Sigma), pyrilamine (Sigma), cimetidine (Belupo), impromidine (Smith Kline Beecham), (R) α-methylhistamine (Dr.Arrang), Nα, Nα -dimethylhistamine (Smith Kline Beecham), 1-methylhistamine (Smith Kline Beecham) and 3-methylhistamine (Dr. Arrang), Nα, Nα -dimethylhistamine (Smith Kline Beecham), 1-methylhistamine (Smith Kline Beecham) and 3-methylhistamine (Smith Kline Beecham).

RESULTS

Effects of histamine and N^{α}, N^{α}-dimethylhistamine

Histamine (o.1-100 μM) caused a concentration-dependent contraction of the isolated quinea pig aorta (Figure 1-top, Table 1). This contraction response was significantly reduced by the H_2 receptor antagonist cimetidine (100 μM) and abolished by the H_1 receptor antagonist pyrilamine (1 μM) (Figure 1-top, Table 1). Similarly, Nα, Nα– dimethylhistamine (0.1-100 μM) caused a concentration-dependent contraction of the isolated quinea pig aorta. However, the slope of this response was significantly less than the slope of the response to his-

tamine. Further, like histamine these responses were reduced by the H_2 receptor antagonist cimetidine (100 μM) and abolished by the H_1 receptor antagonist pyrilamine (1 μM) (Figure 1-bottom, Table 3). There was no significant relaxation of the aorta by either histamine or N^{α}, N^{α}-dimethylhistamine in the presence of pyrilamine.

Table 1. Comparison of the E_m (Student's t test), slope of regression lines and EC_{50} values obtained from log concentration-response curves of histamine alone and in the presence of antagonists on guinea pig aorta (n=4).

PARAMETERS	CONTROL	PYRILAMINE	CIMETIDINE
EC_{50}(μM)	4.26	/	3.89
E_m(g)	2.07 ± 0.24g	/	0.36 ± 0.08g P<0.05
slope	31.91 ± 4.27	/	6.59 ± 1.63
r	0.982		0.940

[a]Values given are means ± S.E.M. Differences were considered significant when P<0.05.

Table 2. Comparison of the E_m (Student's t test), slope of regression lines and EC_{50} values obtained from log concentration-response curves of (R) α -methylhistamine alone and in the presence of antagonists on guinea pig aorta constricted with KCl (50 mM) (n=8).

PARAMETERS	CONTROL	PYRILAMINE +CIMETIDINE	PYRILAMINE +CIMETIDINE +IMPROMIDINE	IMPROMIDINE
EC_{50}(μM)	0.12	0,47	/	0.09
E_m(%)	21.64±4.80	35.74±4.84 P<0.05	/	16.55±0.60 P<0.05
slope	−(3.60±0.39)	−(7.10±0.69)	/	−(2.68±0.15)
r	0.980	0.990		0.996

[a]Values given are means ± S.E.M. Differences were considered significant when P<0.05.

Table 3. Comparison of the E_m (Student's t test), slope of regression lines and EC_{50} values obtained from log concentration-response curves of Nα, Nα -dimethylhistamine alone and in the presence of antagonists on guinea pig aorta (n=4).

PARAMETERS	CONTROL	PYRILAMINE	CIMETIDINE
EC_{50}(μM)	6.1	/	690
E_m(g)	2.15 ± 0.43g[a]	/	0.36 ± 0.05g P<0.05
slope	23.35 ± 1.17	/	4.22 ± 1.45
r	0.997		0.898

[a]Values given are means ± S.E.M. Differences were considered significant when P<0.05.

Effects of (R) α-methylhistamine

(R) α-methylhistamine (0.1-100 μM) caused a concentration-dependent relaxation of the isolated quinea pig aorta pre-contracted with KCl (50 mM) (Figure 1-middle, Table 2). When the aorta was preincubated with both the H_1

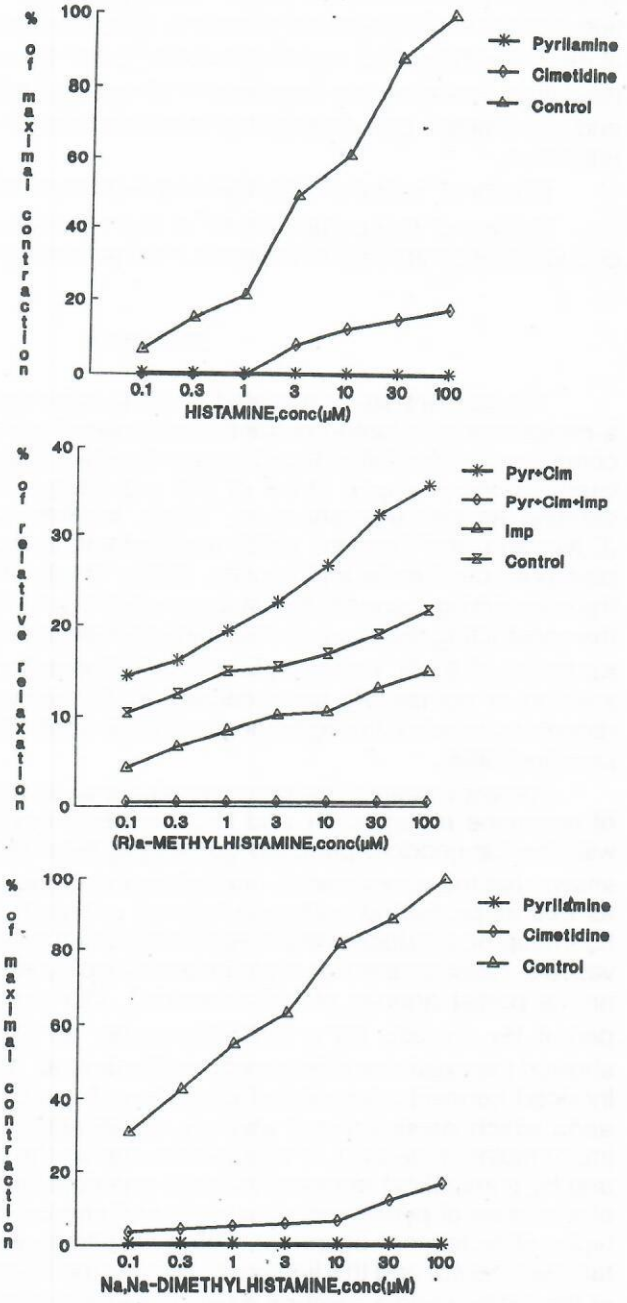

Figure 1. (top) - Concentration-effect related lines for the effects of histamine on the isolated guinea pig aorta in the presence of pyrilamine (1 μM) or cimetidine (100 μM); (middle) - concentration- effect related lines for the effects of (R) α-methylhistamine on the pre-contracted isolated guinea pig aorta (50 mM of KCl) (EC_{100} = 1.46 +/- 0.24 g), in the presence of pyrilamine (1 μM) and cimetidine (100 μM), in the presence of impromidine (10 μM), in the presence of pyrilamine (1 μM), cimetidine (100 μM) and impromidine (10 μM); (bottom) - concentration-effect related lines for the effects of N^{α}, N^{α}- dimethylhistamine on the isolated guinea pig aorta, in the presence of pyrilamine (1 μM) or cimetidine (100 μM).

antagonist pyrilamine (1 μM) and H_2 antagonist cimetidine (100 μM), the relaxation response to (R) a-methylhistamine was significantly increased. Impromidine alone (10 μM) had no significant effect on the (R) α– methylhistamine-induced relaxation. However, the combination of pyrilamine (1 μM), cimetidine (100 μM) and impromidine (10 μM) together abolished the (R) α-methylhistamine-induced relaxation.

Effects of 1-methylhistamine and 3-methylhistamine

Neither of these substances in the concentration range 0.1-100 μM induced either contraction of the aorta or relaxation in pre-contracted preparations.

DISCUSSION

The present study revealed that the administration of histamine caused a concentration-related contraction of isolated quinea pig aorta under resting conditions. Histamine-induced contraction has also been demonstrated in isolated coronary arterial strips of the pig (Hagen and Paegelow, 1980), dog cerebral arteries (Konishi et al., 1981), isolated cat middle cerebral arteries (Edvinsson and Owman, 1975) and isolated aorta of cats, mice and guinea pigs (Van de Voorde and Leusen, 1984). Treatment with pyrilamine abolished the constricting response to histamine while treatment with cimetidine decreased the constricting response to histamine (pyrilamine Kd for H_1=0.8 nM; cimetidine Kd for H_2=0.8 μM, Kd for H_1=0.45 mM). These findings suggest that the constriction of guinea pig aorta caused by histamine is mediated through H_1 -receptors, which is in agreement with the observation of Van de Voorde and Leusen (1984).

Recent research has demonstrated that, in addition to the two main types of histamine receptor, H_1 and H_2, there is a novel H_3 receptor. Experiments with the Langendorff perfused guinea pig heart (Anđelković et al., 1990) have shown that the H_3 agonist Nα-methylhistamine (Ischikawa and Sperelakis, 1987) as well as producing a change in heart rate and contractility mediated by the H_2 -receptor, produced an increase in coronary flow and a decrease in coronary vascular resistance which could be abolished by impromidine (an H_3 -antagonist and a partial agonist of H_2 -receptors). Our previous results with the most potent H_3 agonist, (R) α-methylhistamine, on the isolated quinea pig aorta showed the existence of H_3 receptors (Rosic et al., 1991). (R) α– methylhistamine induced concentration-related relaxation of pre-contracted by KCl guinea pig aorta which persisted and was not significantly changed in the presence of impromidine. Mareover, it was potentiated in the presence of H_1 (pyrilamine) and H_2 (cimetidine) antagonists but it was completely inhibited in the presence of a mixture of pyrilamine, cimetidine and impromidine (antagonists of all three types of histamine receptors). These results suggest the presence of H_3 histamine receptor and this finding is similar to the H_3-mediated relaxation response of the rabbit middle cerebral artery (Ea-Kim and Oudart, 1988) supporting the suggestion that H_3-receptors may be involved in vasodilator responses in ad-

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EFEKTI METILIRANIH DERIVATA HISTAMINA NA IZOLOVANOJ AORTI ZAMORCA

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SADRŽAJ

Efekti histamina i različitih metiliranih derivata histamina i to: (R) α-metilhistamina, N^{α}, N^{α}-dimetilhistamina, 1-metilhistamina i 3-metilhistamina ispitivani su na izolovanoj aorti zamorca. Histamin i N^{α}, N^{α}-dimetilhistamin, (0.1-100 μM) zajedno uzrokuju koncentracijski-zavisnu kontrakciju aorte koja se umanjuje u prisustvu cimetidina (100 μM) i potpuno blokira u prisustvu pirilamina (1 μM). (R) α-metilhistamin produkuje koncentracijsk –i zavisnu relaksaciju prstenova aorte pre-kontrahovane sa KCl (50 mM) koja je potencirana u prisustvu pirilamina (1 μM) i cimetidina (100 μM), ali se u potpunosti blokira dodavanjem impromidina (10 μM), što sugeriše prisustvo H_3 receptora. 1-metilhistamin i 3-metilhistamin ne menjaju značajno niti mirovni vaskularni tonus preparata niti posle prekontrakcije sa KCL (50 mM).

dition to those produced by stimulation of H_2 -and H_1-receptors (Konishi et al., 1981).

However, N^{α}, N^{α}-dimethylhistamine did not relax but contracted isolated guinea pig aorta under resting conditions. Treatment with pyrilamine abolished this concentration-related contractile response but cimetidine decreased N^{α}, N^{α}-dimethylhistamine-induced contractions. These findings suggest that the contractions of isolated quinea pig aorta caused by N^{α}, N^{α}-dimethylhistamine are mediated through H_1-receptors. Treatment with methylated derivatives of histamine ring 1-methylhistamine and 3-methylhistamine did not change significantly the tonus of isolated quinea pig aorta under resting conditions nor the tonic phase of KCl induced pre-contraction which could indicate that methylation of the hystamine ring in the 1 - or 3- position probably leads to the loss of its vasoactive effect.

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