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Effects of histamine and the histamine antagonists mepyramine and cimetidine on human coronary arteries *in vitro*

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1 The effects of histamine have been studied on human isolated coronary artery preparations taken from hearts ranging in age from 9 to 73 years.

2 Histamine in large concentrations (100 μM) contracted arteries which were without tone or spontaneous activity and sometimes induced rhythmic contractile activity. If spontaneous rhythmic activity was present it was enhanced by histamine. The contractile effects of histamine were inhibited by mepyramine but not by cimetidine.

3 Arteries which were contracted by depolarization responded with relaxation to histamine concentrations lower than those required to evoke a contraction; arteries from younger hearts were more sensitive than those from older hearts.

4 Mepyramine potentiated the maximal relaxant effect of histamine in arteries from hearts of all ages but cimetidine had very little effect.

5 In the presence of mepyramine, cimetidine antagonized the relaxant effect of histamine, shifting the concentration-effect curve to the right.

6 It is concluded that human coronary arteries contain both H_1- and H_2-type receptors, the H_1-receptors mediating contraction. The relaxant effects of histamine can only be inhibited by a combination of both H_1- and H_2-receptor antagonists.

Introduction

Histamine-containing mast cells are present in cardiac muscle and coronary arteries (Mannaioni, 1972) from which histamine can presumably be released by physiological and pathological stimuli and perhaps exert a local regulatory action on myocardium and blood flow. Histamine is also present in sympathetic nerves (Ryan & Brody, 1970) and is released into the circulation during sudden withdrawal of sympathetic tone (Heitz & Brody, 1975) and following direct stimulation of sympathetic nerves and spinal nerve roots (Lioy & White, 1973); this may also indicate a local regulatory role in vascular smooth muscle tone. The effects of histamine on the human coronary vasculature are not clear. In perfused human hearts, histamine causes an increase in coronary flow (Kountz, 1932; Kountz, Parsons & Koenig, 1934) while in isolated coronary vessels it has been reported to produce contraction of larger vessels and relaxation of small vessels (Kountz, 1932). Godfraind & Miller (1983) observed contraction, relaxation or no effect in different arterial preparations; these differences might be related to the age of the vessels. Many studies in animal tissues have described the presence of H_1- and H_2-receptors in coronary vessels, the different receptors subserving different functions (for example Broadley, 1975; Giles Heiss, Wilcken, 1977) and similar observations have been made in human coronary arterial segments (Ginsburg, Bristow, Harrison & Stinson, 1980a; Ginsburg, Bristow, Stinson & Harrison, 1980b).

We have investigated further the responses induced by histamine in human coronary arteries of various ages and the effects of the specific histamine-receptor antagonists mepyramine (H_1) and cimetidine (H_2) (Brimblecombe, Duncan, Durant, Ganellin, Parsons & Black, 1973) on these responses.

Methods

Hearts were taken from 8 patients (9–73 years old) within 5 h of sudden death (due to accident or non-cardiac related vascular collapse). None of these

patients had been treated with antihistamines; (one 73 y.o.) was treated for arthritis and one had been anaesthetized. The terminal portions (about 2 cm) of the anterior descending branch of the left coronary artery and of the circumflex coronary artery were removed and cleaned of all loosely adherent tissue. Rings of coronary artery (about 2 mm wide) were suspended in 50 ml organ baths containing physiological solution (mM: NaCl 112, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1, $MgSO_4$ 1.2, $CaCl_2$ 1.25 and glucose 11.5) maintained at 37°C and aerated with a gas mixture of 95% O_2 and 5% CO_2 under a tension of 2 g. Ca^{2+}-free physiological solution was prepared by omission of calcium. Some rings of coronary artery from each heart were stored overnight in physiological solution at 4°C for use the next day.

Contractile responses were measured by means of an isometric transducer coupled to a potentiometric pen recorder. After an equilibration period of 60 min the preparations were contracted maximally in a depolarizing solution (mM: NaCl 17, KCl 100, $NaHCO_3$ 25, KH_2PO_4 1, $MgSO_4$ 1.2, $CaCl_2$ 1.25 and glucose 11.5), then washed in normal physiological solution and further equilibrated for 60 min before exposing the tissues to histamine at a single concentration or by cumulative additions, increasing the bath concentration by about 3 fold at each concentration level. After washing and allowing a 30 min equilibration period, preparations were incubated with either mepyramine 1 μM or cimetidine 1 μM for a further 30 min before being re-exposed to histamine. In other preparations, after the second 60 min equilibration period, the arteries were contracted for a second time in depolarizing solution and when the contraction had stabilized, histamine was added to the bath again, either as a single concentration or in a cumulative manner. After the maximal effect was obtained preparations were washed until the baseline was regained, then equilibrated for 30 min before being incubated with either mepyramine 1 μM, or cimetidine 1 μM or both mepyramine and cimetidine together, for a further 30 min before being contracted for a third time in depolarizing solution and obtaining a second set of responses to histamine. Control experiments to examine histamine responses in the absence of antagonists were performed at the same time as were parallel control experiments to examine the maintenance of tone of the depolarization induced contraction.

Drugs

Histamine HCl (Merck), mepyramine HCl (Specia), cimetidine HCl (S.K.F.) and isoprenaline HCl (Boehringer Ingelheim) were dissolved in distilled water as stock solutions of 10 mM each day and diluted as required.

Statistics

The data are expressed as means ± s.e.mean. Tests of significance have been made using Student's *t* test, *P* values smaller than 0.05 being considered significant.

Results

Contraction and relaxation induced by histamine

Isolated coronary artery preparations taken from 9–24 year old (y.o.) hearts were without spontaneous activity. However preparations taken from older hearts often displayed various patterns of spontaneous activity and rhythmic contractile activity could be induced by exposure to histamine. In preparations which did show spontaneous activity, histamine increased the amplitude of the phasic contractions and the basal tone of the preparation.

Histamine 10 μM produced a phasic contraction of 14 out of 15 arterial preparations taken from 4 hearts (9–45 y.o.) which were without spontaneous activity. These phasic contractions were followed by rhythmic contractions in 7 of the preparations which were maintained over the next 10 min (Figure 1). After washing the rhythmic activity ceased. When added cumulatively (0.01 to 100 μM) to preparations from a 15 y.o. heart, which also had no inherent tone or activity, phasic responses were seen when the concentration reached 10 to 100 μM (6 out of 6 preparations); these responses were not followed by any rhythmic activity (Figure 2). These contractions induced by histamine were completely abolished by mepyramine 1 μM (3 out of 3 preparations) and were either unaffected by cimetidine 1 μM (2 out of 3 preparations) or potentiated (1 preparation).

When arterial preparations from a 45 y.o. heart were previously contracted in depolarizing solution, histamine (0.01 to 1.0 μM) added cumulatively to the organ bath produced a relaxation of tone (Figure 3). The cumulative addition of histamine (0.03 to 3 μM) to arteries taken from 9 and 37 y.o. hearts (Figures 4 and 5) and contracted in depolarizing solution also produced concentration-related reductions in tension.

Arterial preparations from a 73 y.o. heart were contracted in depolarizing solution but these contractions were not relaxed by either histamine (0.01 to 100 μM, Table 1) or isoprenaline (0.1 to 30 μM).

Histamine relaxation of depolarization-induced contraction and the effects of selective antagonists

Arteries from a 37 y.o. heart were relaxed by histamine 0.01 to 1.0 μM, larger concentrations producing a reduced effect. Maximal relaxation was 47.9 ± 6.8% (EC_{50} 0.22 ± 0.10 μM, $n = 5$) (Table 1).

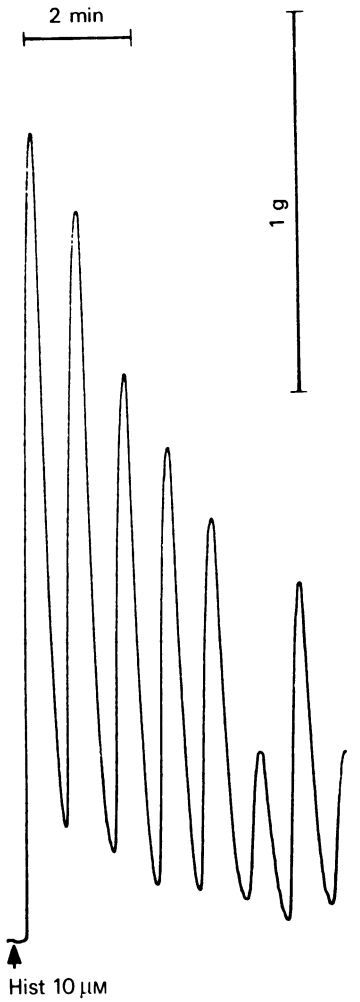

Figure 1 Contraction produced in a 9 y.o. human coronary artery segment by 10 μM histamine, which also induces rhymical contractile activity.

In the presence of cimetidine 0.1 and 1.0 μM, which had no significant effect on the depolarization – induced contraction, the maximal relaxation induced by histamine was 44.1 ± 6.3% (EC_{50} 0.12 ± 0.44 μM) and 41.1 ± 7.5% (EC_{50} 0.13 ± 0.04 μM) respectively. Neither the maximal responses nor the EC_{50} values were significantly changed in the presence of cimetidine (Figure 4).

Arteries from a 9 y.o. heart were relaxed by histamine 0.1 to 1.0 μM, with larger concentrations the relaxation tended to reverse. In these 9 y.o. arteries the maximal relaxation was 27.9 ± 2.0% of the depolarization-induced contraction, the EC_{50} value being 0.2 ± 0.1 μM ($n = 5$) (Table 1). In the presence

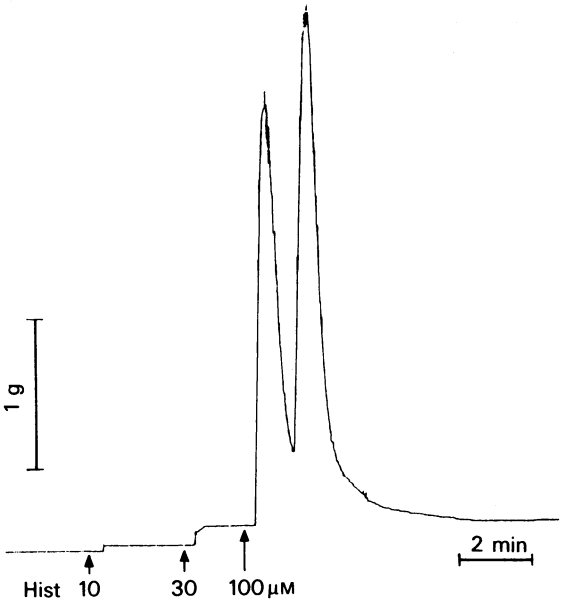

Figure 2 Typical contractions produced in a 15 y.o. human coronary artery segment by cumulative additions of histamine.

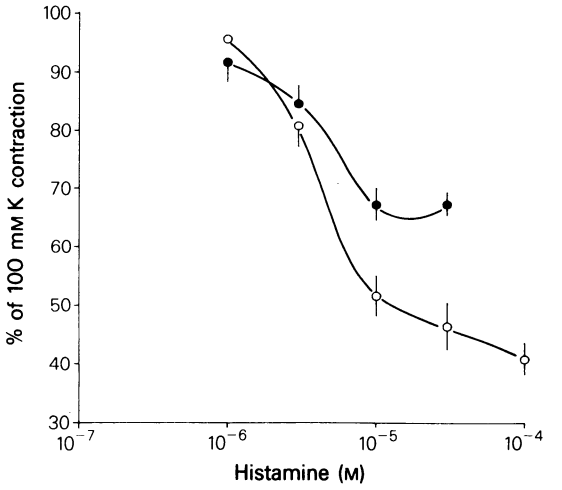

Figure 3 Relaxation of depolarization-induced contractions produced by cumulative additions of histamine to 45 y.o. human coronary artery segments in the absence (●) and in the presence of mepyramine (1 μM; ○). Equilibrium responses to each concentration of histamine (ordinate scale) are expressed as a percentage of the depolarization-induced contraction. Vertical bars represent s.e.mean. Each curve is the mean of 5 observations.

Table 1 Effect of cumulative additions of histamine (0.03 to 10 μM) to human coronary artery preparations contracted by depolarizing solutions (100 mM K+)

Age (yrs)	*Response*	EC_{50} (μM)	*Concentration of histamine* (μM) *producing* *Threshold response*	*Maximal response*	*Maximal effect* (% of depolarization induced contraction)
9	Relaxation	0.2 ± 0.1	0.03 – 0.1	1.0	27.9 ± 2.0 (5)
37	Relaxation	0.2 ± 0.1	0.01	1.0 – 10	47.9 ± 6.8 (5)
45	Relaxation	3.0 ± 0.7	0.3 – 1.0	10	39.5 ± 4.5 (5)
73	Resistant				

Figures in parentheses represent number of arterial preparations.

of mepyramine 1 μM, which itself had no significant effect on depolarization-induced contractions, the maximal relaxation was increased to 54.0 ± 6.3% and the EC_{50} value to 8.6 ± 1.1 μM ($0.02 < P < 0.05$ and $0.001 < P < 0.01$ respectively). Cimetidine 1 μM, added to the organ bath when these preparations were maximally relaxed by histamine in the presence of mepyramine (1 μM), produced a one third ($n = 3$) reversal of the relaxation.

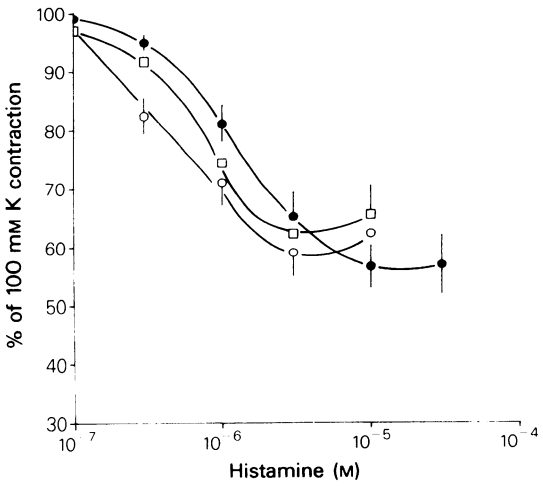

Figure 4 Relaxation of depolarization-induced contractions produced by cumulative additions of histamine to 37 y.o. human coronary artery segments in the absence (●) and in the presence of cimetidine (0.1 μM, ○; 1.0 μM, □). Equilibrium responses to each concentration of histamine (ordinate scale) are expressed as a percentage of the depolarization-induced contraction. Vertical bars represent s.e.mean. Each curve is the mean of 5 observations.

Arteries from a 45 y.o. heart were relaxed by histamine (1 to 10 μM); concentrations greater than 30 μM producing a reduced effect. Maximal relaxation was 39.5 ± 4.5% (EC_{50} 3.0 ± 0.7 μM). In the presence of mepyramine 1 μM the maximal relaxation was significantly increased to 59.3 ± 2.6% ($0.001 < P < 0.01$) without significantly altering the EC_{50}, which was 3.5 ± 1.1 μM (Figure 3). Cimetidine (1 μM) added to the bath when the preparations were maximally relaxed also produced about a one third reversal of the histamine-induced relaxation.

Comparing the relaxant effects of histamine on the arteries from 9 and 45 y.o. hearts the EC_{50} values were significantly different ($0.01 < P < 0.02$), the younger arteries being 16.6 times more sensitive to the relaxant effect of histamine although the maximal responses attained were not significantly different ($0.1 < P < 0.2$). In the presence of mepyramine the arteries had a similar sensitivity to the relaxant effects of histamine and there was no significant difference between the maximal responses attained.

Arteries from a 15 y.o. heart were relaxed by histamine 0.3 to 300 μM in the presence of mepyramine 1 μM. Maximal relaxation was 60.3 ± 1.0% and the EC_{50} value 5.7 ± 0.5 μM (Figure 5). Both the maximal response attained and the EC_{50} value were similar to those of the 9 and 45 y.o. arteries in the presence of mepyramine (compare Figures 3 and 5). In the presence of both mepyramine (1 μM) and cimetidine (1 μM) the concentration-effect curves for histamine were shifted to the right in a non-parallel fashion and the EC_{50} value was increased from 5.7 ± 0.5 to 53.0 ± 5.0 μM (Figure 5).

Discussion

This study has shown that histamine at high concentrations is capable of contracting all (even very old)

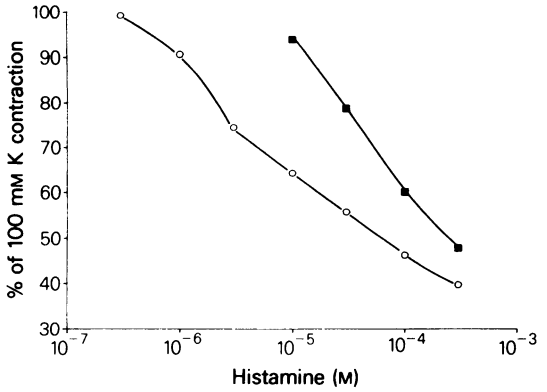

Figure 5 Relaxation of depolarization-induced contractions produced by cumulative additions of histamine to 15 y.o. human coronary artery segments in the presence of mepyramine (1 μM; ○) and in the presence of both mepyramine (1 μM) and cimetidine (1 μM; ■). Equilibrium responses to each concentration of histamine (ordinate scale) are expressed as a percentage of the depolarization-induced contraction. Each curve is the mean of 4 observations, standard errors do not exceed the size of the symbols.

coronary arteries but this was not always an obviously concentration-related effect (Figure 2). These histamine-mediated contractions were inhibited by mepyramine and unaffected or potentiated by cimetidine, indicating that H_1-receptors mediate contraction in these arteries. Ginsburg *et al.* (1980b) have described histamine-mediated concentration-dependent contractions of human isolated coronary arteries taken from hearts of transplant patients. The reasons for these different observations are unknown but may be related to the age and health of the patients. Histamine relaxed depolarized arteries from hearts less than 49 years old. The sensitivity of these arteries to histamine seemed to diminish with age (Table 1). These observations may indicate an age-related change in the relative number or importance of H_1- and H_2-receptors. Age-related changes in the importance of β-adrenoceptors in rat aorta have been reported (Fleisch, 1971; Godfraind & Dieu, 1978; Godfraind, 1979). In the presence of mepyramine, all arteries exhibited an enhanced and approximately equal maximal responsiveness to histamine, indicating that the dilator potential of H_2-receptors was equal at all ages but could be modified by the function of H_1-receptors. Ginsburg *et al.* (1980b) described a weak histamine-mediated relaxation of depolarized arteries of only 15–20%, which occurred in the presence, but not in the absence of the H_1- antagonist pyrobutamine (1 μM). The difference between their observations and the present ones could be due to non-physiological factors, such as age (not reported by Ginsburg *et al.*, 1980b) or pathological changes, since their arteries were obtained from pathological hearts.

Histamine-mediated relaxation was not antagonized by cimetidine alone (Figure 4), however in the presence of mepyramine, cimetidine shifted histamine-mediated concentration-effect curves to the right. This would seem to indicate some type of interaction between H_1- and H_2-receptors to produce dilatation that can only be inhibited by a combination of H_1- and H_2-receptor antagonists. Such an effect has also been reported in dog coronary arteries *in vivo* (Giles *et al.*, 1977) and in guinea-pig isolated hearts by some (Reinhardt, Wiemann & Schumann, 1976) but not all observers (Ecran, Bökesoy & Türker, 1974; Levi & Kuye, 1974; Broadley, 1976).

It is of interest to point out the report by Eckel, Gristwood, Nawrath, Owen & Satter (1982) who showed that the positive inotropic action of histamine on human isolated myocardium was antagonized by cimetidine alone, an indication of an H_2-receptor mediated response. The concentration range for the positive inotropic action was similar to the range reported in this paper for histamine induced relaxation. However, these authors did not report an age-related change in cardiac muscle sensitivity to histamine. Therefore, the present observations could be of pathological significance, because the release of histamine has been implicated in cardiac arrhythmias (see Gristwood, Owen and Smith, 1980; Editorial, 1982) an action that could be more important in aged people who show a reduced sensitivity to the relaxant action of histamine and thereby a reduced blood supply to the heart with increased inotropy. This situation could worsen the intensity of ischaemic arrhythmias (Carmeliet, 1982). However, in younger people such a situation could be overcome by coronary vasodilatation.

In summary, the present results show that human coronary arteries contain H_1-receptors which mediate contraction and H_2-receptors which mediate dilatation. The relative importance of each receptor type may depend on the age of the vessel. H_2-receptors appear to be more sensitive than H_1-receptors.

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