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Inhibition of cardiac sympathetic neurotransmission by histamine in the dog is mediated by H_1-receptors

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1 The role of histamine H_1- and H_2-receptors in mediating prejunctional inhibition of cardiac sympathetic neurotransmission and histamine-induced coronary vasodilatation were investigated in perfused dog hearts *in situ*.

2 Intra-arterial injections of histamine into the right coronary artery during the resting state caused slightly positive chronotropic responses in doses larger than 1 μg.

3 Histamine in doses of 0.1 to 10 μg into the right coronary artery reduced the tachycardia resulting from electrical stimulation of the cardiac sympathetic nerves.

4 Intra-coronary infusions of chlorpheniramine (300 μg/min) significantly reduced the histamine-induced depression of cardiac nerve stimulation. The effects of cimetidine (300 μg/min) and metiamide (300 μg/min) were less pronounced.

5 Histamine (1 to 10 μg) further increased heart rate resulting from the continuous intra-coronary infusion of noradrenaline (1 or 3 μg/min).

6 Intra-arterial injections of histamine (0.1 to 10 μg) caused an increase in coronary blood flow in a dose-dependent manner. This was partially inhibited by intra-coronary infusion of chlorpheniramine (10 to 300 μg/min) and by cimetidine (10 to 300 μg/min). The combination of both drugs (10 to 100 μg/min of each) caused a larger inhibition.

7 The present results suggest that the histamine-induced depression of heart rate during cardiac sympathetic nerve stimulation is due to a prejunctional effect mediated mainly by H_1-receptors. Histamine-induced coronary vasodilatation in the dog is mediated both by H_1- and H_2-receptors.

Introduction

During the course of investigations on the prejunctional effects of various substances on cardiac sympathetic nerve function in the perfused dog heart *in situ*, it was observed that the prejunctional inhibitory action of histamine was effectively antagonized by the histamine H_1-receptor antagonist, chlorpheniramine. This finding is of interest since previous studies have suggested that prejunctional inhibition by histamine of sympathetic neurotransmission is mediated by histamine H_2-receptors and not by H_1-receptors. The first report of a role of histamine in controlling sympathetic function via a prejunctional action noted that histamine caused a decrease in the release of labelled noradrenaline from dog saphenous vein strips during nerve stimulation (McGrath & Shepherd, 1976). This decreased release of noradrenaline was blocked by a histamine H_2-receptor blocking drug but not by a H_1-receptor blocker, and was mimicked by a H_2-receptor agonist. Similar results have been obtained in the dog perfused gracilis muscle (Powell, 1979) and in the dog heart (Lokhandwala, 1978a).

The distribution of histamine H_1- and H_2-receptors in the coronary vasculature of different animal species has not been fully established. Information obtained from the guinea-pig and the rabbit (Broadley, 1975; Coruzzi, Bongrani & Bertaccini, 1979; Flynn, Gristwood & Owen, 1979; Sakai, 1980), shows histamine H_1-receptor mediated vasodilatation, H_1-receptor mediated vasoconstriction and/or H_2-receptor mediated vasodilatation. In the dog coronary circulation, however, there is little information concerning the role of histamine H_1- and H_2-receptors in mediation of the vascular effects of histamine. Recently, Konishi, Toda & Yamamoto (1981) showed that relaxation of helically-cut strips of the dog coronary artery induced by histamine was mediated by histamine H_2-receptors. The present study provides evidence for H_1-receptor mediated prejunctional inhibition of cardiac sympathetic nerve function and for both H_1- and H_2-receptor mediated coronary vasodilatation.

Methods

Mongrel dogs of either sex, weighing from 9 to 15 kg, were anaesthetized initially with 30 mg/kg of sodium pentobarbitone intravenously; a constant level of anaesthesia was then maintained by an intravenous infusion (Havard Apparatus Co., 940) of pentobarbitone at a rate of 4 to 6 mg $kg^{-1} h^{-1}$. After endotracheal intubation, artificial respiration was maintained by a respiration pump (Harvard Apparatus Co., 607) with room air at 18 strokes/min and 20 ml/kg tidal volume. The chest was opened along the midline and both vagi were cut at the midcervical level. The right coronary artery was dissected near its origin and a cannula introduced into it for perfusion with the dog's own blood obtained from the right carotid artery. Coagulation of blood was prevented by an initial intravenous injection of sodium heparin, 500 units/kg, and hourly intravenous injections of 100 units/kg. Blood flow through the right coronary artery was measured by an electromagnetic flow meter (Nihon Kohden, MF-27). Heart rate was measured by a cardiotachometer (Nihon Kohden, RT-5) triggered from a surface silver bipolar electrode sutured on the right atrial epicardium, and monitored by a digital meter (Newport, 200B). Systemic blood pressure in the femoral artery was measured by a pressure transducer (Nihon Kohden, MPU-0.5). Changes in all parameters were recorded on an ink-writing oscillograph (Nihon Kohden, RJG-3008G).

The stellate ganglia on both sides were exposed and the ansa subclavia (cardiac sympathetic nerves) were cut. The distal end of the nerves on the right side was stimulated with rectangular pulses of 1 ms duration and supramaximal voltage (5 to 8 V) at 3 Hz delivered by an electronic stimulator (Nihon Kohden, SEN-1101) and isolation unit (Nihon Kohden, SS-101J).

The first series of experiments were concerned with histamine-induced prejunctional inhibition of cardiac sympathetic transmission. These experiments were conducted as follows: (1) the effect of histamine on heart rate was examined during resting state, (2)

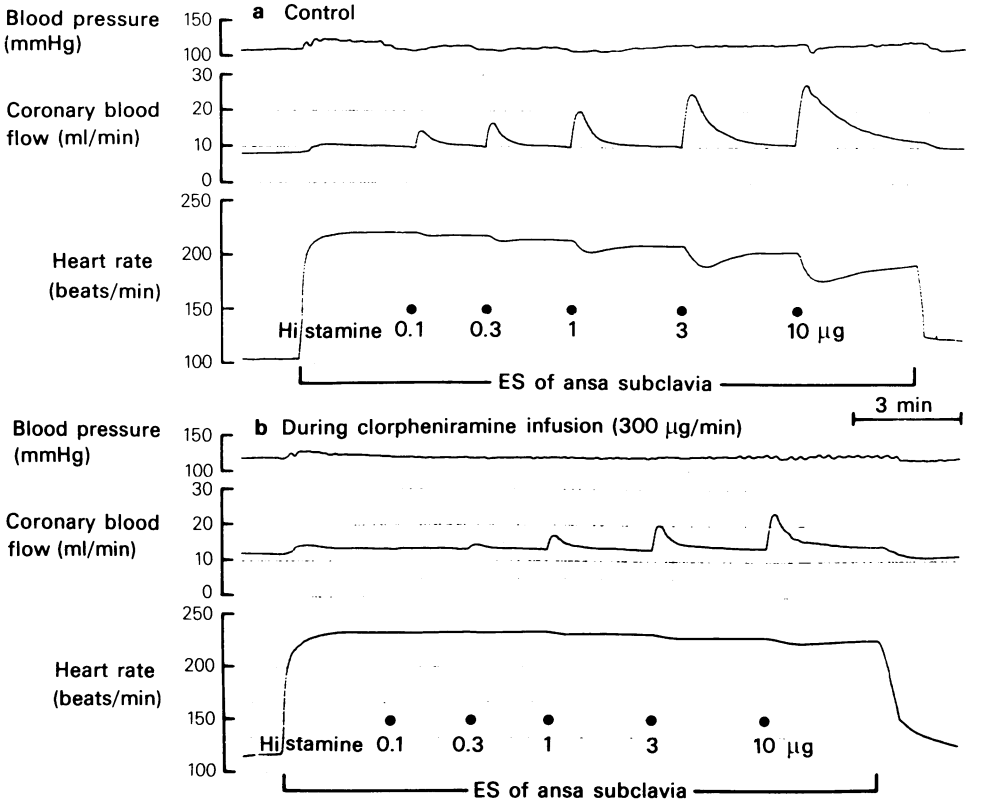

Figure 1 Effect of histamine injections into the right coronary artery on heart rate elevated by electrical stimulation (ES) of the ansa subclavia and on coronary blood flow, before (a) and during an infusion of chlorpheniramine (b) into the same artery. Rectangular pulses of 7 V and 1 ms were applied at 3 Hz. Note that both the histamine-induced depression of tachycardia and the increase in coronary blood flow are inhibited by chlorpheniramine.

the effects of histamine receptor blocking agents were studied on the histamine-induced depression of tachycardia induced by cardiac sympathetic nerve stimulation, (3) the effects of histamine were examined on noradrenaline-induced tachycardia. In the second series of experiments, the effects of histamine receptor blocking drugs on histamine-induced coronary vasodilatation were examined. In the dog heart the sino-atrial node receives a blood supply both from the dorsal right atrial artery (originating from the right coronary artery) and from the ventral left atrial artery (originating from the left coronary artery). The blood supply from the dorsal right atrial artery is dominant in most dog hearts; a minority receive blood mainly from the ventral left atrial artery (James, 1962; Hashimoto, Tanaka, Hirata & Chiba, 1967). Hearts of the former type were used in the first series of experiments and of the latter type for the coronary blood flow investigations.

Drugs used were histamine dihydrochloride (Tokyo Kasei), chlorpheniramine maleate (Yoshitomi), cimetidine (Fujisawa), metiamide (SKF) and noradrenaline hydrochloride (Sigma). Histamine, in volumes of 0.01 to 0.03 ml, was injected by a microinjector into a rubber tube connected to the perfusion circuit. The other drugs were infused at rates of 0.1 or 0.3 ml/min into the right coronary artery by means of an infusion pump (Harvard Apparatus Co., 975E). Statistical analysis was performed by Student's *t* test.

Results

Effect of histamine on heart rate at rest

When doses of 0.1 to 10 μg of histamine were injected into the right coronary artery, a slightly positive chronotropic response was produced in doses larger than 1 μg. The basal heart rate was 119 ± 4 beats/min (mean ± s.e.mean, $n = 10$) and increases in heart rate induced by 1, 3 and 10 μg of histamine were 4 ± 1, 6 ± 2 and 11 ± 2 beats/min, respectively. In a dose of 10 μg, histamine passed into the systemic circulation and lowered blood pressure

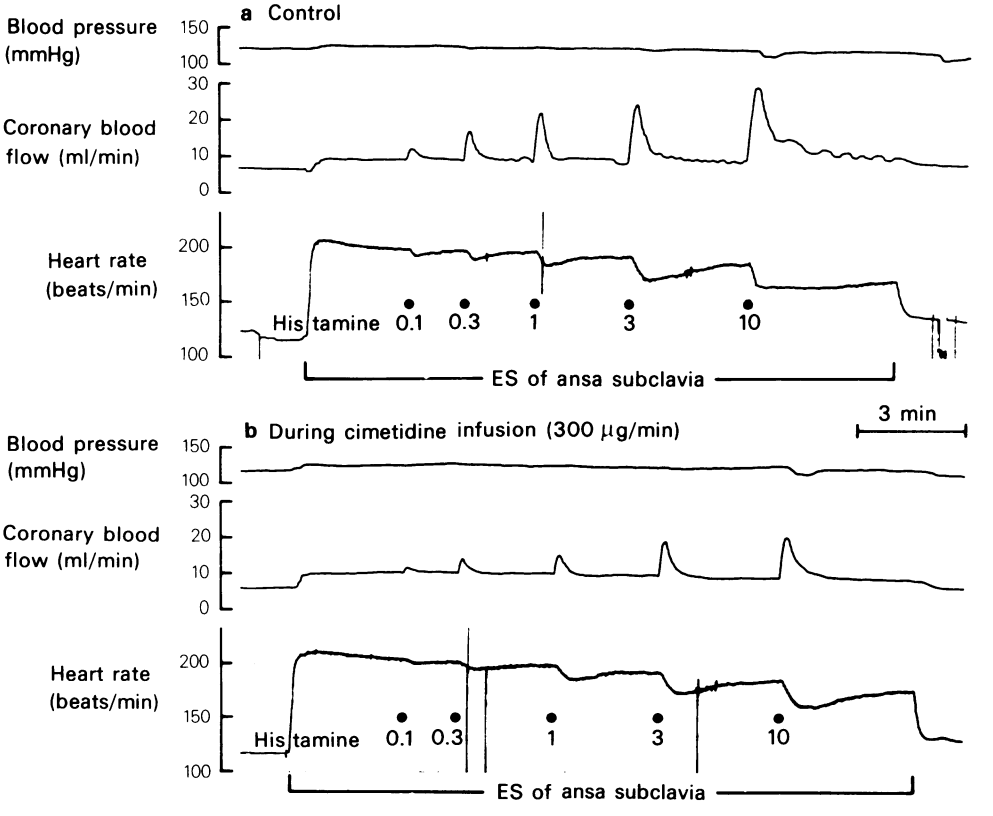

Figure 2 Effect of injections of histamine into the right coronary artery on heart rate elevated by electrical stimulation (ES) of the ansa subclavia and on coronary blood flow before (a) and during an infusion of cimetidine (b) into the same artery. Rectangular pulses of 5 V and 1 ms were applied at 3 Hz. Note that the histamine-induced depression of tachycardia is unaffected by cimetidine but that the increase in coronary blood flow is inhibited.

by 14 ± 4 mmHg from a basal mean blood pressure of 112 ± 5 mmHg, $n = 10$.

Effect of histamine during cardiac sympathetic nerve stimulation: effects of chlorpheniramine, cimetidine and metiamide

Continuous electrical stimulation of the cardiac sympathetic nerves produced a marked increase in heart rate, which stabilized at approximately 200 beats/min 3 to 5 min after the beginning of the stimulation. After stabilization, doses of 0.1 to 10 μg of histamine were injected into the right coronary artery at 3 to 5 min intervals. This caused a dose-dependent depression of the neurally induced tachycardia.

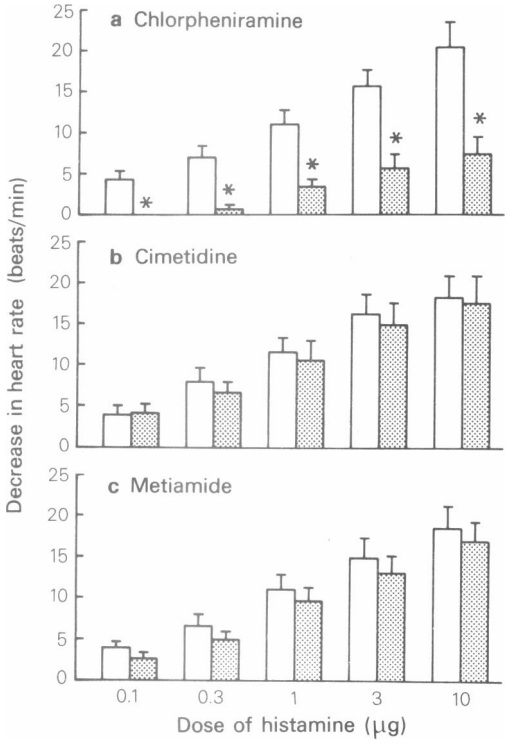

Figure 3 Effects of chlorpheniramine (a), cimetidine (b) and metiamide (c) on the histamine-induced depression of tachycardia resulting from cardiac sympathetic nerve stimulation. Histograms show decreases in heart rate induced by histamine injected into right coronary artery before (open columns) and during infusions of 300 μg/min of each antagonist (stippled columns) into the same artery, and are the means of each 7 experiments. Vertical bars show s.e.mean. Stimulation-elevated heart rate before and after the antagonists were 215 ± 6 and 227 ± 9 beats/min in (a), 206 ± 11 and 205 ± 12 beats/min in (b) and 224 ± 11 and 225 ± 7 beats/min in (c). *$P < 0.01$.

The influence of chlorpheniramine, cimetidine or metiamide on the histamine-induced reduction in tachycardia was observed in each of 7 experiments. Each antagonist (300 μg/min) was continuously infused into the right coronary artery. Chlorpheniramine significantly inhibited the histamine-induced depression of heart rate (Figures 1 and 3a). With cimetidine and metiamide, the histamine-induced effect was weakly attenuated in some animals and unaffected in others (Figure 2). Statistical significance ($P < 0.05$) for the effects of both antagonists was not obtained (Figure 3b,c).

Effect of histamine on heart rate during infusion of noradrenaline

A marked increase in heart rate together with a slight rise in blood pressure, resulted from the continuous infusion of noradrenaline (1 or 3 μg/min) into the right coronary artery. After a stable elevation of heart rate (to 210 ± 3 beats/min) was achieved, doses of 1 to 10 μg of histamine were injected into the right coronary artery. Increases in heart rate produced by histamine in doses of 1, 3 and 10 μg were 7 ± 4, 15 ± 4 and 17 ± 5 beats/min, respectively, (5 experiments).

Effects of chlorpheniramine and cimetidine on histamine-induced increases in coronary blood flow

Intra-arterial injections of histamine (0.1 to 10 μg) caused dose-dependent increases in coronary blood flow. The basal blood flow was 7.7 ± 0.6 ml/min (15 experiments) and the peak flow rates produced by 0.1, 0.3, 1, 3 and 10 μg of histamine were 12.1 ± 0.7, 14.8 ± 0.8, 18.1 ± 0.9, 22.6 ± 1.1 and 25.4 ± 1.1 ml/min, respectively.

Continuous infusions of chlorpheniramine (10 to 100 μg/min) into the right coronary artery inhibited histamine-induced increases in coronary blood flow in a dose-dependent manner. Increasing dose of chlorpheniramine up to 300 μg/min caused no further inhibition (Figure 4a). Cimetidine (10 to 300 μg/min by infusion), also inhibited the histamine-induced increase in coronary blood flow. A similar inhibition was obtained with doses over 30 μg/min (Figure 4b). Infusions of a combination of chlorpheniramine and cimetidine (10 to 100 μg/min of each) caused a larger inhibition of histamine-induced increases in coronary blood flow (Figure 4c).

Discussion

Several investigators have examined prejunctional actions of various substances on cardiac sympathetic nerve function in the intact dog preparation, by using changes in heart rate during cardiac nerve stimula-

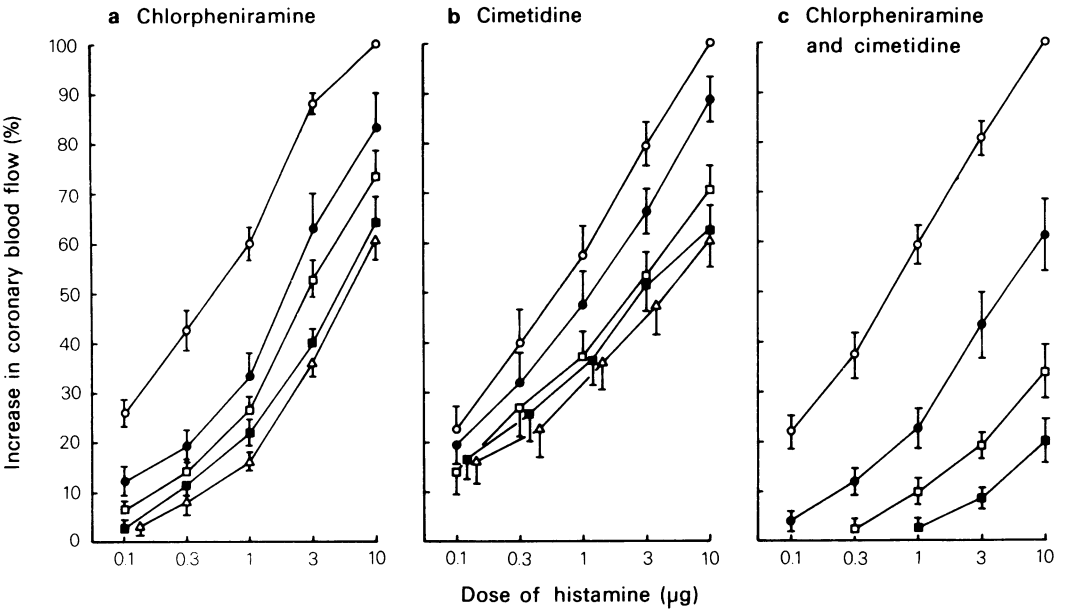

Figure 4 Dose-response curves to intra-arterial injection of histamine for increases in coronary blood flow before (○) and during continuous infusions of chlorpheniramine (a) and cimetidine (b) into the right coronary artery [(●) 10; (□) 30; (■) 100; (△) 300 μg/min,] and also during an infusion of a combination of both chlorpheniramine and cimetidine (c) [(●) 10; (□) 30; (■) 100 μg/min of each]. All values are expressed a percentage of the increase in blood flow induced by 10 μg of histamine in the control periods and are the means of 5 experiments each. Vertical bars show s.e. mean.

tion as an index of noradrenaline release (reviewed by Lokhandwala, 1978b). In these studies, drugs were commonly administered intravenously. In the present study drugs were administered directly into the right coronary artery perfusing the sino-atrial node innervated with the right cardiac sympathetic nerves. Drug action can thus be restricted to its perfusing area i.e. the right atrium and right ventricle. Accordingly, drug-induced changes in heart rate can be observed without modification by their systemic haemodynamic effects. In preliminary experiments we confirmed the fact that the administration of tetrodotoxin into the right coronary artery caused a marked depression of the tachycardia induced by cardiac sympathetic nerve stimulation. Such an action of tetrodotoxin, administered into the sino-atrial node artery, on the positive chronotropic response to right stellate ganglion stimulation was reported by Hashimoto & Chiba (1969). Thus, the preparation used in the present study seems to be a useful *in situ* approach for studying cardiac prejunctional mechanisms.

Histamine caused a definite depression of the tachycardia induced by cardiac sympathetic nerve stimulation, whereas it induced a positive chronotropic response during the resting state and during infusions of noradrenaline. These results suggest that the depression of tachycardia during nerve stimulation is due to prejunctional inhibition by histamine of cardiac sympathetic neurotransmission. The prejunctional inhibitory action of histamine was effectively inhibited by chlorpheniramine. This result suggests that H_1-receptors are involved in this prejunctional inhibitory action of histamine. This result does not accord with previous findings on the prejunctional inhibitory action of histamine, e.g. on sympathetic nerves in dog saphenous strips (McGrath & Shepherd, 1976), in perfused gracilis muscles of dogs (Powell, 1979) and in dog hearts (Lokhandwala, 1978a). These investigators suggested that the histamine receptor involved was of the H_2-type. Recently, Rand, Story & Wong-Dusting (1982) using guinea-pig isolated atria demonstrated that the inhibitory effect of histamine on stimulation-induced release of labelled noradrenaline was abolished by cimetidine (10 μmol/l) and also by mepyramine (1 μmol/l), a histamine H_1-receptor antagonist. They assumed that the abolition of the histamine effect with high concentrations of both cimetidine and mepyramine might have been due to non-specific effects of both antagonists. However, it seems from their data that lower concentrations (0.01 and 0.1 μmol/l) of mepyramine attenuated the response to histamine although they did not abolish it. Their

results may thus be in accordance with our own. From these results we emphasize the predominance in this preparation of histamine H_1-receptors in mediating histamine-induced prejunctional inhibition of cardiac sympathetic nerves.

Intra-arterial injections of histamine increased coronary blood flow in the right coronary artery. This was partially inhibited by chlorpheniramine and by cimetidine. More pronounced inhibition could be achieved by a combination of both drugs. These results suggest that histamine-induced increases in coronary blood flow are mediated by activation of both histamine H_1- and H_2-receptors. Recently, Konishi *et al.*, (1981), using the helical strips of dog coronary artery, reported that the relaxant response to histamine was mediated by histamine H_2-receptors since it was attenuated by cimetidine but was not influenced by chlorpheniramine. It is obvious that such isolated preparations consist of relatively large size vessels. Taken together, it may be suggested that histamine H_2-receptors are located in relatively large coronary vessels and H_1-receptors are located in more distal and small vessels.

The question arises whether the increased metabolic activity caused by the cardiac stimulant effect of histamine is related to its coronary vasodilatation. Powell & Brody (1976) reported that histamine caused a positive chronotropic effect mediated by histamine H_1-receptors in the intact dog. Also, Chiba (1977) reported that in the isolated, blood-perfused atrium of the dog, positive inotropic and chronotropic responses to histamine were mediated by histamine H_1-receptors. In the present experiments, histamine caused coronary vasodilatation even in a small dose of 0.1 μg and positive chronotropic response in doses over 1 μg. The vasodilator response to small doses of histamine was effectively inhibited by chlorpheniramine. Thus, histamine H_1-receptors located in the coronary vasculature may primarily mediate vasodilatation although the possibility of involvement of a metabolic vasodilatation resulting from cardiac stimulation mediated by histamine H_1-receptors cannot be ruled out with cardiac stimulatory doses of histamine.

In conclusion, we suggest that the prejunctional inhibitory action of histamine on cardiac sympathetic neurotransmission in the dog is mediated mainly by H_1-receptors; however, the coronary vasodilator action of histamine is mediated by both H_1- and H_2-receptors.

References

BROADLEY, K.J. (1975). The role of H_1- and H_2-receptors in the coronary vascular response to histamine of isolated and perfused hearts of guinea-pigs and rabbits. *Br. J. Pharmac.*, **54,** 511–521.

CHIBA, S. (1977). Absence of blocking effect of burimamide and metiamide on positive chronotropic and inotropic responses to histamine in isolated dog atrium. *Jap. J. Pharmac.*, **27,** 267–273.

CORUZZI, G., BONGRANI, S. & BERTACCINI, G. (1979). Histamine receptors in the heart and coronary vessels of rabbits. *Pharmac. Res. Commun.*, **11,** 517–528.

FLYNN, S.B., GRISTWOOD, R.W. & OWEN, D.A.A. (1979). Differentiation of the roles of histamine H_1- and H_2-receptors in the mediation of the effects of histamine in the isolated working heart of the guinea-pig. *Br. J. Pharmac.*, **65,** 127–137.

HASHIMOTO, K. & CHIBA, S. (1969). Pharmacological isolation of sinoatrial activity from effects of neural excitation by use of tetrodotoxin. *J. Pharmac. exp. Ther.*, **170,** 91–96.

HASHIMOTO, K., TANAKA, S., HIRATA, M. & CHIBA, S. (1967). Responses of the sino-atrial node to change in pressure in the sinus node artery. *Circulation Res.*, **21,** 297–304.

JAMES, T.N. (1962). Anatomy of the sinus node of the dog. *Anat. Record*, **143,** 251–265.

KONISHI, M., TODA, N. & YAMAMOTO, M. (1981). Different mechanisms of action of histamine in isolated arteries of the dog. *Br. J. Pharmac.*, **74,** 111–118.

LOKHANDWALA, M.F. (1978a). Inhibition of sympathetic nervous system by histamine: Studies with H_1- and H_2-receptor antagonists. *J. Pharmac. exp. Ther.*, **206,** 115–122.

LOKHANDWALA, M.F. (1978b). Presynaptic receptor system on cardiac sympathetic nerves. *Life Science*, **24,** 1823–1832.

MCGRATH, M.A. & SHEPHERD, J.T. (1976). Inhibition of adrenergic neurotransmission in canine vascular smooth muscle by histamine. *Circulation Res.*, **39,** 566–573.

POWELL, J.R. (1979). Effects of histamine on vascular sympathetic neuroeffector transmission. *J. Pharmac. exp. Ther.*, **208,** 360–365.

POWELL, J.R. & BRODY, M. (1976). Identification and specific blockade of two receptors for histamine in the cardiovascular system. *J. Pharmac. exp. Ther.*, **196,** 1–14.

RAND, M.J., STORY, D.F. & WONG-DUSTING, H.K. (1982). Effect of histamine on the resting and stimulation-induced release of [^{3}H]-noradrenaline in guinea-pig isolated atria. *Br. J. Pharmac.*, **75,** 57–64.

SAKAI, K. (1980). Role of histamine H_1- and H_2-receptors in the cardiovascular system of the rabbit. *J. cardiovasc. Pharmac.*, **2,** 607–617.

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