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EFFECTS OF HISTAMINE ON THE RESTING AND STIMULATION-INDUCED RELEASE OF [^{3}H]-NORADRENALINE IN GUINEA-PIG ISOLATED ATRIA

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1 The sympathetic transmitter stores of guinea-pig isolated atria were labelled with [^{3}H]-noradrenaline. The effects of histamine (0.3 to 100 μmol/l) on resting and stimulation-induced (S-I, 2 Hz for 10 s) release of radioactivity were investigated.

2 Histamine, in low concentrations (0.3 and 1 μmol/l) had no effect on resting release but inhibited S-I release of radioactivity. The inhibition was abolished by the H_2-receptor antagonist, cimetidine (10 μmol/l) and also by the H_1-receptor antagonist, mepyramine (1 μmol/l).

3 The inhibitory actions of histamine on S-I release were not due to indirect effects involving α-adrenoceptors, β-adrenoceptors, muscarinic cholinoceptors or prostaglandin synthesis.

4 Histamine in a high concentration (100 μmol/l) increased the resting and S-I release of radioactivity. The increase in resting release was abolished by the neuronal uptake blocking drug cocaine (30 μmol/l) but the increase in S-I release was only partially blocked by cocaine.

Introduction

Histamine is present in large quantities in blood vessels and cardiac tissue. Although in these tissues it is mostly stored in mast cells, there is evidence that some histamine is present in sympathetic nerve trunks (Giotti, Guidotti, Mannaioni & Zilletti, 1966; Feigin & Prager, 1969; Howland & Spector, 1972; Ryan & Brody, 1970; 1972). The development of the concept of modulation of transmitter noradrenaline release mediated by the action of endogenous substances acting on specific prejunctional receptors (see reviews by Starke, 1977; Westfall, 1977) led to the exploration of a similar role for histamine. Thus the ability of histamine to depress responses to sympathetic nerve stimulation by a prejunctional mechanism has been demonstrated in canine blood vessels (McGrath & Shepherd, 1976) and heart (Lokhandwala, 1978). The histamine receptors involved were characterized by these workers as the H_2-type since the inhibitory effects of histamine were attenuated by the H_2-receptor antagonist, metiamide.

In the present study, the effects of histamine in a wide range of concentrations on the resting and stimulation-induced release of transmitter noradrenaline in guinea-pig isolated atria were examined, and the effects of antagonists investigated.

Methods

Guinea-pigs of either sex (300–500 g) were stunned by a blow to the head and exsanguinated. The hearts were rapidly removed and the atria were dissected free and mounted in an organ bath containing 2.5 ml of Krebs-Henseleit solution of the following composition (mmol/l): NaCl 118, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 0.45, KH_2PO_4 1.03, $CaCl_2$ 2.5, D-(+)-glucose 11.1, disodium edetate 0.065. The solution in the organ bath and in the reservoirs supplying the organ bath was gassed with a mixture of 5% CO_2 in O_2 and maintained at a temperature of 37°C. The force and rate of spontaneous atrial contractions were recorded either on a Brush 250 or a Grass 79D pen recorder using a high-compliance strain gauge transducer: the basal tension was adjusted to about 10 mN. After an equilibration period of 60 min, the atria were incubated with [^{3}H]-noradrenaline (4 μCi/ml, 0.38–0.4 μmol/l, depending on the specific activity) for 20 min. The solution in the organ bath was then repeatedly exchanged with noradrenaline-free Krebs-Henseleit solution for 60 min to remove loosely bound tritiated compounds.

The atrial intramural nerves were field stimulated with monophasic square wave pulses of 1 ms duration and supramaximal voltage (about 15 V/cm) delivered through platinum wire electrodes placed down the sides of the organ bath. After the washout period, two periods of field stimulation consisting of 20 pulses at a frequency of 2 Hz were given 22 min apart. The efflux of radioactivity into the bathing solution was measured in samples of the bathing fluid after 1 min periods of contact with the atria. For each

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period of stimulation, three consecutive samples were taken immediately before stimulation to determine the resting efflux and a further three consecutive samples were taken, beginning with the start of stimulation. The resting efflux of radioactivity preceding each period of stimulation was taken as the mean amount of radioactivity present in the three samples of bathing solution collected immediately before stimulation was applied. The stimulation-induced efflux of radioactivity was calculated by subtracting the mean amount of radioactivity in the resting samples from the amounts in the three samples collected during and after electrical stimulation was applied, and summing the increases.

In each experiment, resting and stimulation-induced effluxes in the second period in the presence of histamine were calculated as percentages of the corresponding efflux during the first periods in the absence of histamine (McCulloch, Rand & Story, 1974; Hope, McCulloch, Rand & Story, 1978). This procedure takes account of variation between tissues in the absolute amounts of radioactivity released; furthermore, statistical comparison with a matching set of control preparations takes account of changes in resting and stimulation-induced effluxes due to time alone.

When the effect of histamine on the resting and stimulation-induced efflux of radioactivity was investigated, it was added to the bathing solution at least 15 min before the second period of stimulation and was present for the remainder of the experiment. In experiments to assess the effect of histamine in the presence of other drugs, the stated drug was added 30 min before the first stimulation period and was present for the remainder of the experiment.

For the determination of radioactivity, 1 ml aliquots of the samples collected from the organ bath were added to scintillation vials, together with 0.2 ml

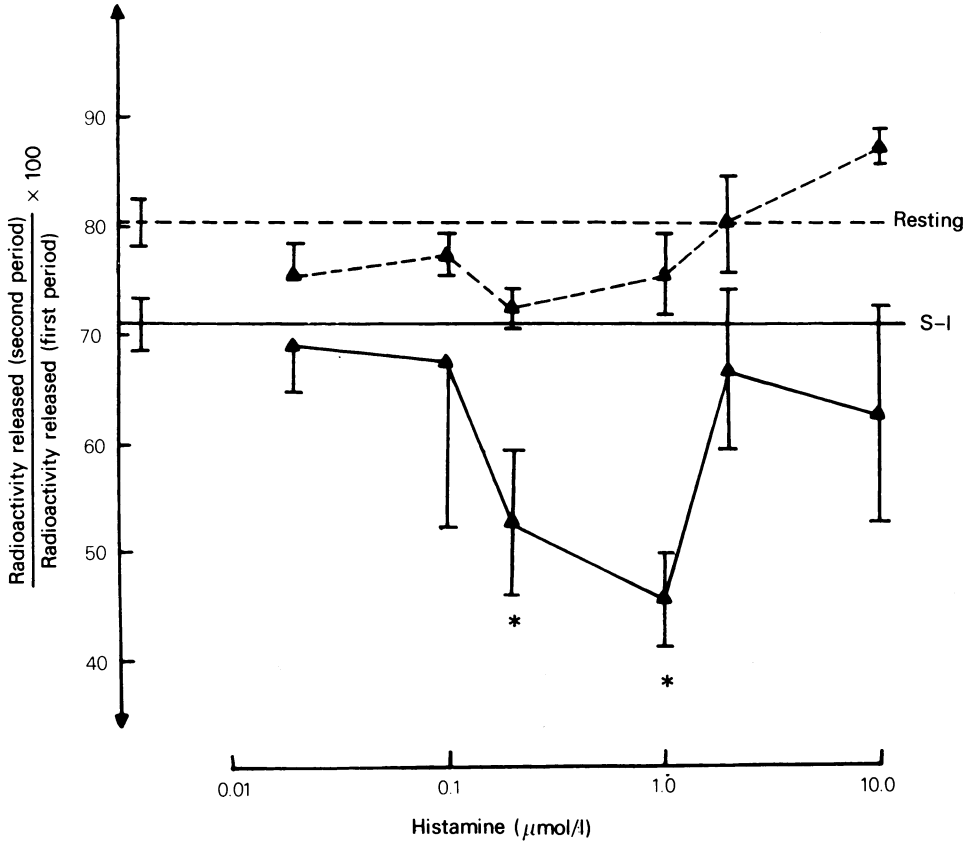

Figure 1 Effect of histamine (0.03 to 10 μmol/l) on resting (▲---▲) and stimulation-induced (S-I, ▲—▲) efflux of radioactivity from guinea-pig isolated atria previously incubated with [^{3}H]-noradrenaline. The vertical axis shows the amount of radioactivity released in the second periods as percentages of those in the first periods. The horizontal lines indicate the means of control experiments ($n = 16$). The points (▲) are the means of 4 to 8 experiments in which histamine was present during the second period of stimulation. The vertical bars indicate s.e.mean. *Significantly different from control experiments: $P < 0.01$.

of 6 mol/l HCl and 10 ml of a liquid scintillation solution of the following composition: 5.5 g of 2,5-diphenyloxazole (PPO), 0.1 g of 1,4-bis-2-(5-phenyl-oxazolyl)-benzene (POPOP) and 333 ml of Triton X made up to 1 litre with toluene. The radioactivity was measured in a Packard model 3330 or 3380 liquid scintillation spectrometer.

Statistical analysis of data

Unpaired, 2-tailed Student's *t* tests were used to test for differences between means. The probability levels associated with the calculated values of *t* are given in the text.

Drugs and radiochemicals

The following drugs were used: atropine sulphate (BDH); cimetidine (Smith, Kline & French Laboratories); cocaine hydrochloride (Drug Houses of Australia); histamine acid phosphate (BDH); indomethacin (Upjohn); mepyramine maleate (May & Baker); phentolamine mesylate (Ciba); propranolol hydrochloride (ICI).

Tritiated noradrenaline (1-[7,8-^{3}H]-noradrenaline) was obtained from the New England Nuclear Corporation or the Radiochemical Centre (Amersham) and the specific activity of the samples ranged from 10.0 to 10.4 Ci/mmol.

Results

Effects of histamine on resting and stimulation-induced (S-I) effluxes of radioactivity and on atrial rate

The mean resting efflux of radioactivity before the first period of stimulation was 8290 d/min (s.e.mean = 299, $n = 16$) and the mean S-I efflux for the first period of stimulation was 10784 d/min (s.e.mean = 1350, $n = 16$). The corresponding effluxes for the second period, expressed as percentages of those in the first period were: resting efflux, 80.2% (s.e.mean = 1.9, $n = 16$) and S-I efflux, 71.0% (s.e.mean = 2.3, $n = 16$).

The effects of histamine in concentrations ranging from 0.03 to 10 μmol/l on the resting and S-I effluxes are shown in Figure 1 and the effects of 100 μmol/l histamine are shown in Figure 2. The only significant effect of histamine on resting efflux was with the highest concentration (100 μmol/l), which produced about a two fold increase (Figure 2).

The S-I efflux was significantly reduced by histamine in concentrations of 0.3 and 1.0 μmol/l to about 70% of control values (Figure 1). In contrast, in the highest concentration (100 μmol/l), histamine markedly increased S-I efflux (Figure 2).

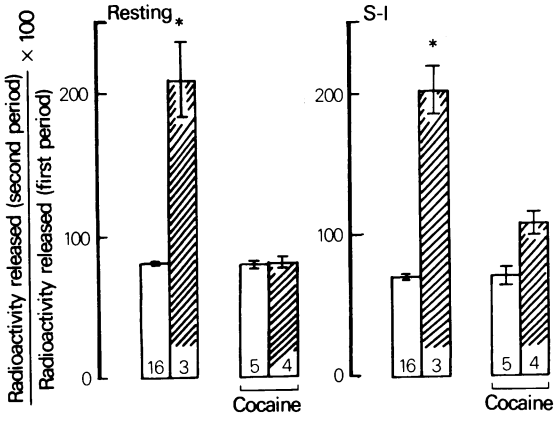

Figure 2 Effects of 100 μmol/l histamine in the absence and presence of cocaine (30 μmol/l) on resting and stimulation-induced (S-I) efflux of radioactivity from guinea-pig isolated atria. The columns show the mean amounts of radioactive effluxes in the second periods as percentages of those in the first periods. Open columns are control experiments; hatched columns are with histamine present during the second period of stimulation. Vertical bars indicate s.e.mean. The number of experiments performed is shown within each column. *Significantly different from the corresponding control experiments: $P < 0.01$.

Histamine increased the resting rate of atrial contractions in a concentration-dependent manner (Figure 3a). The threshold concentration for the effect was in the range 0.03 to 0.1 μmol/l. The effect of histamine in increasing atrial rate was paralleled by less marked increases in the force of atrial contractions (Figure 3a).

In contrast to the dual effect of histamine on the S-I efflux of radioactivity (as shown in Figure 1), only inhibition of the positive chronotropic response to stimulation was observed in the presence of histamine in the concentration range 0.03 to 100 μmol/l (Figure 3b). The positive inotropic response to stimulation was also reduced by histamine throughout this concentration range.

Effect of cocaine on responses to histamine

Experiments were carried out in the presence of cocaine (30 μmol/l) to determine the effects of blockade of neuronal uptake on the changes in resting and S-I effluxes produced by histamine. In the presence of cocaine the resting and S-I effluxes in the first period were 8169 (s.e.mean = 373, $n = 5$) and 15211 d/min (s.e.mean = 1901, $n = 5$), respectively. Those in the second period were 79.8% (s.e.mean = 2.6, $n = 5$) and 71.4% (s.e.mean = 6.5, $n = 5$), respectively, of those in the first period, these

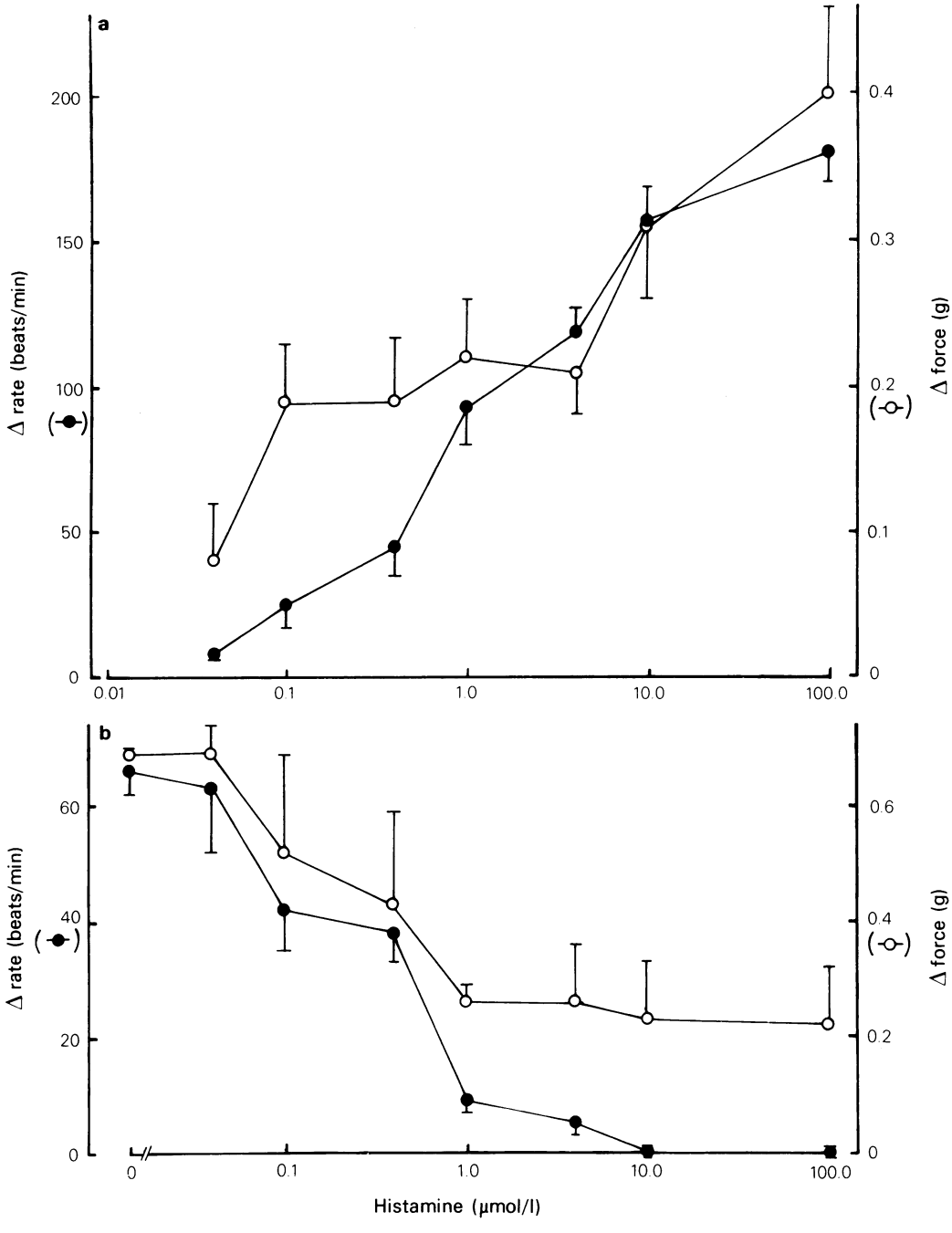

Figure 3 (a) The effect of histamine (0.01 to 100 μmol/l) on the resting rate (●) and force (○) of atrial contractions. The data are expressed as increases in the basal rate and force of contractions. (b) Positive chronotropic (●) and inotropic (○) responses to field stimulation of atrial intramural sympathetic nerves (2 Hz for 10 s periods) on the absence and presence of various concentrations of histamine. The points plotted are means obtained from 4 to 6 atrial preparations. The vertical bars indicate s.e.mean.

values being not significantly different from those obtained in the absence of cocaine ($P > 0.05$).

The increase in resting efflux that was produced by 100 μmol/l histamine in the absence of cocaine was abolished in the presence of cocaine. The increase in S-I efflux produced by this concentration of histamine was considerably reduced by cocaine. These results are shown in Figure 2. The effects of other concentrations of histamine on resting and S-I effluxes were not significantly affected by cocaine ($P > 0.05$).

Effects of mepyramine and cimetidine on the inhibition of S-I efflux produced by histamine

Mepyramine and cimetidine were used in an attempt to characterise the receptor site on which histamine (1 μmol/l) acted to reduce S-I efflux.

Neither mepyramine (0.01, 0.1 and 1 μmol/l) nor cimetidine (3.0 and 10 μmol/l), when introduced 30 min before the first period of stimulation, had any significant effect ($P > 0.05$) on resting or S-I effluxes in the first and second periods of stimulation compared to experiments in the absence of these drugs.

The inhibitory effect of histamine (1 μmol/l) on S-I efflux was abolished by 1 μmol/l of mepyramine and by 10 μmol/l of cimetidine. In the presence of lower concentrations of these two drugs, histamine still significantly decreased S-I efflux. These results are summarized in Figure 4.

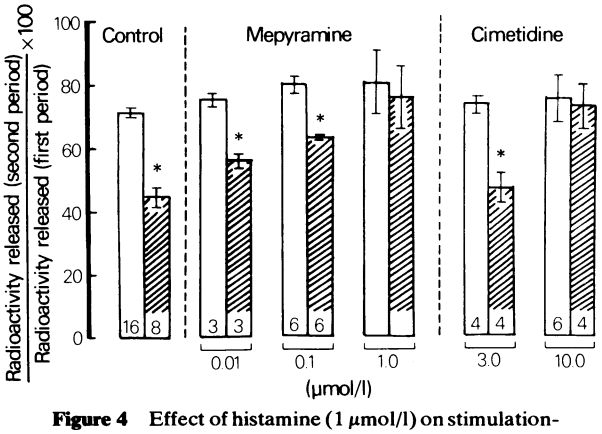

Figure 4 Effect of histamine (1 μmol/l) on stimulation-induced (S-I) efflux of radioactivity from guinea-pig isolated atria in the presence of mepyramine (0.01, 0.1 and 1 μmol/l) or cimetidine (3 and 10 μmol/l). Vertical columns show the amount of radioactivity released in the second period of stimulation as a percentage of that in the first period. Open columns are control experiments; hatched columns are with histamine present during the second period of stimulation. Vertical bars denote s.e.mean. The number of experiments performed is shown within each column. *Significantly different from the corresponding control experiments: $P < 0.01$.

In agreement with previous findings by other workers (Steinberg & Holland, 1975; Levi, Allan & Zavecz, 1976) mepyramine had no effect on the increase in rate of atrial beating produced by histamine, but caused a small decrease in the increased force of beating produced by histamine. On the other hand, cimetidine decreased both the increased rate and force of atrial beating caused by histamine.

Effect of phentolamine, propranolol, atropine and indomethacin on histamine-induced inhibition of S-I efflux

As shown in Table 1, the α-adrenoceptor antagonist, phentolamine (5 μmol/l), added 30 min before the first period of stimulation, significantly increased the S-I efflux of radioactivity in the first period. However, in the presence of phentolamine, histamine (1 μmol/l) still produced the same degree of inhibition of S-I efflux as it did in the absence of phentolamine. Further, propranolol (0.03 μmol/l), atropine (0.3 μmol/l) and indomethacin (3 μmol/l) did not substantially alter the inhibitory effect of 1 μmol/l histamine on S-I efflux.

Discussion

Histamine exerts profound pharmacological actions on cardiovascular tissues by acting on the H_1- and H_2-receptors on the effector cells (see reviews by Owen, 1977; Altura & Halevy, 1978). Recently, a prejunctional site of histamine action in modulating noradrenergic neurotransmission has been suggested since histamine has been shown to modify the responses of blood vessels (McGrath & Shepherd, 1976) and heart (Lokhandwala, 1978) to sympathetic nerve stimulation. The prejunctional receptors involved appeared to be of the H_2-type as the effects of histamine were abolished by the H_2-receptor antagonist metiamide.

We have investigated further the histamine-mediated prejunctional inhibition of sympathetic transmission in cardiac tissue by using guinea-pig isolated atria in which sympathetic transmitter stores were labelled with [^{3}H]-noradrenaline. Histamine, in concentrations of 0.3 and 1 μmol/l inhibited the release of radioactivity evoked by field stimulation of the atrial intramural sympathetic nerves, whereas with a concentration of 100 μmol/l, stimulation-induced release was markedly enhanced. These effects of histamine on the S-I efflux of radioactivity do not appear to be consequences of either the effects of the drug on the basal rate and force the atrial contractions or to its effects on the stimulation-evoked chronotropic or inotropic responses. Thus, histamine produced concentration-dependent increases in the

Table 1 Effects of phentolamine, propranolol, atropine and indomethacin on the inhibition of S-I efflux of radioactivity produced by histamine (1 μmol/l)

Antagonist (present for S_1 and S_2)	*S-I efflux*			
	Control		*Histamine*	
	S_1 (d/min)	$S_2/S_1 \times 100\%$	S_1 (d/min)	$S_2/S_1 \times 100\%$
None	10784 ± 1350	71.0 ± 2.3	10828 ± 1720	*45.5 ± 4.4
	$n = 19$		$n = 8$	
Phentolamine (5 μmol/l)	†26776 ± 2191	81.4 ± 4.8	†23607 ± 5256	*55.8 ± 5.3
	$n = 8$		$n = 4$	
Propranolol (0.03 μmol/l)	9096 ± 592	67.0 ± 5.2	9573 ± 1494	*48.3 ± 4.1
	$n = 3$		$n = 3$	
Atropine (0.3 μmol/l)	14069 ± 833	75.1 ± 2.2	12287 ± 1564	*52.1 ± 1.8
	$n = 7$		$n = 6$	
Indomethacin (3 μmol/l)	9114 ± 2084	87.5 ± 9.0	10412 ± 2095	*44.2 ± 14.0
	$n = 6$		$n = 4$	

The various antagonists were introduced 30 min before the first period of stimulation (S_1) and then remained present throughout. Where indicated, histamine was also present for the second period of stimulation (S_2). The results are expressed as mean ± s.e.; n is given in parentheses.
†Significant effect ($P < 0.05$) of the antagonist on S-I efflux in S_1 compared to the corresponding control experiments; *significant effect ($P < 0.05$) of the antagonist on S-I efflux in the presence of histamine.

rate and force of atrial contractions whereas the resting efflux of radioactivity was unaffected by histamine, except with the highest concentration tested (100 μmol/l) when it increased abruptly. Further, the positive inotropic and chronotropic responses to stimulation were progressively reduced by successively higher concentrations of histamine, whereas the S-I efflux of radioactivity was reduced by low concentrations of histamine and enhanced by a high concentration.

The concentrations of histamine that inhibited transmitter noradrenaline release in guinea-pig atria were similar to those found by McGrath & Shepherd (1976) to produce this effect in isolated strips of canine blood vessels. Lokhandwala (1978) showed that in the dog heart *in situ*, histamine in concentrations too low to elicit postjunctional responses, nevertheless inhibited responses of the heart to sympathetic nerve stimulation.

In a concentration of 100 μmol/l, histamine increased both resting and stimulation-induced effluxes of radioactivity. The increase in resting efflux was completely blocked by cocaine, whereas, in the presence of cocaine, the enhanced S-I efflux was still present, although reduced. It appears therefore that histamine, when present in high concentrations, may enter noradrenergic nerve terminals by a cocaine-sensitive mechanism and displace noradrenaline from the transmitter stores. The enhancement of stimulation-induced transmitter release by a high concentration of histamine occurs by a mechanism which is only partly impaired by neuronal uptake block with cocaine. The lack of effect of histamine in concentrations of 3 and 10 μmol/l on transmitter release may be due to a balance between the inhibitory effect obtained with lower concentrations and the facilitatory effect with higher concentrations.

The inhibitory effect of histamine on stimulation-induced efflux was abolished in the presence of a high concentration of mepyramine (1 μmol/l) but was still produced in the presence of lower concentrations of mepyramine which could be expected to block responses involving histamine H_1-receptors (0.01 and 0.1 μmol/l). The histamine H_2-receptor antagonist, cimetidine, was without effect in a concentration of 3 μmol/l, whereas a concentration of 10 μmol/l cimetidine also abolished the inhibitory effect of histamine on stimulation-induced efflux. The pA_2 value for antagonism by cimetidine of responses to histamine acting on postjunctional H_2-receptors is 6.1 in guinea-pig atria (Brimblecombe, Duncan, Durant, Emmett, Ganellin & Parsons, 1975). Thus, although the findings with histamine antagonists in the present experiments could be construed to indicate that the inhibitory effect of histamine on transmitter noradrenaline release involved histamine H_2-receptors, the abolition of the effect with high concentrations of both cimetidine and mepyramine may have been due to non-specific effects of both antagonists.

Histamine (1 μmol/l) does not appear to act on prejunctional α-adrenoceptors to inhibit transmitter release since phentolamine in a concentration sufficient to block prejunctional α-adrenoceptors (5 μmol/l) had no effect on the inhibitory action of histamine on transmitter noradrenaline release. Histamine has ganglion stimulating activity (Brimble & Wallis, 1973) and may conceivably have acted on vagal ganglion cells in the atria resulting in acetylcholine release and muscarinic inhibition of S-I efflux

(Story, Allen, Glover, Hope, McCulloch, Rand & Sarantos, 1975). The involvement of a cholinergic mechanism in the inhibitory action of histamine was eliminated by showing that it was not affected by atropine. The presence of indomethacin (3 μmol/l) also had no effect on the inhibitory action of histamine, thus eliminating the possibility that histamine was acting by induction of prostaglandin synthesis; prostaglandins are known to have prejunctional inhibitory actions (Starke, 1977; Westfall, 1977).

The question arises whether the prejunctional inhibitory action of histamine could modulate the noradrenergic nerve activity *in vivo* under normal physiological conditions. Studies have shown the presence of histamine in sympathetic nerve trunks (Ryan & Brody, 1970; 1972). Also, in perfused tissues prelabelled with radioactive histamine, radioactivity was detected in the perfusate during reflex vasodilatation or on withdrawal of sympathetic tone (Tuttle, 1967; Beck, Schon, Pollard & Wyse, 1971; Heitz & Brody, 1975), and on stimulation of sympathetic nerves (Tuttle & McCleary, 1970) or of spinal nerve roots (Lioy & White, 1973). However, release of endogenous histamine by nerve stimulation or its presence in sympathetic nerve endings has not been shown conclusively, and histamine is not generally regarded as a neurotransmitter in peripheral autonomic nerves (Green, Johnson & Weinstein, 1978). In the guinea-pig heart, histamine is present in large quantities, but is mostly contained in mast cells (Giotti *et al.*, 1966) from where it is released in pathological states such as anaphylactic shock. Our finding that neither cimetidine nor mepyramine alone influence resting or stimulation-induced noradrenaline release indicates that histamine is not normally involved in prejunctional modulation of transmitter release. The mast cell histamine in guinea-pig atria, however, has been shown to be released rapidly in anaphylaxis (and in fact, calculated to reach concentrations as high as 1 to 10 μmol/l in the extracellular fluid) (Penna, Illanes, Ubilla & Mujica, 1959; Feigen & Prager, 1969). Under such conditions it is possible that histamine released from mast cells may lead to inhibition of transmitter noradrenaline release and that this effect may contribute to the overall anaphylactic reaction.

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