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Effect of histamine on the resting and stimulation-induced release of [^{3}H]noradrenaline from rat isolated atria

M. J. RAND, D. F. STORY, H. K. WONG-DUSTING*, *Department of Pharmacology, University of Melbourne, Victoria, 3052, Australia*

The effect of histamine on the mammalian heart is well documented (see reviews by Owen 1977; Altura & Halevy 1978). Histamine causes tachycardia in most species but there are considerable species differences in the sensitivity of the heart to histamine. Thus the guinea-pig (Owen 1977; Trzeciakowski & Levi 1981) and human heart (Levi et al 1978) are both highly sensitive to the positive chronotropic effects of histamine, while the canine heart is unresponsive to histamine in concentrations sufficient to alter other physiological parameters such as blood pressure and gastric secretion (Lokhandwala 1978a). In the rat heart, histamine in low concentrations has been reported to have no effect, or negative inotropic and chronotropic effects unrelated to the activation of histamine receptors (Bartlet 1963; Dai 1976; Laher & McNeill (1980).

Noradrenergic nerve terminals are known to be endowed with receptors on which agonists can act to produce inhibition or facilitation of transmitter release (see review by Starke 1981). Recently, the presence of prejunctional histamine receptors, the activation of which inhibits stimulation-induced noradrenaline release, has been reported in the canine heart (Lokhandwala 1978b) and the guinea-pig heart (Wong-Dusting et al 1979; Rand et al 1982). It is likely that in the guinea-pig heart under most circumstances, the prejunctional inhibitory effect of histamine on noradrenergic transmission would be masked by its marked postjunctional effects. However, it seemed possible that in the rat heart an inhibitory effect of histamine on transmitter noradrenaline release may be of greater relevance in view of the lack of postjunctional effects of the amine. In the present study we have therefore investigated the effects of histamine on the stimulation-induced release of [^{3}H]noradrenaline from rat isolated atria.

Methods

Rats of either sex (200–250 g) were stunned by a blow to the head and exsanguinated. The hearts were rapidly removed and the atria dissected free and mounted in an organ bath containing 2·5 ml of Krebs-Henseleit solution. 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 37 °C.

After an equilibration period of 30 to 60 min, the atria were incubated for 20 min in [^{3}H]noradrenaline (6·7 μCi ml^{-1}; 0·67 μmol litre^{-1}). The solution in the organ bath was then repeatedly exchanged with noradrenaline-free Krebs-Henseleit solution for 50 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^{-1}) 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. The resting efflux (efflux of radioactivity per min immediately preceding stimulation) and the stimulation-induced efflux of radioactivity were calculated for each period of stimulation as described by Rand et al (1982).

The effect of histamine on the resting and stimulation-induced efflux of radioactivity was determined by adding it to the bathing solution 15 min before the second period of stimulation and replacing it whenever the bathing solution was renewed for the remainder of the experiment. In some experiments,

Table 1. Effect of cocaine (30 μmol litre^{-1}) on the enhancement of resting efflux of radioactivity by histamine (100 μmol litre^{-1}) in rat atria. Cocaine was added 30 min before the first period of stimulation and then remained throughout the experiment. Histamine was added 15 min before the second period of stimulation. R_1 is the resting efflux before the first period of stimulation. In each experiment the resting efflux before the second period of stimulation (R_2) was calculated as a percentage of that for the first period. The results are expressed as mean ± standard error; n is the number of experiments. * denotes a significant effect ($P < 0.01$) by histamine on the resting efflux compared to experiments in its absence.

Drug present in S_1 and S_2	Drug present in S_2	Resting efflux of radioactivity: R_1 (d min^{-1})	$\frac{R_2}{R_1} \times 100\%$
—	—	11 520 ± 1532 (n = 11)	89·2 ± 6·1
—	Histamine 100 μmol litre^{-1}	12 435 ± 1110 (n = 8)	*167·5 ± 14.2
Cocaine 30 μmol litre^{-1}	—	11 728 ± 383 (n = 6)	82·8 ± 3.7
Cocaine 30 μmol litre^{-1}	Histamine 100 μmol litre^{-1}	11 965 ± 785 (n = 5)	90·6 ± 5.1

* Correspondence.

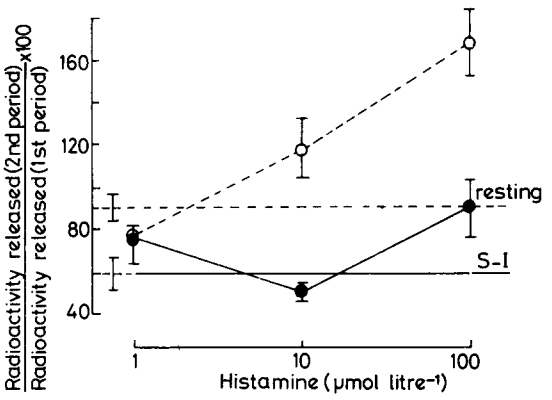

FIG. 1. The effect of histamine (1, 10 and 100 μmol litre^{-1}) on the resting and stimulation-induced (S-I) efflux of radioactivity from rat isolated atria which had previously been incubated in [^{3}H]noradrenaline. The atrial intramural sympathetic nerves were stimulated at a frequency of 2 Hz in two 10 s periods with a 22 min interval between them. The horizontal axis represents the histamine concentration (μmol litre^{-1}). The vertical axis shows the resting (○- - -○) and stimulation-induced (●——●) effluxes of radioactivity for the second periods of stimulation expressed as percentages of those for the first periods; the mean control resting (- - -) and S-I (——) efflux values are represented by horizontal lines. Each point represents a mean value from 4 to 8 experiments. Vertical bars represent standard error of means. The only point that differs significantly from the corresponding control value is that for the resting efflux in the presence of 100 μmol litre^{-1} histamine for which $P < 0.001$.

cocaine was added 30 min before the first stimulation period and was present for the remainder of the experiment.

In each experiment, resting and stimulation-induced effluxes for the second period of stimulation were calculated as percentages of the corresponding effluxes for the first period of stimulation. This procedure takes into account the variation between tissues in the absolute amount 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 (Hope et al 1978; McCulloch et al 1974).

The following drugs were used: cocaine hydrochloride (Drug Houses of Australia); histamine acid phosphate (British Drug Houses). Tritiated noradrenaline ((−)-[7,8-^{3}H]noradrenaline) was obtained from the New England Nuclear Corporation or the Radiochemical Centre (Amersham) and the specific activity was 10 Ci mmol^{-1}.

The Krebs-Henseleit solution was of the following composition (mmol litre^{-1}): 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.

Unpaired, 2-tailed Student's *t*-tests were used to test for statistically significant differences between means. Probability levels corresponding to the calculated values of *t* are given in the text; $P < 0.05$ was taken to indicate a statistically significant difference between means.

Results

The mean resting efflux of radioactivity before the first period of stimulation was 11 520 d min^{-1} (s.e. mean = 1532, n = 11) and the mean stimulation-induced (S-I) efflux for the first period of stimulation was 18 295 d min^{-1} (s.e. mean = 2945, n = 11). The corresponding effluxes for the second period expressed as percentages of those in the first period were: resting efflux, 89·2% (s.e. mean = 6·1, n = 11) and S-I efflux, 59.0% (s.e. mean = 7·5, n = 11). The effects of histamine (1, 10 and 100 μmol litre^{-1}) on the resting and S-I efflux of radioactivity compared with control experiments are shown in Fig. 1. Histamine had no significant effect on S-I efflux in the concentration range studied; however, there was an apparently concentration-dependent increase in the resting efflux of radioactivity in the presence of histamine.

The effect of histamine (100 μmol litre^{-1}) on the resting efflux of radioactivity was investigated in the presence of cocaine (30 μmol litre^{-1}) to block the neuronal amine uptake mechanism. These results are summarized in Table 1. Whereas in the absence of cocaine, 100 μmol litre^{-1} histamine enhanced the resting efflux of radioactivity almost 2-fold, in the presence of cocaine histamine had no significant effect on resting efflux. In the presence of cocaine, histamine again had no significant effect on the S-I efflux of radioactivity.

Discussion

It has been reported that the atrial myocardium of the rat, unlike that of other species such as guinea-pig and man, is not endowed with receptors for histamine (Bartlet 1963; Dai 1976; Laher & McNeill 1980). From the present study it would appear that there are also no histamine receptors associated with the terminal noradrenergic nerves of rat atria. Thus histamine (1 μmol litre^{-1}) which had previously been shown to inhibit stimulation-induced transmitter noradrenaline release in both the canine heart in-situ (Lokhandwala 1978b) and in guinea-pig isolated atria (Wong-Dusting et al 1979; Rand et al 1982) had no effect on transmitter efflux in rat isolated atria.

In a high concentration (100 μmol litre^{-1}), histamine markedly increased the resting efflux of [^{3}H]noradrenaline from rat atria. This effect appeared to be due to displacement of [^{3}H]noradrenaline from transmitter stores, as it was abolished by blockade of neuronal uptake with cocaine. Release of endogenous noradrenaline from rat isolated atria by high doses of histamine has also been suggested by Laher & McNeill (1980), who found that the chronotropic effect of large doses of histamine was blocked by the β-adrenoceptor antagonist propranolol or reserpine pretreatment, but not by the histamine H_1-receptor antagonist promethazine or the H_2-receptor antagonist cimetidine.

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5-Hydroxytryptamine uptake inhibitors antagonize the antireserpine effects of noradrenaline uptake inhibitors

J. Maj*, Z. Rogóz, G. Skuza, *Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

Imipramine is an inhibitor of noradrenaline (NA) and 5-hydroxytryptamine (5-HT) uptake, whereas desipramine, its desmethyl metabolite, mainly inhibits the uptake of NA. The antireserpine action of imipramine does not occur if its metabolism is inhibited by proadifen (Maj et al 1981, 1982a). That negative effect may result from the absence of the desmethyl metabolite and/or from the much increased the brain level of imipramine at which its inhibitive action on 5-HT uptake is brought into prominence and can be an antagonistic factor towards the noradrenergic mechanism. This is supported by the finding that 5-HT uptake inhibitors attenuate the antireserpine action of NA uptake inhibitors (Maj et al 1982c). That action, however, has been observed in the hypothermia test and consequently can be of central as well as peripheral origin. In the present study we have, therefore, taken locomotor activity as a criterion, assuming that its changes should result from central action. In order to simulate the conditions occurring after administration of imipramine we injected concurrently a NA uptake inhibitor and a 5-HT uptake inhibitor. As selective NA uptake inhibitors we used desipramine and maprotiline (Maitre et al 1974) and as selective 5-HT inhibitors, citalopram (Christensen et al 1977; Pawlowski et al 1981), fluoxetine (Fuller et al 1975; Slater et al 1979), fluvoxamine (Claassen et al 1977; Maj et al 1982b) and zimelidine (Ross et al 1976). None of them shows the antireserpine or antitetrabenazine action, but only citalopram and zimelidine were studied in the locomotor activity test.

* Correspondence.

Methods

The experiments were on Albino Swiss, male mice (25–30 g) having had free access to food and water. The locomotor activity was measured over 1 h when the mice were individually placed in photoresistor actometers. The NA and 5-HT uptake inhibitors were given i.p. singly or together 5 h before the experiments at the doses, generally accepted as effective and chosen on the ground of preliminary experiments. Reserpine (2 mg kg^{-1}) was administered s.c. 4 h before the experiment. All drugs were injected as solutions in 0·9% NaCl (saline). There were 8–10 mice to a group. The statistical significance was determined with Student's *t*-test.

Drugs given were: citalopram hydrobromide (Lundbeck), desipramine hydrochloride (Ciba-Geigy), fluoxetine hydrochloride (Lilly), fluvoxamine maleate (Philips-Duphar D.V.), maprotiline hydrochloride (Ciba-Geigy), reserpine (Serpasil-amp., Ciba-Geigy), zimelidine dihydrochloride (Astra).

Results and discussion

All the NA or 5-HT uptake inhibitors tested, when given alone (at doses given in Table 1) 5 h before experiment did not affect locomotor activity in normal mice (data not shown).

Desipramine antagonized the reserpine hypoactivity (Table 1). Citalopram, fluoxetine, fluvoxamine and zimelidine were ineffective in this respect. Each of them abolished or much attenuated the antireserpine action of desipramine.

Maprotiline counteracted sedation in reserpinized