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Are the prejunctional histamine receptors on sympathetic nerve terminals in guinea-pig isolated atria activated during anaphylaxis in vitro?

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In isolated atria from sensitized guinea-pigs, antigenic challenge with ovalbumin induces an anaphylactic reaction in which there is an increased rate and force of contraction. At the same time, stimulation-induced release of [^{3}H]noradrenaline is inhibited by 40%. Cimetidine decreased the tachycardia occurring during anaphylaxis but had no effect on the inhibition of stimulation-induced transmitter release. It is known that antigenic challenge of sensitized guinea-pig atria releases histamine from mast cells; this histamine acts postjunctionally to increase heart rate. However, the inhibition of noradrenergic transmitter release is not due to the stimulation of prejunctional inhibitory histamine receptors.

Noradrenergic nerve terminals are endowed with a variety of receptors on which agonists can act to produce inhibition or facilitation of transmitter release (see review by Starke 1981). Inhibition of noradrenergic transmission by action on specific prejunctional receptors has been demonstrated for α-adrenoceptor agonists, dopamine, 5-hydroxytryptamine, histamine, muscarinic agonists, γ-aminobutyric acid, opioids, prostaglandins and adenosine. However, with a few exceptions, the role of these receptors in physiological or pathological processes has yet to be established (Starke 1981).

Histamine has been shown to act prejunctionally to inhibit noradrenergic transmission in blood vessels (McGrath & Shepherd 1976; Powell 1979) and cardiac tissues (Lokhandwala 1978; Wong-Dusting et al 1979; Rand et al 1982).

In guinea-pig atria, cimetidine had no effect on the stimulation-induced release of noradrenaline in concentrations that were sufficient to block the prejunctional inhibitory effect of exogenously applied histamine (Rand et al 1982). Therefore it appears unlikely that the prejunctional histamine receptors are involved in the normal physiological control of noradrenergic transmission in this tissue. Nevertheless, prejunctional histamine receptors may be activated when large amounts of histamine are released in tissues and into the circulation in pathological states, such as anaphylactic reactions.

Histamine is present in the heart of the guinea-pig, mostly in mast cells, and is released during in vitro anaphylactic reactions from the whole perfused heart (Feigen & Prager 1969; Capurro & Levi 1975), and the isolated atria (Penna et al 1959; Liebig et al 1975) in sufficient amounts to produce profound effects by acting on postjunctional histamine receptors. In this paper, we report studies undertaken to determine whether histamine released during in vitro anaphylactic reactions in guinea-pig atria acts prejunctionally to modify noradrenergic transmission.

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METHODS

Guinea-pigs of either sex, 250–300 g, were sensitized by two intraperitoneal injections of ovalbumin on two consecutive days. Fifteen to 30 days after sensitization, the guinea-pigs were killed and the hearts removed. The atria were dissected free and mounted in an organ bath containing 2·5ml of Krebs-Henseleit solution 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. The solutions in the organ bath and the reservoirs supplying the organ bath were gassed with a mixture of 5% CO_2 in O_2 and were maintained at a temperture 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^{-1}, 0·4 μmol litre^{-1}) 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 intramural nerves in the atria were field

stimulated with monophasic square wave pulses of 1 ms duration and supramaximal voltage (about 15 V cm^{-1}) delivered through two platinum wire electrodes placed each side of the atria and 1 cm apart. 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 collections of the bathing fluid after 2 min periods of contact with the atria. For each period of stimulation, six consecutive collections of the bathing solution were taken with the 10 s period of stimulation being delivered during the fourth collection period. The resting efflux of radioactivity preceding each period of stimulation was taken as the mean amount of radioactivity present in the three collections of bathing solution taken immediately before stimulation was applied. The stimulation-induced efflux of radioactivity was calculated by subtracting the resting efflux from the amounts in the three collections made during and after electrical stimulation was applied, and summing the increases.

Antigenic challenge was accomplished by injecting ovalbumin into the organ bath immediately after collecting the third resting sample of bathing solution before the second stimulation period. Each sensitized atrial preparation was challenged only once with antigen to avoid desensitization. The atria were electrically stimulated 90 s after addition of antigen. In experiments in which the atria were subjected to antigenic challenge in the presence of cimetidine, this drug was added 30 min before the first period of stimulation and remained present for the remainder of the experiment.

In each experiment, the stimulation-induced efflux for the second period of stimulation was calculated as a percentage of that for the first period (McCulloch et al 1974; Hope et al 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 stimulation-induced efflux due to time alone.

For the determination of radioactivity, 1 ml aliquots of the solution collected from the organ bath were added to scintillation vials, together with 0·2 ml of 6 mol $litre^{-1}$ 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-phenyloxazolyl)-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.

In the statistical analysis of the data, the unpaired, 2-tailed Student's *t*-test was used to compare differences between means. The probability levels associated with the calculated values of *t* are given in the text.

The following drugs were used: cimetidine (Smith, Kline & French Laboratories); ovalbumin (Grade V, crystalline egg albumin, Sigma).

Tritiated noradrenaline ([(−)-(7,8-^{3}H]noradrenaline) was obtained from the New England Nuclear Corporation; the specific activity was 10·4 Ci $mmol^{-1}$.

RESULTS

In control experiments with no antigenic challenge but using sensitized atria from guinea-pigs which had been injected with two 5 mg doses of ovalbumin on consecutive days, the mean stimulation-induced (S-I) efflux for the first period of stimulation was 10985 d min^{-1} (s.e. mean 1720, n = 4). The S-I efflux for the second period, expressed as a percentage of that in the first period (% S_2/S_1), was 90·8% (s.e. mean = 9·8, n = 4). The increase in atrial rate in response to nerve stimulation at 2 Hz for 10 s was 84 beats min^{-1} (s.e. mean = 4, n = 8). The mean S-I efflux for the first period of stimulation and % S_2/S_1 in atria from guinea-pigs which had not been pretreated with ovalbumin were 11970 d min^{-1} (s.e. mean = 1100, n = 8) and 79·8% (s.e. mean = 2·3, n = 8), respectively, these values being not significantly different ($P > 0·5$ and 0·1, respectively) from those obtained in atria from pretreated guinea-pigs.

The degree of anaphylaxis, as determined from the increase in atrial rate, and the effect of antigenic challenge on the S-I efflux of radioactivity depended on the doses of ovalbumin used for sensitization and on the concentration of ovalbumin introduced into the organ bath for challenge. In all cases, during anaphylaxis, there was little or no further increase in atrial rate in response to stimulation. This was presumably due to the effect of challenge increasing the resting rate to near maximal levels. The effects of various combinations of sensitizing dose and challenge concentration of ovalbumin on atrial rate and on S-I efflux are summarized in Table 1.

Ovalbumin challenge of sensitized atria resulted in marked increases in the rate and force of contractions which peaked within 90 s. In atria from guinea-pigs sensitized with two 5 mg doses of ovalbumin, challenge with 0·4 μg ml^{-1} produced a mean increase in rate of 161 beats min^{-1}. Progressively smaller chronotropic effects occurred on challenge with the same concentration of ovalbumin in atria

Table 1. Effect of antigenic challenge of guinea-pig atria previously incubated with [^{3}H]noradrenaline on the rate of beating and on the efflux of radioactivity released in response to field stimulation (2 Hz, 10 s) of intramural autonomic nerves. Atria were taken from guinea-pigs sensitized to ovalbumin by administering the dose indicated on two consecutive days. The atria were subjected to antigenic challenge by adding ovalbumin to the organ bath 90 s before the second period of stimulation. The stimulation-induced (S-I) efflux of radioactivity for the second period of stimulation is expressed as a percentage of that for the first period ($S_2/S_1 \times 100\%$). The results are expressed as mean and standard error (s.e.m.). The number of preparations (n) is indicated for each group. An asterisk (*) denotes a significant difference in S-I efflux from that in atria from sensitized guinea-pigs (2 × 5 mg doses of ovalbumin) but in which there was no challenge.

Sensitizing doses (mg)	Challenge concn. (μg ml^{-1})	n	Peak increase in atrial rate on challenge (beats min^{-1}) mean	s.e.m.	SI-efflux of radioactivity ($S_2/S_1 \times 100\%$) mean	s.e.m.
none	none	8	—		79·8	2·3
5	none	4	—		90·8	9·8
5	0·4	6	161	11	*53·2	5·7
1	0·4	3	79	33	51·3	26·0
0·1	0·4	4	47	10	78·5	6·0
10	40	5	166	28	98·2	17·0
10	400	3	168	3	*232·6	16·3

from guinea-pigs sensitized with two 1 mg and two 0·1 mg doses of the antigen (Table 1). The increases in force of contractions were not expressed quantitatively since in spontaneously beating atria, changes in force are dependent on changes in rate (Koch-Weser & Blinks 1963).

The effect of anaphylaxis on transmitter noradrenaline efflux was dependent on the degree of anaphylaxis. Thus, in atria from guinea-pigs sensitized with 5 mg doses of ovalbumin, challenge with 0·4 μg ml^{-1} decreased % S_2/S_1 to a mean of 53·2%. Challenge with the same concentration in atria from guinea-pigs sensitized with 1 mg doses of ovalbumin also reduced S-I efflux; however, the effect was more variable and the reduction was not significant. On lowering the sensitizing doses further to 0·1 mg, challenge with 0·4 μg ml^{-1} was without effect on S-I efflux (Table 1).

In an attempt to produce more severe anaphylaxis, guinea-pigs were sensitized with two 10 mg doses of ovalbumin and the atria were subsequently challenged with the antigen in concentrations of 40 or 400 μg ml^{-1}. As shown in Table 1 the chronotropic response to challenge in both cases was of a similar magnitude to that produced with 5 mg sensitization and 0·4 μg ml^{-1} challenge. However, ovalbumin challenge with 40 μg ml^{-1} had no effect on S-I efflux and efflux was markedly enhanced on challenge with 400 μg ml^{-1}.

In a separate series of experiments in which electrical stimulation was not given, it was established that antigenic challenge (0·4 μg ml^{-1}) of atria from guinea-pigs previously sensitized with ovalbumin (2 × 5 mg) had no effect on the resting efflux of radioactivity.

Since prejunctional inhibitory histamine receptors have been shown to be present in guinea-pig atria (Wong-Dusting et al 1979; Rand et al 1982), and large amounts of mast cell histamine are released from this tissue during anaphylaxis (Capurro & Levi 1975; Feigen & Prager 1969; Levi 1972), the effect of the specific H_2-receptor antagonist cimetidine on the decrease in S-I efflux of radioactivity during anaphylaxis was investigated. These experiments were performed using atria taken from guinea-pigs which had been sensitized with two 5 mg doses of ovalbumin. The concentration of antigen used as a challenge was 0·4 μg ml^{-1}.

The histamine H_2-receptor antagonist cimetidine, in a concentration of 30 μmol litre^{-1}, did not significantly affect the increase in heart rate or the inhibition of S-I efflux of radioactivity during the anaphylactic response (Table 2). A higher concentration of cimetidine (100 μmol litre^{-1}) reduced the chronotropic response to antigenic challenge but still

Table 2. The effect of antigenic challenge with ovalbumin (0·4 μg ml^{-1}) on the resting rate of contractions and on the stimulation-induced (S-I) efflux of radioactivity in isolated atria from guinea-pigs previously sensitized with ovalbumin (2 × 5 mg). S-I efflux in the second period (S_2) of stimulation is expressed as a percentage of that in the first (S_1) period. The increase in resting rate produced by ovalbumin challenge was determined immediately before the second period of stimulation. Ovalbumin challenge was given 90 s before the second period of stimulation. In some experiments, cimetidine (30 or 100 μmol litre^{-1}) was introduced 30 min before the first period of stimulation and then remained present throughout. The number of experiments (n) is shown in each case. An asterisk (*) denotes a significant effect ($P < 0·01$) compared to the corresponding control value. The obelisk (†) denotes a significantly smaller increase in resting heart rate compared to the other two values ($P < 0·01$). The mean absolute value of S_1 in the control group was 10895 (s.e. mean = 1720); the mean values of S_1 in each test group were not significantly different from this value ($P > 0·05$).

	n	Peak increase in rate on challenge (beats min^{-1}) mean	s.e.m.	S-I efflux of radioactivity ($S_2/S_1 \times 100\%$) mean	s.e.m.
Control	4	—	—	90·8	9·8
Ovalbumin challenge	6	161	11	53·2*	5·7
Cimetidine (30 μmol litre^{-1})	3	—	—	81·0	5·5
Ovalbumin challenge + cimetidine (30 μmol litre^{-1})	6	137	11	53·4*	5·0
Cimetidine (100 μmol litre^{-1})	3	—	—	80·0	6·4
Ovalbumin challenge + Cimetidine (100 μmol litre^{-1}	4	68†	21	54·8*	4·4

had no significant effect on the inhibition of S-I efflux (Table 2).

DISCUSSION

The presence of prejunctional histamine receptors, activation of which decreases transmitter noradrenaline release, has been demonstrated in both blood vessels (McGrath & Shepherd 1976; Powell 1979) and in cardiac tissues (Lokhandwala 1978; Wong-Dusting et al 1979; Rand et al 1982). In guinea-pig atria, exogenous histamine was shown to decrease the stimulation-induced release of radiolabelled noradrenaline; however, there was no evidence for the activation of the inhibitory prejunctional histamine receptors by endogenous histamine (Rand et al 1982). We have now examined the effect of anaphylactically released mast cell histamine on stimulation-induced noradrenaline release.

Histamine released into the solution perfusing or bathing guinea-pig isolated hearts or atria during in vitro anaphylactic reactions has been shown to reach a maximal level within 2 min of antigenic challenge and to be one of the principal mediators of the increased rate and force of myocardial contractions, atrioventricular block and decreased coronary flow (Feigen & Prager 1969; Penna et al 1959; Levi 1972; Capurro & Levi 1975). The degree of anaphylaxis in response to antigenic challenge is known to be dependent on both the sensitizing and the challenging doses of antigen. Therefore, in the present study, anaphylactic reactions were induced in guinea-pig atria with different sensitizing and challenging doses of ovalbumin, and the effects on transmitter noradrenaline release were investigated.

Radiolabelled noradrenergic transmitter efflux was reduced in atria from guinea-pigs sensitized with two 5 mg doses of ovalbumin immediately following challenge with 0·4 μg ml^{-1} of the antigen. However, when the degree of anaphylaxis was reduced by decreasing the sensitizing doses of ovalbumin to 1 mg, challenge produced inconsistent effects on transmitter efflux and with further reduction in the sensitizing doses to 0·1 mg, there was no effect of challenge on the stimulation-induced release.

There was no apparent correlation between the inhibition of transmitter release and the effects of the anaphylactic challenge on the rate and force of atrial contractions. Thus sensitization with 5 mg doses and subsequent challenge with 0·4 μg ml^{-1} ovalbumin produced similar increases in atrial rate to sensitization with 10 mg doses and challenge with 40 and 400 μg ml^{-1}; however, transmitter efflux was respectively decreased, unchanged and enhanced. Presumably in each of these cases the rate increases were maximal or near maximal and the differing effects of challenge on transmitter efflux reflect differing amounts of mediator release. Furthermore, we have previously shown that changes in mechanical activity of the heart do not affect transmitter noradrenaline release (Rand et al 1982).

The effect of cimetidine on the decrease in transmitter efflux during anaphylaxis was examined to determine whether prejunctional histamine receptors were being activated. The conditions chosen for sensitization and challenge in these experiments (sensitization with two 5 mg doses and challenge of the atria with 0·4 μg ml^{-1} of ovalbumin) appeared to produce a near maximal anaphylactic response. However, it was not possible to use conditions which produced submaximal anaphylaxis due to the inconsistent effects on rate and transmitter efflux. Cimetidine had no effect on the inhibition of stimulation-induced efflux during anaphylaxis even in a concentration of 100 μmol $litre^{-1}$. Previously we have shown that 10 μmol $litre^{-1}$ cimetidine abolished the prejunctional inhibitory action of exogenous histamine (Wong-Dusting et al 1979; Rand et al 1982). Furthermore, in the present experiments, the postjunctional chronotropic response was reduced but not abolished by a high concentration of cimetidine (100 μmol $litre^{-1}$). Thus it appears that the inhibition of noradrenergic transmission occurring during the anaphylactic reaction is not mediated to any appreciable extent by histamine, and that the postjunctional chronotropic response is only partially due to activation of histamine H_2-receptors. Other substances, namely prostaglandins and SRS-A, are known to be released during anaphylaxis and these may act postjunctionally and prejunctionally as well as modulating the effects of histamine (Liebig et al 1975; Allan & Levi 1980; Levi & Burke 1980).

Thus, although there is inhibition of transmitter noradrenaline release during anaphylaxis in guinea-pig isolated atria, this does not appear to be due to activation of prejunctional histamine receptors.

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