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EFFECT OF SYMPATHOMIMETIC AMINES ON THE HISTAMINE FORMING CAPACITY OF HUMAN LEUCOCYTES

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1 The effects of α- and β-adrenoceptor stimulants on the histamine-forming capacity (HFC) of human isolated leucocytes have been studied, *in vitro*.

2 It was confirmed that antigen significantly stimulates the HFC of human leucocytes.

3 β-Adrenoceptor stimulants, such as isoprenaline and salbutamol (10^{-6}-10^{-3} M) significantly inhibited the HFC of human leucocytes in the presence and absence of antigen. At concentrations lower than 10^{-6} M, this effect was not observed. In general the degree of inhibition of HFC by β-adrenoceptor stimulants followed their potency as β-adrenoceptor stimulants.

4 α-Adrenoceptor stimulants significantly stimulated leucocyte HFC; noradrenaline within a limited concentration of 10^{-6} M, while stimulation was seen consistently with phenylephrine at concentrations of 10^{-7}-10^{-4} M. Adrenaline, which stimulates both α- and β-adrenoceptors, produced small inhibition, no effect, or a degree of stimulation.

5 Phentolamine, an α-adrenoceptor blocking agent, produced an effect opposite to that of the α-adrenoceptor stimulants, i.e. a significant inhibition of the HFC of human isolated leucocytes.

Introduction

Following an initial observation by Schild (1936) that adrenaline produced a significant inhibition of histamine release from guinea-pig isolated perfused lung, this phenomenon has been re-discovered in a number of different actively and passively sensitized preparations. In virtually every variety of preparation studied to date, some inhibition of antigen-induced histamine release by sympathomimetic amines has been found (in many instances the release of slow reacting substance of anaphylaxis was also inhibited); the extent of inhibition appeared to vary with the potency of the drug as a β-adrenoceptor stimulant (Assem & Schild, 1969, Koopman, Orange & Austen, 1970).

Antigen, besides releasing histamine, also stimulates its formation in sensitized human leucocytes (Assem, Schild & Vickers, 1972; Assem & Feigenbaum, 1972; Assem & Feigenbaum, 1973). Observing this, we tested the effect of adrenaline and other sympathomimetic amines on antigen-induced formation as well as release, to determine whether the extent of the inhibition of formation also varied as a function of their efficacy as β-adrenoceptor stimulants. We also pursued the possibility that these amines would inhibit the histamine formation which is independent of antigenic stimulation, which would suggest a physiological regulatory mechanism by the autonomic nervous system affecting histamine formation. Subsequent studies have attempted to elucidate possible biochemical and receptor mechanisms involved in the anti-anaphylactic effect of these drugs.

Methods

Effect of sympathomimetic amines on antigen-induced stimulation of HFC in sensitized leucocytes

In the initial experiments where antigen-challenged leucocyte preparations were used, blood samples were obtained from patients severely allergic to the house-dust mite, *Dermatophagoides pteronyssinus*. Appropriate concentrations of this antigen were prepared from a stock solution supplied by Bencard Laboratories. [^{14}C]-L-histidine labelled in the 2 position of the imidazole ring was obtained from the Radiochemical Centre, Amersham.

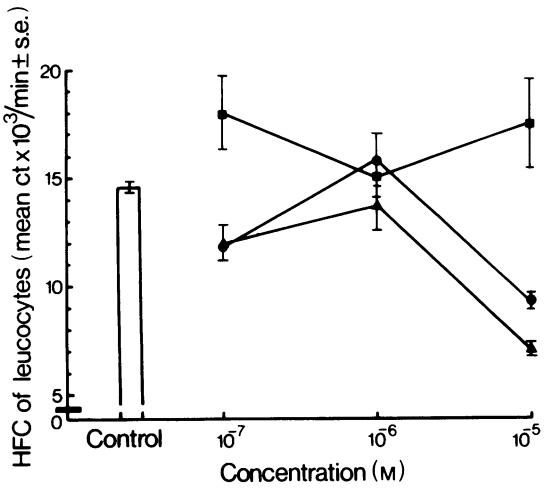

Fig. 1 The effect of isoprenaline (▲), adrenaline (■) and noradrenaline (●) on the histamine-forming capacity (HFC) of leucocytes isolated from the same blood sample of a non-allergic subject. Each point represents the mean count in three to four aliquots of leucocytes.

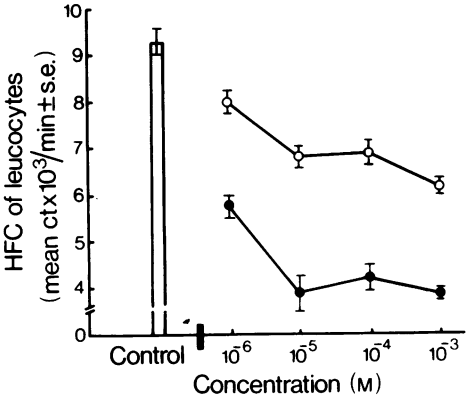

Fig. 2 Inhibition of histamine-forming capacity (HFC) of isolated leucocytes (four replicates in each point) from a non-allergic subject by isoprenaline (●) and salbutamol (○).

Leucocytes were isolated by a modification of the method of Lichtenstein & Osler (1964), as described by Assem, Turner-Warwick, Cole & Shaw (1971). Histamine forming capacity (HFC) was measured as histidine decarboxylase activity by the isotope dilution method of Kahlson, Rosengren & Thunberg (1963). The leucocytes (10^7 cells in 0.5 ml Tyrode solution) were incubated for 15 min at 37°C under nitrogen in beakers containing 2.9 ml of a mixture of [^{14}C]-L-histidine, 10^{-4} M aminoguanidine sulphate (used to inhibit the catabolism of newly formed histamine), and 400 μg of non-labelled histamine dihydrochloride to prevent the effect of the methylating enzyme system on newly formed histamine. Following the first incubation, drug and antigen in 0.4 ml Tyrode solution pH 7.4, or Tyrode solution alone, was added to the samples before a second incubation. In some experiments sodium metabisulphite or ascorbic acid was added to prevent oxidation of sympathomimetic drugs. This incubation continued for a further 30 min at the completion of which 40 mg of unlabelled histamine dihydrochloride was added as a carrier. The proteins were then precipitated with perchloric acid, and removed by centrifugation of the samples. With the pH of the supernatant adjusted to 6.5, the samples were applied to columns containing phosphate buffered Dowex resin (pH 6.5, type 50 WX 4). After washing the columns with excess buffer, histamine was eluted with concentrated hydrochloric acid. The rest of this procedure was carried out as described by Kahlson *et al.* (1963).

Effect of sympathomimetic amines on histamine formation in unchallenged isolated leucocytes

To determine the effect of sympathomimetic amines on histamine formation by isolated leucocytes occurring independently of challenge by antigen, blood samples were obtained from normal volunteers with no history of allergy. The samples were treated as above except that the Tyrode solution added had a pH of 6.5, and no antigen was added.

Results

Table 1 shows the effect of isoprenaline on the stimulation of histamine formation by antigen. Antigen challenge of leucocytes enhanced HFC confirming earlier findings. Isoprenaline produced a striking inhibition of this effect in the concentrations used. The results of 4 experiments showing the inhibition by isoprenaline of antigen-induced stimulation of HFC are summarized in Table 1. Isoprenaline also inhibited HFC in absence of antigen.

Since the inhibition by sympathomimetics of

antigen-induced histamine release from leucocytes seems to be due to stimulation of β-adrenoceptors in basophil leucocytes (the main blood cells involved in histamine release), we investigated the possibility that the inhibition of HFC by these compounds is due to a similar mechanism. In 10^{-5} M concentration, isoprenaline inhibited HFC more potently than did noradrenaline, whilst adrenaline had no definite inhibitory effect (Figure 1). In contrast, the potency of inhibition of histamine release was:

isoprenaline > adrenaline > noradrenaline

(Assem & Schild, 1969, 1971a). Salbutamol, a β-receptor agonist, generally recognized as being less potent than isoprenaline, also inhibited HFC less potently (Figure 2).

HFC of leucocytes was either stimulated or depressed by adrenaline 10^{-7}-10^{-5} M, and inhibited by 10^{-4} M. Noradrenaline in concentrations of 10^{-7}, 10^{-5} and 10^{-4} M inhibited HFC, whilst 10^{-6} M stimulated HFC (Table 2).

Phenylephrine, which stimulates α-receptors more selectively than does noradrenaline, produced in 10^{-7}-10^{-4} M concentrations an increase in HFC.

As expected, phentolamine antagonized the stimulatory effect of phenylephrine, and propranolol antagonized the inhibitory effect of isoprenaline. However, the effects of the two antagonists were not simply due to 'blockade' of

Table 1 Effect of isoprenaline on HFC of antigen-challenged and non-antigen-challenged leucocytes.

		Isoprenaline		*Control*			
Experiment	*Concentration (M)*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*
1 With Ag (sensitized cells)				(W/out Ag)		(With Ag)	
1**	10^{-5}	16.6	6.7	14.4	3.4	60.1	2.9
	10^{-4}	20.2	15.5				
2**	10^{-5}	0.7	0.6	11.4	2.7	89.7	10.8
	10^{-4}	2.1	1.1				
3**	4×10^{-5}	17.6	2.8	52.6	3.9	64.6	3.5
4**	10^{-4}	71.5	3.1	126	4.2	184.6	17.8
2. Without Ag (non-sensitized cells)							
5*	10^{-8}	507	32	380	16		
	10^{-7}	349	46				
	10^{-6}	428	46				
	10^{-5}	389	33				
6*	10^{-8}	632	86	489	34		
	10^{-7}	439	67				
	10^{-6}	547	48				
	10^{-5}	490	12				
7*	10^{-6}	649	7	656	31		
	10^{-5}	545	62				
	10^{-4}	615	50				
8***	10^{-6}	5755	217	9165	440		
	10^{-5}	3964	315				
	10^{-4}	4244	303				
	10^{-3}	3925	55				
9***	10^{-7}	12080	297	14581	226		
	10^{-6}	13738	1156				
	10^{-5}	6990	219				

The amount of isotope-labelled histamine formed during the 30 min incubation is expressed as mean counts per min in similarly-treated aliquots (4 replicates each).
Anti-oxidant compounds: * none used; ** sodium metabisulfite 0.1 mg/ml; *** ascorbic acid 10^{-5} M.

Table 2 Effect of adrenaline, noradrenaline and phenylephrine on HFC of non-antigen-challenged leucocytes.

Adrenaline

Experiment	Concentration (M)	Mean	s.e.	Control Mean	Control s.e.
1*	10^{-5}	283	25	392	23
2*	10^{-5}	303	58	404	24
	10^{-4}	282	20		
3**	10^{-7}	18040	1687	14581	226
	10^{-6}	15032	926		
	10^{-5}	17545	2147		
4*	10^{-7}	19059	1223	17379	621
	10^{-6}	17408	874		
	10^{-5}	22978	4857		

Noradrenaline

Experiment	Concentration (M)	Mean	s.e.	Control Mean	Control s.e.
5*	10^{-8}	301	12	380	16
	10^{-7}	279	9		
	10^{-6}	538	14		
	10^{-5}	343	35		
6**	10^{-7}	12051	846	14581	226
	10^{-6}	15850	1286		
	10^{-5}	9340	375		

Phenylephrine

Experiment	Concentration (M)	Mean	s.e.	Control Mean	Control s.e.
7	10^{-6}	120	3.8	91.1	12.9
	10^{-5}	147	16.1		
8	10^{-5}	182	14.1	125	7.9
9	10^{-8}	746	9	767	45
	10^{-7}	1676	69		
	10^{-6}	1208	63		
	10^{-5}	1099	45		
	10^{-4}	808	16		

The amount of histamine formed is expressed as mean counts per min in similarly treated aliquots.
Anti-oxidant compounds: * none used; ** ascorbic acid 10^{-5} M.

α- and β-receptors respectively, since in the absence of agonists, phentolamine also inhibited HFC, and propranolol stimulated it.

Discussion

The present study and our previous studies show that sympathomimetic drugs modulate anaphylactic histamine release as well as HFC in human leucocytes. The effects of sympathomimetic amines on the HFC of human isolated leucocytes differed in some respects from their effects on histamine release from the same cells (Assem & Schild, 1971a).

α-Adrenoceptor stimulants, such as noradrenaline and phenylephrine, while having no stimulatory effect on histamine release from leucocytes, were found to stimulate leucocyte HFC. Noradrenaline stimulated HFC only in

10^{-6} M concentrations, whilst it produced inhibition at higher concentrations.

β-Adrenoceptor stimulants (isoprenaline and salbutamol) inhibited both histamine release and HFC in allergen-challenged sensitized leucocytes. However, they also inhibited spontaneous HFC whilst having no effect on spontaneous histamine release.

Adrenaline, which is a potent stimulant of both α- and β-receptors, consistently inhibited antigen-induced histamine release, whilst its effect on HFC varied from small inhibition to small stimulation.

Phentolamine and other α-adrenoceptor blocking drugs, while having little if any effect on antigen-induced or spontaneous histamine release, either from normal leucocytes or sensitized leucocytes, significantly inhibited the antigen-induced and spontaneous HFC.

β-Receptor blocking drugs potentiated the antigen-induced stimulation of HFC. These drugs inhibited histamine release incompletely (Assem & Schild, 1971b), which suggests that they possess a partial agonist effect on leucocyte (basophil) β-receptors. While propranolol and practolol both acted as antagonists to isoprenaline in histamine release studies (Assem & Schild, 1971b), only propranolol antagonized the effect of isoprenaline on HFC, practolol having little or no effect. Propranolol therefore not only blocked the action of isoprenaline but, like a physiological antagonist, itself produced the opposite effect to that of isoprenaline.

There is some evidence that the inhibition by β-adrenoceptor stimulants of HFC in leucocytes involves the adenylate (adenyl) cyclase system and cyclic AMP. This view is in agreement with the various effects of β-receptor stimulants, including inhibition of histamine release (Assem, 1971). The evidence in the case of HFC is as follows:

(1) Inhibition of HFC is associated with β-activity, α-activity producing the reverse effect. Cyclic AMP formation is also stimulated by β- (Assem, 1971) but not α-adrenoceptor stimulants. Robison, Butcher & Sutherland (1967) suggested that cyclic AMP formation may be reduced by α-adrenoceptor stimulants. Moreover, the release of slow-reacting substance of anaphylaxis is inhibited by β-stimulants, and potentiated by α-stimulants (Koopman *et al.*, 1970).

(2) Cyclic AMP and its dibutyryl derivative inhibit HFC (Assem, unpublished observations) the latter being more potent in HFC experiments with intact cells, presumably because of its ability to penetrate cell membranes and to reach cytoplasmic histidine decarboxylase (the enzyme responsible for HFC).

Nothing is known at present about the mechanism of stimulation of HFC by α-adrenoceptor stimulants and by β-receptor blocking agents. The mechanism of inhibition of HFC by α-receptor blocking drugs is also not known. It is interesting that phentolamine and thymoxamine, two α-receptor blocking drugs, potentiate the increase in cyclic AMP formation in human leucocytes in response to isoprenaline (Alston, Patel & Kerr, 1974).

The inhibition of histamine release and HFC in human leucocytes by β-adrenoceptor stimulants may contribute to the effectiveness of these drugs in asthma. Although significant changes in leucocyte HFC over the short period of incubation in our experiments (30 min) were produced only by relatively high concentrations, inhibition of HFC in lung tissue and bronchial passages may be obtained more readily. Antigen-induced histamine release from sensitized leucocytes is less readily inhibited by isoprenaline than the release of histamine from sensitized lung tissue (Assem & Schild, 1969). Furthermore, repeated administration of β-stimulants, particularly by inhalation, may have a more pronounced effect in lung than the short incubation of leucocytes *in vitro*. It is conceivable, therefore, that in addition to bronchodilator effects of these drugs, the inhibition of histamine release and formation may also be of benefit.

The evidence for the possible 'physiological' role of adrenergic and other autonomic mechanisms in 'regulating' the anaphylactic reaction, and for similar regulatory mechanisms in other types of allergic reactions has been recently reviewed (Assem, 1974).

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