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Antagonism by β-adrenoceptor blocking agents of the antianaphylactic effect of isoprenaline

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Summary

1. β-Adrenoceptor blocking agents antagonized the inhibitory (antianaphylactic) effect of isoprenaline on antigen-induced histamine release in isolated, passively sensitized, human lung and actively sensitized guinea-pig lung suggesting that the effect is exerted through β-adrenoceptors. No antagonism was obtained with the human allergic leucocyte preparation.

2. The antianaphylactic effect of isoprenaline was antagonized not only by propranolol but also by the 'selective' β-adrenoceptor blocking agents, practolol and butoxamine.

3. The classification of β-adrenoceptors is discussed. Certain similarities between antianaphylactic and lipolytic β-adrenoceptors are noted.

Introduction

Sympathomimetic amines inhibit antigen-induced histamine release in a variety of preparations, including actively sensitized guinea-pig lung (Schild, 1936; Assem & Schild, 1971), passively sensitized guinea-pig lung (Assem, Pickup & Schild, 1970), passively sensitized human and monkey lung (Assem & Schild, 1969; Ishizaka, Ishizaka, Orange & Austen, 1970) and actively sensitized human leucocytes (Lichtenstein & Margolis, 1968; Assem & Schild, 1971). This effect, which subsequently will be referred to as 'antianaphylactic', is exerted in some preparations by remarkably low concentrations of catecholamines, and it follows their order of activity as β-adrenoceptor stimulants (Assem & Schild, 1969). The antianaphylactic effect of isoprenaline is antagonized by propranolol (Assem & Schild, 1969; Koopman, Orange & Austen, 1970) supporting the notion that β-adrenoceptors are involved.

The object of this investigation was to investigate the action of β-adrenoceptor blocking agents in more detail. There is evidence that several types of these agents can be distinguished, exemplified by propranolol which produces a generalized block of β-adrenoceptors, practolol which blocks β-adrenoceptors in the heart but not in smooth muscle (Barrett, Crowther, Dunlop, Shanks & Smith, 1968) and butoxamine which blocks β-adrenoceptors in smooth muscle but not in the heart (Burns & Lemberger, 1965). We have investigated the effects of these three blocking agents hoping to shed some light on the type of receptor involved in the antianaphylactic effect of isoprenaline.

Methods

The methods used for measuring histamine release from chopped lung by biological assay on guinea-pig ileum have been previously described (Assem & Schild, 1968). Histamine releases are expressed in terms of the histamine content of the tissue. Each release figure is the mean of at least duplicate determinations on separate samples of the same lung. Histamine release from human leucocytes was also measured by biological assay.

Histamine release from passively sensitized human lung

Passive sensitization of chopped human lung was carried out as described by Assem & Schild (1968), using serum from patients severely allergic to the house-dust mite *Dermatophagoides pteronyssinus*. Antigen extracted from the mite was kindly provided by Dr. K. Maunsell ; an optimal concentration of this antigen for histamine release (50 μg/mg) was used throughout.

Histamine release from leucocytes of allergic patients

Leucocytes were isolated from heparinized blood samples collected from patients allergic to *D. pteronyssinus*. In this test a modification of the method described by Lichtenstein & Osler (1964) was used (Assem & McAllen, 1970). Leucocytes were challenged with suboptimal concentrations of mite extract ranging from 0·16 to 4·0 μg/ml.

Histamine release from sensitized chopped guinea-pig lung

Passively sensitized lung. Chopped guinea-pig lung was passively sensitized by incubation with homologous antiovalbumin or antidinitrophenyl (DNP) sera, the latter being provided by Dr. D. Colquhoun. Optimum concentrations of antigen were generally used.

Actively sensitized lung. Guinea-pigs were actively sensitized by injecting intraperitoneally single doses of 20 mg ovalbumin in incomplete adjuvant (Difco). They were killed after 2–4 weeks and their lungs isolated and perfused with Tyrode solution. The lungs were chopped and challenged with ovalbumin in a concentration of 10 μg/ml.

Inhibition by sympathomimetic amines of antigen-induced histamine release

This was carried out as described by Assem & Schild (1969, 1971). Histamine release was estimated biologically on guinea-pig ileum suspended in Tyrode solution (mM: NaCl 137, KCl 2·7, $CaCl_2$ 1·8, $MgCl_2$ 1, NaH_2PO_4 0·42, $NaHCO_3$ 12) in jacketed baths of 1 ml volume. Automatic apparatus modified from that of Boura, Mongar & Schild (1954) was used.

Potential sources of artifact, such as interference by isoprenaline with the bioassay of histamine, were avoided by using drug concentrations which in the final assay solutions (10–100 fold dilutions of supernatants) would not produce interference. The threshold for interference by isoprenaline was usually $>4 \times 10^{-7}$M. Because of variability in the interference threshold, this was assessed in each experiment. In lung experiments interference rarely occurred, but in leucocytes, where relatively high concentrations of sympathomimetics were required, interference was more frequent, in which case the results were discarded.

Effects of adrenoceptor blocking drugs

Propranolol, practolol, and butoxamine were used for blocking β-adrenoceptors and isoprenaline was used as agonist. β-Adrenoceptor blocking agents were applied either simultaneously with or before the agonist. One of the factors in this choice was the magnitude of spontaneous histamine release from the tissue used. This was relatively great in the passively sensitized human lung preparation, which was incubated for a relatively long period (usually overnight) with the sensitizing serum. On the other hand, the spontaneous histamine release from actively sensitized guinea-pig lung was small and longer preincubation with the blocking agents (10 min–1 h) was possible. Phentolamine was used for blocking α-adrenoceptors. Precautions essentially similar to those previously mentioned were taken in order to avoid artifacts produced through the interference of blocking agents with the biological assay of histamine.

The following drugs were used: isoprenaline sulphate (Burroughs Wellcome), propranolol hydrochloride (ICI), practolol hydrochloride (ICI), butoxamine hydrochloride (Burroughs Wellcome), phentolamine hydrochloride (Ciba), disodium cromoglycate (Fisons).

Results

Antagonism of isoprenaline by propranolol

Isoprenaline inhibits antigen-induced histamine release *in vitro* in both the passively sensitized human lung and actively sensitized guinea-pig lung (Assem & Schild, 1971). Propranolol antagonized the inhibitory effects of isoprenaline in both preparations.

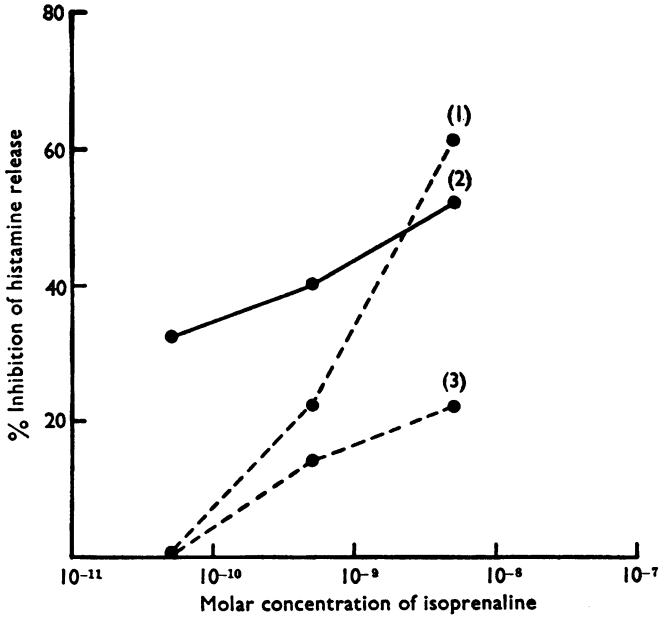

FIG. 1. Chopped human lung, passively sensitized with serum of patient with *D. pteronyssinus* allergy. Histamine release by antigen alone was 11% of content. Isoprenaline and propranolol added at same time as antigen. Abscissa, concentration of isoprenaline (M); ordinate, percentage inhibition of histamine release in presence of various concentrations of propranolol. (1) 5×10^{-9}M propranolol; (2) no propranolol; (3) 5×10^{-8}M propranolol.

In the experiment shown in Fig. 1 propranolol (5×10^{-8}M) administered with isoprenaline strongly antagonized the inhibitory effect of isoprenaline in human lung. Whilst the higher concentration (5×10^{-8}M) produced simple antagonism of isoprenaline a lower concentration (5×10^{-9}M) produced a mixed effect with a steep dose-response curve showing both antagonism and potentiation.

When a typical α-adrenoceptor blocking agent, phentolamine (10^{-6}M), was tested in the human lung it failed to antagonize the antianaphylactic effect of isoprenaline.

In view of the potentiation of isoprenaline inhibition by low concentrations of propranolol, we investigated the effect of propranolol alone. In experiments carried out in the actively sensitized guinea-pig lung preparation, propranolol was administered 10 min before antigen. Propranolol alone produced a definite antianaphylactic effect (Fig. 2). A similar effect was obtained in passively sensitized human lung (Table 1).

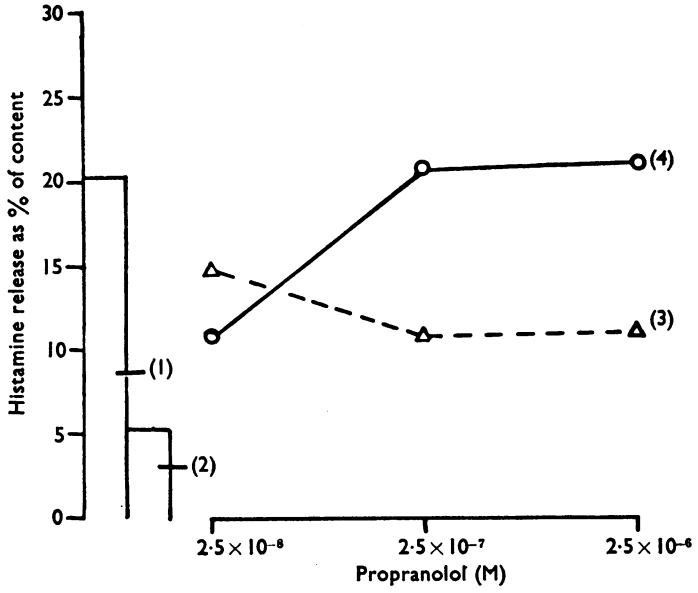

FIG. 2. Chopped guinea-pig lung, actively sensitized with ovalbumin. Effect on histamine release by antigen (Ag, 10 μg/ml ovalbumin) (1) of isoprenaline (10^{-7}M) alone (2), propranolol alone (3) and mixtures of isoprenaline (10^{-7}M) and propranolol (4). Propranolol added 10 min before antigen; isoprenaline added with antigen.

TABLE 1. *Comparison of the effect of propranolol on inhibition by isoprenaline and by disodiumcromoglycate of antigen-induced histamine release from passively sensitized human lung*

	Addition to antigen	Concentration (M)	Histamine release (% of tissue content ± S.E.M.)	Inhibition (%)
	None		47·7±1·4 (4)	
A.	Isoprenaline	10^{-9}	23·9±3·7 (4)	50
B.	Disodium cromoglycate	3×10^{-5}	18·1±5·3 (4)	62
	A+B		13·3±3·9 (4)	72
C.	Disodium cromoglycate	$1{\cdot}5 \times 10^{-4}$	27·2±3·8 (3)	43
	A+C		14·1±5·3 (4)	70
D.	Propranolol	10^{-7}	40·4±1·8 (2)	15
	A+D		42·0±0·4 (4)	12
	B+D		19·4±3·2 (3)	59

All drugs were applied simultaneously with antigen.

These results indicate a complex situation in which isoprenaline and propranolol both inhibit anaphylactic histamine release on their own, but antagonize each other when combined. Figure 2 shows this antagonism in an experiment on actively sensitized guinea-pig lung. Isoprenaline and propranolol alone inhibited histamine release, isoprenaline by about 75%, propranolol by 25–50% ; but when the two

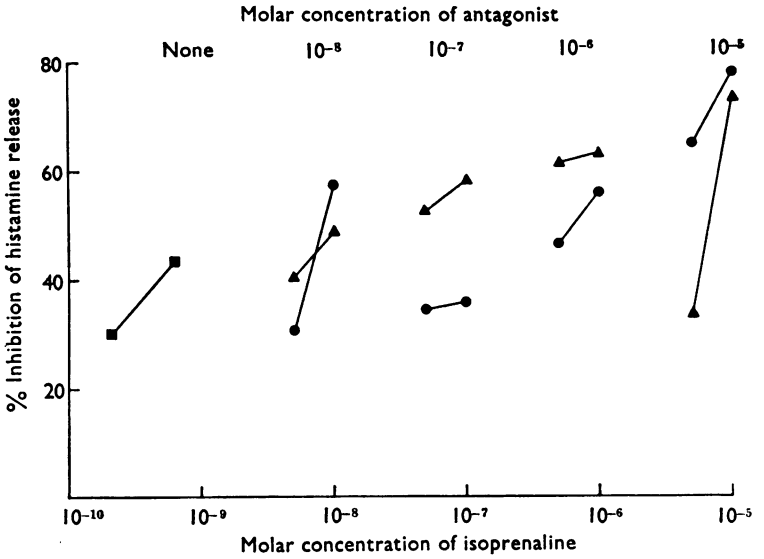

FIG. 3. Chopped actively sensitized (ovalbumin) guinea-pig lung. Antagonism by practolol and butoxamine of isoprenaline inhibition of histamine release by antigen. β-Adrenoceptor blocking agent added 1 h before antigen, isoprenaline added at the same time as antigen. Isoprenaline alone: quadruplicate samples, isoprenaline+β-adrenoceptor blocking agent: duplicate samples. Practolol (●); butoxamine (▲); No antagonist (■).

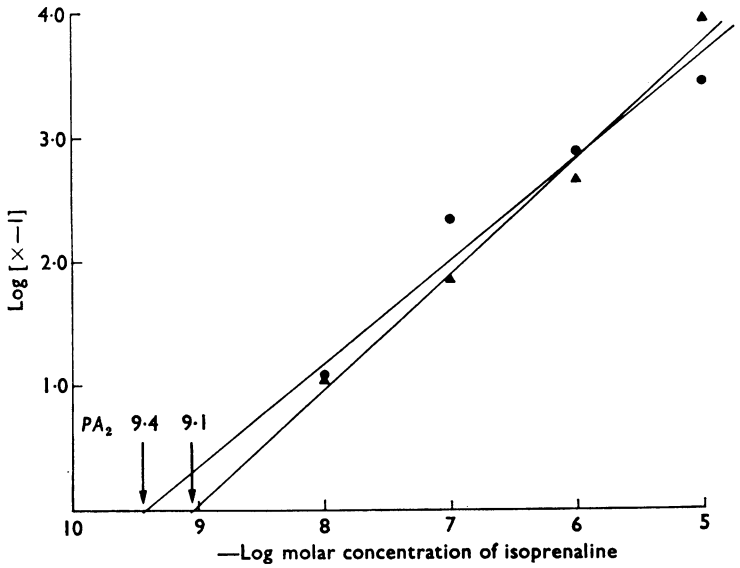

FIG. 4. Plot of log $(x-1)$, where x=dose ratio, against pA_x for isoprenaline combined with butoxamine (▲) and practolol (●). The slopes (n) of the calculated regression lines were 0·83 for practolol and 0·93 for butoxamine. Data derived from Fig. 4.

TABLE 2. *Effect of propranolol and practolol on inhibition by isoprenaline of antigen-induced histamine release from passively sensitized human lung*

Exp. no.	Net uninhibited histamine release (% of tissue content ± S.E.M.)	Isoprenaline (M)	Inhibition of histamine by isoprenaline alone (% ± S.E.M.)	Inhibition of histamine release by isoprenaline in presence of β-adrenoceptor blocking agents (%)					
				Propranolol (M)			Practolol (M)		
				5×10^{-9}	5×10^{-8}	5×10^{-7}	5×10^{-9}	5×10^{-8}	5×10^{-7}
1	10·7 ± 1·5 (4)	10^{-9}	64 ± 13	46	46	5	67	50	27
2	11·0 ± 1·0 (4)	5×10^{-9}	52 ± 7				67	37	
		5×10^{-10}	40 ± 11				35	18	
		5×10^{-11}	32 ± 18				0	0	
3	42·0 ± 0·4 (4)	10^{-10}	70 ± 3		31				
		10^{-9}	88 ± 2		32				
		10^{-8}	100 ± 0		44				

drugs were combined their inhibitory effects cancelled out, so that histamine release was now equal to that by antigen alone.

We investigated whether propranolol produces its effects by way of β-adrenoceptors by comparing its antagonism of isoprenaline with the effect of disodium cromoglycate which is not considered to act through β-adrenoceptors. Table 1 shows the results of an experiment in passively sensitized human lung in which approximately equiactive inhibitory concentrations of isoprenaline (10^{-9}M) and cromoglycate (3×10^{-5}M and $1{\cdot}5 \times 10^{-4}$M) were used. Propranolol produced no antagonism of cromoglycate but it strongly antagonized isoprenaline. Isoprenaline and cromoglycate exhibited no mutual antagonism, their combined effects being greater than that of either drug alone.

Other β-adrenoceptor blocking agents

Since propranolol is considered an 'unspecific' β-adrenoceptor blocking agent two further blocking agents, practolol and butoxamine, believed to act preferentially on β_1- and β_2-adrenoceptors respectively, were investigated. Figure 3 shows the results obtained in actively sensitized guinea-pig lung when various concentrations of the two blocking agents were administered combined with isoprenaline. They were added 1 h before isoprenaline which was administered with the antigen. It is seen

TABLE 3. *Inhibition of histamine release from actively sensitized guinea-pig lung by practolol alone administered before antigen*

Experiment	Uninhibited histamine release (% of tissue content)	Practolol (M)	Inhibition of histamine release (%)
1	7	10^{-6}	18
		10^{-5}	53
		10^{-4}	52
2	13	10^{-8}	17
		10^{-7}	5
		10^{-6}	24
		10^{-5}	2

TABLE 4. *Effect of propranolol, practolol and butoxamine on inhibition by isoprenaline of antigen-induced histamine release from sensitized human leucocytes*

Antigen* (μg/ml)	Uninhibited histamine release (% tissue content)	Isoprenaline (M)	Inhibition by isoprenaline alone (%)	Inhibition (%) of histamine release by isoprenaline in the presence of propranolol	practolol	butoxamine
					$2{\cdot}5 \times 10^{-8}$M	
4	57	$2{\cdot}5 \times 10^{-7}$	29	55	52	
		$2{\cdot}5 \times 10^{-6}$	36			
0·8	61	$2{\cdot}5 \times 10^{-7}$	11	52	32	
		$2{\cdot}5 \times 10^{-6}$	76	86	52	
					10^{-9}M	
0·8	56	10^{-6}	60	45	70	
4	63	10^{-6}	27			
		10^{-5}	39	32	47	
					5×10^{-8}M	
4	31	3×10^{-6}	94		88	
20	82	$2{\cdot}5 \times 10^{-7}$	8		11	
		$2{\cdot}5 \times 10^{-6}$	16		20	
					10^{-9}M	
4	62	10^{-6}	52		50	49
		10^{-7}	5		17	31

**Dermatophagoides pteronyssinus.*

that both drugs antagonized isoprenaline as shown by a concentration dependent shift of the log dose-response curve. Since the individual regression coefficients showed no systematic trend, they were averaged assuming a common slope. Dose ratios (x) were measured graphically on this assumption and the plots of log $(x-1)$ against pA_x (Arunlakshana & Schild, 1959) are shown in Fig. 4. The graphical pA_2 value for butoxamine was $pA_2=9{\cdot}1$ and for practolol $pA_2=9{\cdot}4$, the slopes of the regression lines being 0·83 for practolol and 0·93 for butoxamine.

The results of further experiments in which propranolol and practolol antagonized the antianaphylactic effect of isoprenaline in the passively sensitized human lung preparation are shown in Table 2. The β-adrenoceptor blocking agents were applied at the same time as isoprenaline. Both drugs antagonized the antianaphylactic effect of isoprenaline. The antagonism increased with the concentration of the blocking agent and decreased with the concentration of isoprenaline.

Practolol resembled propranolol in producing some antianaphylactic effect of its own as shown in Table 3. Butoxamine had no such action.

Human leucocyte preparation

As pointed out before (Assem & Schild, 1971) the human leucocyte preparation is more resistant to the antianaphylactic action of isoprenaline than human lung or guinea-pig lung. It is nevertheless possible to show the inhibitory action of isoprenaline in the leucocyte preparation as first demonstrated by Lichtenstein & Margolis (1968), particularly if suboptimal antigen concentrations and relatively high isoprenaline concentrations are used. The interactions of the three β-adrenoceptor blocking agents with isoprenaline in the leucocyte preparation are shown in Table 4. It is seen that they failed to antagonize the antianaphylactic effect of isoprenaline and in some cases potentiated it.

Discussion

Our experiments confirm previous findings (Assem & Schild, 1969; Koopman *et al.*, 1970; Assem, 1971) that β-adrenoceptor blocking agents antagonize the antianaphylactic effect of isoprenaline; they thus support the notion (Assem *et al.*, 1970; Assem & Schild, 1969, 1971) that the inhibitory effect of isoprenaline on anaphylactic histamine release is exerted through β-adrenoceptors. Since these receptors are believed to be a heterogenous group (Furchgott, 1967) it is necessary to examine further the type of receptor involved. These experiments show that not only a 'general' β-adrenoceptor blocking agent, such as propranolol, but also 'selective' agents such as butoxamine and practolol, are highly active in antagonizing this effect of isoprenaline. How then can the antianaphylactic β-adrenoceptor be classified?

The usual way of classifying receptors is by means of antagonists. Arunlakshana & Schild (1959) suggested that pA_x values could be used to classify receptors in different tissues since similar receptors would be expected to give the same pA_x with a competitive antagonist, the underlying assumption being that pA_x values represent affinities of antagonists for receptors. Classification by pA_x has given reasonably consistent results with muscarinic acetylcholine antagonists or with H1-type antihistamines (Ash & Schild, 1966), but in other cases difficulties have arisen. These could be due to one or other of the following reasons. (1) Genuine heterogeneity of receptors. (2) Receptors appear homogeneous when tested with one

antagonist, heterogeneous when tested with another (this may be the case for β-adrenoceptors). (3) The antagonism is not competitive as shown by non-parallel log dose-response curves. (4) The antagonism is apparently competitive as shown by parallel log dose-response curves but pA_2 values do not correspond to affinity constants.

With reference to (4) the use of pA_2 for receptor classification is clearly justifiable when the antagonism is of a simple competitive type in which case pA_2 represents the logarithm of the affinity constant of the antagonist. Frequently, however, the displacement of the dose-response curves along the log dose axis is less than expected and the plot of log $(x-1)$ against pA_x, although linear, has a slope which is less than 1. Under these conditions the graphically determined pA_2 value does not represent an affinity constant and cannot be used in a meaningful way for purposes of classifying receptors.

The point is illustrated in Table 5 which gives the pA_2 values for isoprenaline of two β-adrenoceptor blocking agents in different preparations. At first glance the pA_2 values seem to suggest that pronethalol acts on the same receptor in heart and tracheal smooth muscle, whilst butoxamine has different receptors in the two preparations, that is case (2) above. However, inspection of the slopes of the regression lines (m) shows that in the trachea they deviate markedly from the theoretical value 1 so that no definite conclusions can be drawn. Similar considerations apply to the practolol and methoxamine used in this work. Whilst in the present experiments the slopes of the regression lines for butoxamine and practolol were not markedly different from 1, this is not so in smooth muscle preparations where very low n values (0·4–0·48) obtained for both butoxamine and practolol (Patil, 1968; Levy & Wilkenfeld, 1970), so that precise comparisons between different receptor systems are again not feasible.

In these circumstances we have attempted only a rough qualitative classification of receptor affinities of the three β-adrenoceptor blocking agents tested, using data from the literature based partly on activity comparisons of these three agents in intact animals. Only two grades of strong (s) and weak (w) receptor affinity have been distinguished. Table 6 brings out the known qualities of butoxamine and practolol in showing their respective low affinities for chronotropic cardiac receptors and vasodilator receptors, but rather surprisingly it brings out certain common

TABLE 5. pA_2 *values for pronethalol and methoxamine with isoprenaline*

	Pronethalol		Methoxamine	
	pA_2	Slope n	pA_2	Slope n
Cardiac†	7·4	1*	5·2	0·94
Tracheal‡ relax.	7·26	0·61	6·25	0·52

*Estimated; †Blinks (1967); ‡Patil (1968).

TABLE 6. *Activity of β-adrenoceptor blocking agents against isoprenaline*

	Heart rate	Fall in arterial blood pressure	Lipolysis	Antianaphylaxis
Propranolol	s[2]	s[2]	s[7]	s[6]
Practolol	s[1]	w[4]	s[4]	s[6]
Butoxamine	w[1]	s[3]	s[5]	s[6]

s=strong; w=weak. [1]Parratt & Wadsworth (1970); [2]Black, Duncan & Shanks (1965); [3]Levy (1966); [4]Barrett *et al.* (1968); [5]Burns & Lemberger (1965); [6]present work; [7]Nakano, Kusakari & Berry (1966).

features of the lipolytic and antianaphylactic receptors for both of which all three drugs have strong affinities. The table suggests a tripartite classification of the β-adrenoceptors in the heart, in vascular smooth muscle, and those with lipolytic and antianaphylactic activity; it is not claimed, however, that this is more than an *ad hoc* classification applicable to these particular β-adrenoceptor blocking agents and receptor systems. Perhaps the most interesting finding is the resemblance between lipolytic and antianaphylactic receptors, which may reflect similarities between β-adrenoceptors on fat cells and mast cells.

Two of the three β-adrenoceptor blocking agents tested exhibited some degree of agonist activity producing an isoprenaline-like inhibitory effect on histamine release when administered alone. This was not entirely surprising since these drugs often have agonist activity, possibly because of their general structural resemblance to β-adrenoceptor agonists. When the blocking agents were combined with isoprenaline the inhibitory action of the latter was, nevertheless, antagonized. Only in the relatively insensitive leucocyte preparation were the effects of isoprenaline generally potentiated. Beta-adrenoceptor blocking agents may act as partial agonists antagonizing the more potent agonist isoprenaline by preventing its access to receptors. In some of our experiments both propranolol and isoprenaline exhibited inhibitory activity but when administered together their inhibitory effects cancelled out completely. We have no explanation for this type of complete cancellation of effect which cannot be readily accounted for by straightforward receptor theory.

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