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Reversal by pronethalol of dibenamine blockade: a study on the seminal vesicle of the guinea-pig

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1. The guinea-pig seminal vesicle has been shown to be a very suitable test object for the study of mechanisms involving α-adrenoceptive receptors, because no β-receptors were found in this preparation.

2. Adrenaline, noradrenaline and phenylephrine were directly acting agonists, their ED50 values being $7{\cdot}1 \times 10^{-6}$M, $1{\cdot}5 \times 10^{-5}$M and $2{\cdot}7 \times 10^{-5}$M, respectively.

3. Pretreatment with reserpine had no influence on the contractions caused by adrenaline, noradrenaline and phenylephrine but abolished or greatly reduced the contractions caused by dopamine. Cocaine enhanced the effects of adrenaline, noradrenaline and phenylephrine and reduced those of dopamine.

4. Pronethalol ($6{\cdot}8 \times 10^{-5}$M) reversed the α-receptor blockade by dibenamine, ergotamine and phentolamine of responses to adrenaline, noradrenaline and phenylephrine; it did not affect the blockade by dibenamine of responses to histamine.

5. Reversal of the blockade by dibenamine was observed only when its concentration was such that it caused a parallel shift of the dose-effect curves of the agonists to the right; higher concentrations, which caused an unsurmountable depression of the maximal contraction, were not antagonized by pronethalol.

6. It is assumed that the reversal is dependent on a direct action on α-receptors, " spare receptors " being probably involved.

Eltherington & Horita (1960) observed that after blockade of the vascular α-receptors by phenoxybenzamine, injection of the β-receptor blocking agent dichloroisoprenaline (DCI) caused a return of the adrenaline pressor response to approximately 50% of the control value. Moreira & Osswald (1965), Gulati, Gokhale & Udwadia (1965), Tuttle (1965) and Garrett, Malafaya-Baptista & Osswald (1966) confirmed this observation on the blood pressure and demonstrated that this action of DCI extended to the nictitating membrane and to the femoral blood flow; it was also observed with other β-receptor blocking agents. Although tentative explanations have been suggested, the mechanism of this phenomenon remains controversial, in view of the complexity of the cardiovascular parameters.

The same reversal was observed *in vitro* by Olivares, Smith & Aronow (1967) and Kohli & Ling (1967) on rabbit aortic strips and by Patil, Tye, May, Hetey & Miyagi

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(1968) on the rat vas deferens. These structures, however, are not preparations of choice, because aortic strips and the vas deferens have both α- and β-receptors.

The purpose of the present investigation was to study the reversal of dibenamine blockade in the guinea-pig seminal vesicle, an organ which has only α-receptors (Guimarães, 1968).

Methods

Male guinea-pigs weighing 600–900 g were killed by a sharp blow on the head and the seminal vesicles removed. Since excessive distension of the seminal vesicle prevents maximal contractions, the vesicles were allowed partly to drain through the open proximal end before suspending them in the bath. Each vesicle was placed in a 50 ml. muscle bath chamber and connected to an isotonic lever adjusted to give approximately 9-fold magnification and counter-weighted to exert 0·5–1 g resting tension. The contractions were recorded on a kymograph. The bathing fluid was oxygenated Tyrode solution at 37° C of the following composition (g/l.): NaCl 8·0 ; KCl 0·2 ; $CaCl_2$ 0·2 ; $MgCl_2$ 0·01 ; $NaHCO_3$ 1·0 ; NaH_2PO_4 0·05 ; glucose 1·0. Drugs were added by means of a 1 ml. calibrated syringe or a pipette ; the volume added never exceeded 0·5 ml. One minute after addition, the drugs were washed out by draining and refilling the bath twice with fresh Tyrode solution.

Antagonists were added to the muscle chamber before the agonist. The time of contact varied with the antagonist used: 25 min for dibenamine, 20 min for ergotamine and phentolamine and 15 min for pronethalol. In experiments in which prevention of blockade by pronethalol was studied, pronethalol ($6{\cdot}8 \times 10^{-5}$M) was added to the bath 5 min before the blocking drug.

Dose-response curves were obtained by the single dose method, with repeated washings between drug additions. Relative maximal contractions and ED50 were determined.

The power to block adrenoceptive receptors was determined as pA_2 (Schild, 1947) for surmountable antagonists and as pM_{50} for unsurmountable antagonists. pM_{50} is the negative logarithm of the molar concentration of the antagonist required to cause a 50% reduction in the amplitude of the response to the maximal effective dose of the agonist. To determine pM_{50}, dose-response curves of the agonist were obtained by the single dose method. After the maximal effective concentration was found, dibenamine was added. In preliminary trials six seminal vesicles were used ; the concentrations of dibenamine present in the baths were 1×10^{-8}M, $2{\cdot}5 \times 10^{-8}$M, 5×10^{-8}M, 1×10^{-7}M, $2{\cdot}5 \times 10^{-7}$M and 5×10^{-7}M, respectively. The results showed the range of concentrations of dibenamine required for the estimation of pM_{50}. In the final experiments two concentrations of dibenamine were used ; one concentration that reduced the response to the maximal effective dose to slightly less than 50% and one concentration that reduced the response to the maximal effective dose to slightly more than 50%. Only one concentration of dibenamine was used for each of the two seminal vesicles obtained from one guinea-pig. When the observed reduction in contraction was not sufficiently close to 50%, the experiment was discarded.

A stock solution of dibenamine (10 mg/ml.) was prepared in propylene glycol. Stock solutions of the other drugs were prepared in 0·9% NaCl solution (containing approximately 0·05 N HCl in the case of the catecholamines) and renewed once a week. All stock solutions were kept at 4° C when not in use. The catecholamine

solutions contained 0·1% sodium bisulphite as an antioxidant. Final dilutions of all drugs were made with 0·9% NaCl solution before the experiment.

Drugs used were: acetylcholine chloride (Roche), (−)-adrenaline (Höchst), barium chloride (Merck), cocaine hydrochloride (Uquipa), dibenamine hydrochloride (N,N-dibenzyl-β-chloroethylamine, Koch-Light), histamine hydrochloride (Fluka), (−)-isoprenaline (Cilag-Chemie), (−)-noradrenaline (Höchst), phentolamine hydrochloride (Ciba), (−)-phenylephrine hydrochloride (Boehringer-Sohn), pronethalol hydrochloride (I.C.I.) and reserpine phosphate (Ciba). All doses were calculated in terms of the drug base, and are expressed as molar concentrations.

Results

Effects of adrenaline, noradrenaline, phenylephrine, dopamine and isoprenaline

Concentrations of adrenaline from 1×10^{-6} to $7{\cdot}1 \times 10^{-5}$M, of noradrenaline from $2{\cdot}3 \times 10^{-6}$ to $1{\cdot}6 \times 10^{-4}$M, of phenylephrine from $4{\cdot}2 \times 10^{-6}$ to $3{\cdot}2 \times 10^{-4}$M and of dopamine from 1×10^{-5} to 1×10^{-3}M, produced concentration-dependent contractions of the guinea-pig vesicles. Dose-response curves obtained by plotting these data were parallel for adrenaline, noradrenaline and phenylephrine and their projections on the abscissa were of the order of 1·7 logarithmic units (Fig. 1).

Dopamine dose-response curves showed great variations in their slope from experiment to experiment. Isoprenaline produced no response in resting preparations in concentrations from 1×10^{-7} to 1×10^{-3}M.

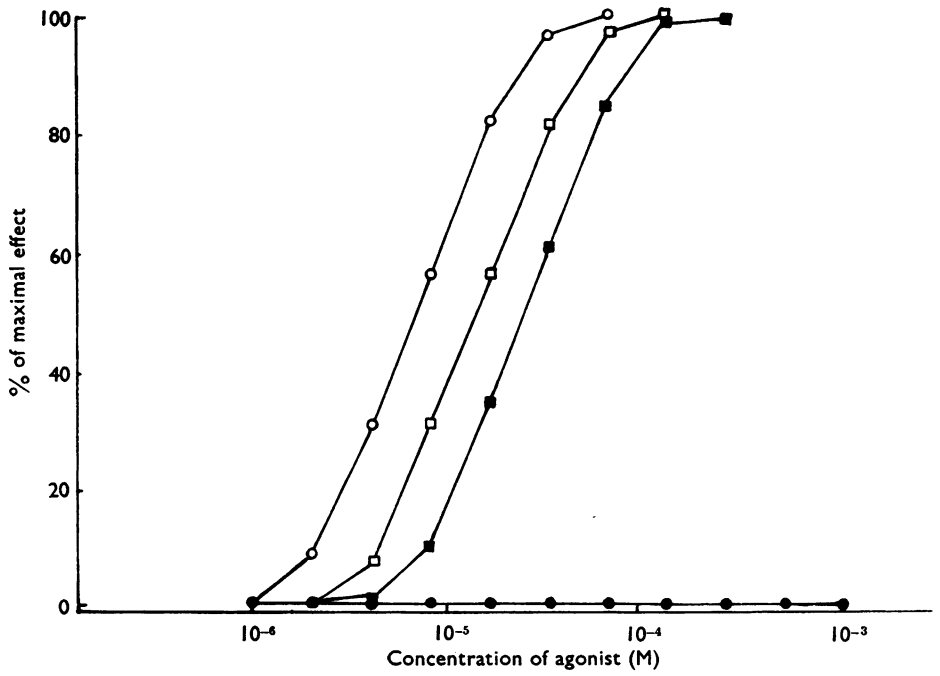

FIG. 1. Dose-response curves for adrenaline (○——○), noradrenaline (□——□), phenylephrine (■——■) and isoprenaline (●——●) on the guinea-pig isolated seminal vesicle. Ordinate, height of contractions as percentage of maximal effects; abscissa, molar concentration of the agonists. Isoprenaline had no agonistic properties; the ED50 values of adrenaline, noradrenaline and phenylephrine were $7{\cdot}1 \times 10^{-6}$, $1{\cdot}5 \times 10^{-5}$ and $2{\cdot}7 \times 10^{-5}$M, respectively.

Maximal contractions were obtained with adrenaline, noradrenaline and phenylephrine, which produced a shortening of the initial length of the preparation of about 10%.

The time which elapsed between drug addition to the bath and the beginning of the contraction was about 14·7 sec for the minimal effective concentrations of adrenaline, noradrenaline and phenylephrine and of about 5·2 sec for their maximal effective concentrations. The corresponding latencies for dopamine were about 21 and 11 sec, respectively. When the amines were removed by washing, the vesicles rapidly relaxed to the original baseline.

In a series of thirty-one experiments on seminal vesicles from eighteen guinea-pigs, the ED50 values calculated for adrenaline, noradrenaline and phenylephrine showed that the order of potency was adrenaline>noradrenaline>phenylephrine (Fig. 1).

When isoprenaline (1×10^{-7} to 1×10^{-3}M) was added to the bath during the contractions produced by exposure to sympathomimetic agonists, acetylcholine (6×10^{-6} to 5×10^{-5}M), histamine (1×10^{-5} to 1×10^{-4}M), or barium chloride (5×10^{-5} to 5×10^{-4}M), no inhibition of the contractile response was obtained. Exposure of seminal vesicles to isoprenaline (up to 1×10^{-3}M) did not reduce the sensitivity to sympathomimetic agonists.

Dopamine elicited responses with a slower rise time, a phenomenon which is observed with indirectly acting sympathomimetic amines.

Effects of α-receptor blocking agents

Dibenamine, ergotamine and phentolamine antagonized the contractor effects of adrenaline, noradrenaline, phenylephrine and dopamine; the pA_2 or the pM_{50} values calculated for each agonist were almost identical (Table 1).

Dibenamine, in concentrations ranging from 4×10^{-8} to 3×10^{-7}M, antagonized the contractor effects of adrenaline, noradrenaline, phenylephrine and dopamine, but the maximal contractor effect of these agonists returned when the agonist concentration was increased. In concentrations from 3×10^{-7} to 3×10^{-6}M, dibenamine produced a concentration-dependent unsurmountable depression of the maximal contractor effects of adrenaline, noradrenaline, phenylephrine and dopamine. In these concentrations dibenamine also antagonized histamine; the pM_{50} value was $6{\cdot}74 \pm 0{\cdot}12$ ($n=4$). Contractile responses to acetylcholine were not depressed by dibenamine (up to 5×10^{-6}M).

Ergotamine (5×10^{-9} to $3{\cdot}4 \times 10^{-6}$M) antagonized all sympathomimetic amines; it had the highest pA_2 value (Table 1). There was a parallel shift of the dose-response curves to the right. The lowest doses of ergotamine were easily washed

TABLE 1. *Antagonism by α-receptor blocking agents of the contractor effects of sympathomimetic amines*

Agonist	Dibenamine	Ergotamine	Phentolamine
	pM_{50} ± S.E.M.	pA_2 ± S.E.M.	pA_2 ± S.E.M.
Adrenaline	7·15±0·06 (5)	8·13±0·07 (6)	7·07±0·03 (7)
Noradrenaline	7·26±0·05 (8)	8·10±0·08 (4)	7·18±0·04 (5)
Phenylephrine	7·22±0·07 (5)	8·06±0·05 (6)	7·12±0·06 (4)

The numbers in parentheses refer to the number of experiments from which the means were calculated. S.E.M. = Standard error of the mean.

out, but this was not the case with high concentrations. Phentolamine (5×10^{-8} to 2×10^{-5}M) also produced a parallel shift of the dose-response curves to the right.

Effects of pronethalol on the responses to sympathomimetic amines

In concentrations ranging between $4{\cdot}4 \times 10^{-8}$ and $1{\cdot}7 \times 10^{-5}$M, pronethalol did not affect the contractions caused by adrenaline, noradrenaline, phenylephrine and

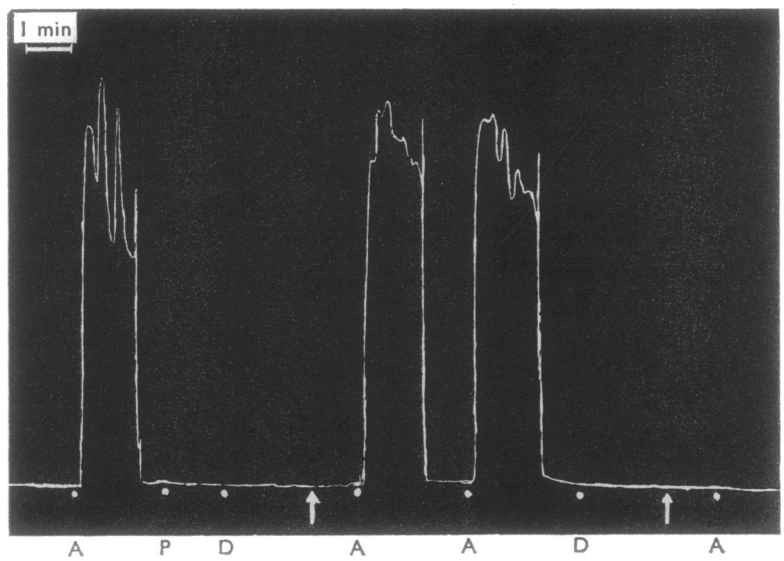

FIG. 2. Guinea-pig seminal vesicle. Effects of (A), adrenaline ($3{\cdot}6 \times 10^{-5}$M); (P), pronethalol ($6{\cdot}8 \times 10^{-5}$M); (D), dibenamine ($1{\cdot}5 \times 10^{-7}$M). At the arrows, washout. The addition of pronethalol to the bath 5 min before the addition of dibenamine prevented the α-receptor blocking action of dibenamine which was kept in the bath for 25 min and washed out 2 min before the addition of adrenaline.

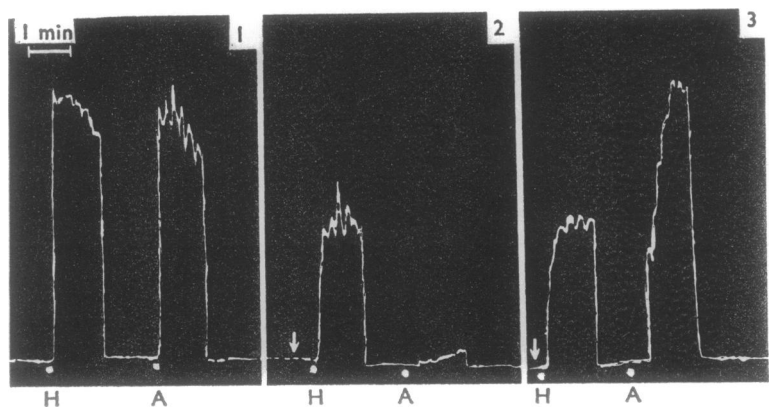

FIG. 3. Guinea-pig seminal vesicle. Effects of (H), histamine (2×10^{-5}M); (A), adrenaline ($3{\cdot}6 \times 10^{-5}$M). Between 1 and 2, dibenamine ($1{\cdot}5 \times 10^{-7}$M) was added to the bath and washed out after 25 min. Between 2 and 3, pronethalol ($6{\cdot}8 \times 10^{-5}$M) was added to the bath and washed out after 15 min. At the arrows, washout.

dopamine, but in higher concentrations, pronethalol depressed the contractile responses elicited by sympathomimetic amines, as well as those elicited by histamine. This blockade was, however, very transient. Concentrations of pronethalol larger than 1×10^{-4}M caused small and irregular contractions and a marked decrease in the sensitivity to sympathomimetic agonists. Potentiation of the effects of adrenaline, noradrenaline, phenylephrine or dopamine by pronethalol was not observed.

Effect of pronethalol on the blockade by the α-receptor blocking agents

Previous exposure of the preparations to pronethalol ($6{\cdot}8 \times 10^{-5}$M) protected the adrenoceptive receptors from blockade by dibenamine (Fig. 2). When pronethalol was added after α-receptor blockade had developed, reversal of this blockade was observed. The preparations were again responsive to adrenaline, noradrenaline and phenylephrine, but not to histamine (Fig. 3). Similar results were obtained with ergotamine or phentolamine as α-blocking agents. The dibenamine blockade was reversed by pronethalol only when the concentration of dibenamine was not greater than 3×10^{-7}M.

Effects of reserpine pretreatment

Intramuscular injection of reserpine (3 mg/kg) 24 hr before the experiment did not significantly change the responses of the seminal vesicles to adrenaline, noradrenaline and phenylephrine, but abolished or reduced the actions of dopamine. The influence of pronethalol on the blockade caused by dibenamine, ergotamine and phentolamine was not modified by reserpine pretreatment.

Effects of cocaine

Cocaine (10^{-6} to 10^{-5}M) caused a potentiation of the contractions due to adrenaline and noradrenaline; a maximal contraction was produced by one-third of the concentration which was required for maximal contraction before the addition of cocaine. The effect of phenylephrine was less enhanced and that of dopamine was depressed by cocaine. Cocaine did not alter the reversal by pronethalol of the α-receptor blockade caused by dibenamine, ergotamine and phentolamine.

Discussion

Adrenaline, noradrenaline, phenylephrine and dopamine produced concentration-dependent contractions of guinea-pig isolated seminal vesicles. Calculations of the ED50 values from the dose-response curves showed the order of potency to be: adrenaline>noradrenaline>phenylephrine. This order of potency agrees with that suggested by Ahlquist (1948) for the activation of α-adrenoceptive receptors. Furthermore, three different α-receptor blocking agents inhibited the contractile responses elicited by the sympathomimetic amines. The pA_2 and pM_{50} values calculated for each agonist were of the same order of magnitude and supply additional evidence that the sympathomimetic agents are activating identical receptors (Schild, 1947; Arunlakshana & Schild, 1959).

Isoprenaline, in a large range of concentrations, did not have relaxing effects, either in resting preparations or in preparations previously contracted by sympathomimetic amines, histamine or acetylcholine; moreover, the β-receptor blocking drug, pronethalol, did not affect the contractions induced by adrenaline. These facts con-

firm the lack of β-adrenoceptive receptors in the guinea-pig seminal vesicles (Guimarães, 1968). Similar results were obtained by Clark, Lish & Dungan (1961) in the rat seminal vesicle.

The reversal by pronethalol of the fall in blood pressure caused by adrenaline after blockade with dibenamine and phenoxybenzamine has not been satisfactorily explained. Gulati *et al.* (1965) ascribed the reappearance of the pressor action of adrenaline and noradrenaline to a direct reversal of the blocking action of pronethalol on α-receptors occupied by phenoxybenzamine, while Moreira & Osswald (1965) and Garrett *et al.* (1966) suggested that the reversal was due to the occupation of unoccupied α-receptors by adrenaline diverted from the β-receptors by the blocking effects of pronethalol. These interpretations were based on results obtained by measurement of arterial blood pressure which may be influenced by multiple factors. The results presented by Olivares *et al.* (1967), Kohli & Ling (1967) and Patil *et al.* (1968) were based on experiments with sympathomimetic agonists on structures possessing both α- and β-receptors.

Our results, obtained on the guinea-pig isolated seminal vesicle, an organ without β-receptors, show: (1) that the reversal phenomenon is also evident in this tissue and does not depend on a blockade of the β-receptors ; (2) that the reversal is not based on chemical antagonism of dibenamine by pronethalol, because it occurs not only with dibenamine but also with ergotamine and phentolamine ; (3) that the reversal is specific for sympathomimetic drugs, since histamine blockade by dibenamine is not antagonized ; (4) that the reversal is not dependent on storage sites and storage mechanisms, since reserpine pretreatment and cocaine has no influence on its development ; (5) that the reversal is probably dependent on an action on α-receptors, since only high concentrations of pronethalol, which produce transient α-blockade, are able to produce this reversal.

Since the reversal was obtained only with concentrations of dibenamine which cause a parallel shift of the dose-effect curves to the right and was not observed in the presence of higher concentrations of dibenamine, which produce unsurmountable antagonism, one could speculate, as Garrett *et al.* (1966) have done, that " spare receptors " are involved in the reversal phenomenon.

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