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Research Papers

The nature of the adrenergic receptors of the trachea of the guinea-pig

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The adrenergic receptors of the guinea-pig isolated trachea have been characterised as β-receptors by established criteria. No evidence was obtained that any α-receptors are present. (−)-Isoprenaline was 17 times more potent than (−)-adrenaline which was 10 times more potent than (−)-noradrenaline. High concentrations of piperoxan, thymoxamine and dihydrogenated ergot alkaloids did not antagonise the catecholamines, while phentolamine and phenoxybenzamine potentiated them, isoprenaline almost as much as noradrenaline. Propranolol, pronethalol and the 3,4-dichloro-analogues of (±)-noradrenaline, adrenaline and isoprenaline each specifically antagonised the catecholamines, isoprenaline moreso than noradrenaline. The characteristics of this blockade by pronethalol and propranolol fulfilled established criteria for competitive antagonism; propranolol (pA_2 against noradrenaline 6·56 ± 0·21) was 18·6 (11·4 to 30·5) times more potent than pronethalol, (pA_2 against noradrenaline 5·29 ± 0·07).

MOST analyses of the actions of sympathomimetics, and of the interactions of this group of drugs with others, have been made on tissues with α-receptors. The neglect of the β-receptor may stem from the lack of a convenient and reliable test tissue.

Quantitative studies on the catecholamines are made difficult if the drugs have two opposite actions on the test tissue, or if they interact with two types of receptor. Such complexities have been shown to exist in intestinal muscle. Axelsson, Bueding & Bulbring (1961) found adrenaline to have both inhibitory and excitatory actions on the electrical activity of the guinea-pig taenia coli. Ahlquist & Levy (1959), Furchgott (1960) and Wilson (1964) have each presented evidence for the presence of both α- and β-adrenergic receptors in the small intestine of the dog, rabbit and guinea-pig respectively; in each animal, interaction between catecholamines and either α- or β-receptors produced inhibition of the intestine. An investigation of the adrenergic receptors of the bronchioles of the anaesthetised dog by Castro de la Mata, Penna & Aviado (1962) showed that sympathomimetic bronchodilation was subserved by β-receptors but that α-receptors subserving bronchoconstriction were present and could be revealed by β-receptor blockade with dichloroisoprenaline.

The isolated paired tracheal chain preparation of the guinea-pig, described by Foster (1960) as a development of the preparations of Castillo & de Beer (1947) and Akcasu (1952) would provide a convenient and reliable test tissue for pharmacological analyses if it were shown to be equipped with β-receptors only.

Experimental

METHODS

Pairs of tracheal chain preparations (Foster, 1960) were suspended in identical jacketed organ baths containing 5 ml of Krebs solution at 37·5°,

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bubbled with 95% oxygen and 5% carbon dioxide. Relaxations were recorded on smoked paper with isotonic balanced balsa wood levers, magnifying 20 times; the tissue supported a load of 200 to 240 mg. The load was removed from the tissue while it was washed, and replaced 5 min before the next drug addition. Washing was effected by displacement of the bath fluid with fresh pre-warmed and pre-bubbled Krebs solution. Drug-induced relaxant responses were recorded for 15 min after which 25 min of washing at 5 min intervals was necessary for recovery of full inherent tone.

(+)-Ascorbic acid, 200 μg/ml, was always included in the Krebs solution to delay catecholamine autoxidation.

All experiments were designed to yield quantitative information. They consisted of repeated recording of the log concentration: effect curves of one or more catecholamines before and after exposure of the tissue to α- or β-blocking agents. Two methods were used to record the log concentration:effect curve; the normal sequential method in which the tissue was washed to recovery between each dose of agonist, and the cumulative method of Ariëns & de Groot (1954) in which the concentration of agonist in the bath was increased fourfold every 15 min without washing. After recording the first relaxation fully only the last 7 min of each accumulated relaxation was recorded.

Near the beginning and end of each experiment a maximum relaxation was produced by addition of a suitable dose of either aminophylline or a catecholamine. These drugs produced equal maximum relaxations. Relaxations produced by drugs were expressed as a proportion (%) of this maximum possible relaxation; doses as final bath concentration of base.

DRUGS

Drugs used were: (–)-adrenaline bitartrate, aminophylline (theophylline ethylenediamine dihydrate), (+)-ascorbic acid, (±)-dichloroadrenaline hydrochloride, (±)-dichloroisoprenaline hydrochloride, (±)-dichloronoradrenaline hydrochloride, dihydroergotamine ethanesulphonate, Hydergine (a mixture of equal parts of the methanesulphonates of dihydroergocornine, dihydroergocristine and dihydroergokryptine), (–)-isoprenaline bitartrate dihydrate, (–)-noradrenaline bitartrate monohydrate, papaverine hydrochloride, phenoxybenzamine hydrochloride, phentolamine hydrochloride, piperoxan hydrochloride, pronethalol hydrochloride, propranolol hydrochloride, sodium nitrite, thymoxamine hydrochloride.

Results

JUSTIFICATION OF CUMULATIVE METHOD FOR CONSTRUCTION OF LOG CONCENTRATION: EFFECT CURVES

Log concentration: effect curves for either noradrenaline or isoprenaline were constructed by both the sequential and cumulative methods on each member of a pair of tracheal chain preparations. In this way comparison of the curves obtained could be made both within and between members. Fig. 1 illustrates and compares the methods and shows the similarity of

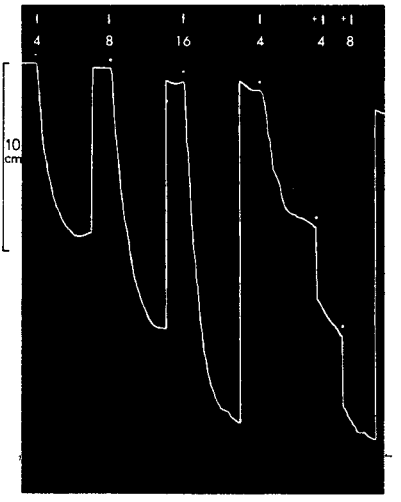

FIG. 1. The method used in recording sequential and cumulative log concentration: effect curves for isoprenaline(I). The figures refer to the final bath concentration in ng/ml. The sequential method involves three 15 min records of drug-induced relaxations, each followed by 25 min of washing. The cumulative method involves three 15 min relaxations; the first is recorded in full, the drum is switched off for the first 8 min of each accumulated relaxation to gain end-point definition. 25 min of washing restores full inherent tone. A frontal writing point was used in making this record.

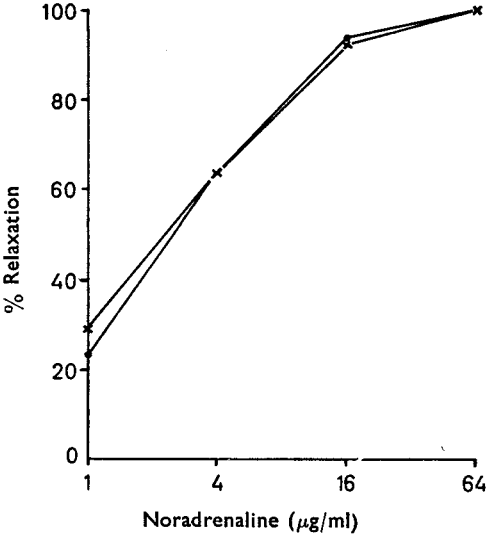

FIG. 2. Comparison of log concentration: effect curves for noradrenaline obtained with the paired tracheal chain preparation. Percentage relaxation is plotted against final bath concentration of noradrenaline (in μg/ml) on a log scale. ●——● = sequential method on one member, ×——× = cumulative method on the other member of the pair of preparations.

responses obtained by the two methods to twofold increases in concentration of isoprenaline. Fig. 2 compares the curves for noradrenaline obtained by both methods over a wider concentration range between members. No systematic difference between the methods was found in either the slope of the curve or the ED50 for either agonist. Small differences were apparent but, whether assessed cumulatively or sequentially, noradrenaline was as often more potent as it was less; the small differences were also more apparent within, than between, chains (due to slight changes in sensitivity which occur with lapse of time), and were similar in magnitude to spontaneous changes which occur when log concentration: effect curves are repeated on the same pair of preparations by the sequential method only (the maximum observed difference in ED50 was 0·22 log units).

RELATIVE POTENCY OF CATECHOLAMINES

(–)-Noradrenaline, (–)-adrenaline and (–)-isoprenaline are powerful relaxants of the guinea-pig isolated tracheal chain. If an adequate concentration is applied each can evoke the maximum relaxation of which the preparation is capable. If smaller concentrations are applied, a steep sigmoid relationship can be demonstrated between the logarithm of the concentration and the percentage relaxation produced. The mid portions of the log concentration: effect curves of these three catecholamines do not differ significantly in slope. There is a non-significant ($0{\cdot}1 < P < 0{\cdot}25$) tendency for the slope of isoprenaline to be less than that of noradrenaline. The logarithms of the concentrations of (–)-noradrenaline producing a 50% relaxation on 68 different preparations were normally distributed as shown by equality of the mean, the median and mode. The relative molar potencies of these three catecholamines are shown in Table 1, and compared with previously reported values.

TABLE 1. MOLAR POTENCIES OF CATECHOLAMINES RELATIVE TO (−)-NORADRENALINE

(−)-Isoprenaline	(−)-Adrenaline	(−)-Noradrenaline	Reference
280	8·1	1	Hawkins (1952)
32	4·6	1	McDougall & West (1953)
—	11·0	1	Lu & Allmark (1954)
—	15·0	1	Lu & Allmark (1954)
174	10·2	1	Present work with range of standard error.
151–200	9·1–11·5		

Note.—Racemic drugs are assumed to show half the activity of the (−)-form. Standard errors are not equal above and below the mean because they are converted from a logarithmic scale.

There is fair, though not excellent, agreement between these values for the relative potencies of the catecholamines. All agree that (–)-isoprenaline is more potent than (–)-adrenaline and that this is more potent than (–)-noradrenaline.

EFFECTS OF α-BLOCKING AGENTS

Piperoxan in concentrations of 10 to 80 μg/ml, produced a small contraction of the tracheal muscle but no change in its sensitivity to noradrenaline.

Thymoxamine in concentrations of 1 to 64 μg/ml caused a very small (less than $\times 2$) potentiation of noradrenaline. 64 μg/ml also caused a small contraction of the tracheal muscle; lower concentrations did not change its tone.

Dihydrogenated ergot alkaloids. Dihydroergotamine, 2 μg/ml, had no effect on either tone or sensitivity to noradrenaline. Hydergine at 6 to 14 μg/ml had no effect on tone but caused a very small potentiation of noradrenaline. 40 μg/ml caused a small ($\times$ 2) potentiation of noradrenaline without changing tone.

Phentolamine. Concentrations of 5 to 80 μg/ml caused a dose-dependent and large ($\times 20$ at 40 μg/ml) potentiation of noradrenaline. Concentrations of 10 to 80 μg/ml caused a dose-dependent and medium ($\times$ 7 at 40 μg/ml) potentiation of isoprenaline. 80 μg/ml caused a small relaxation of the tracheal muscle; lower concentrations did not change its tone.

Phenoxybenzamine in concentrations of 0·4 to 2 μg/ml caused a slowly-developing and large potentiation of all three catecholamines; noradrenaline was potentiated most. There was no change in tone.

EFFECTS OF β-BLOCKING AGENTS

The dichloro-analogues of isoprenaline, adrenaline and noradrenaline, and pronethalol and propranolol each antagonised the catecholamines and did not antagonise aminophylline, sodium nitrite or papaverine at the concentrations tested.

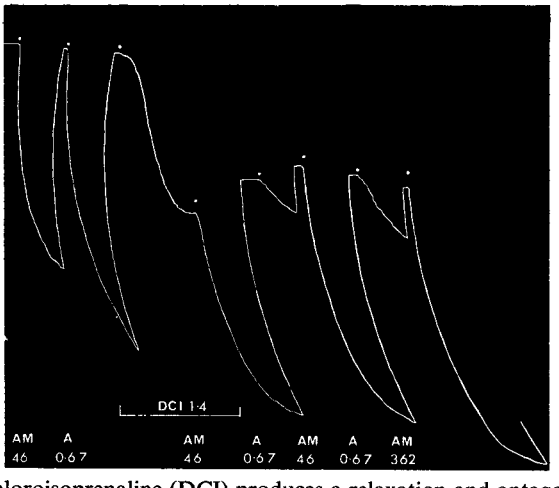

FIG. 3. Dichloroisoprenaline (DCI) produces a relaxation and antagonises adrenaline (A). Aminophylline (AM) is not antagonized. Both the relaxation and antagonism produced by dichloroisoprenaline persist after it is washed from the bath. Concentrations are in μg/ml. A side writing point was used in making this record.

Dichloroisoprenaline was most unsatisfactory because in concentrations of 0·2 to 20 μg/ml it caused a relaxation of the trachea. This relaxation was never maximal but did not seem to be dose-dependent and might

range from 10 to 80% of the existing tone; it also proved slow to wash out. Concentrations of 1 to 20 μg/ml, applied for 35 min, blocked the actions of catecholamines for several hours by an amount which increased with the concentration of dichloroisoprenaline used. This blockade was surmountable and log concentration: effect curves for each catecholamine before and after the blocking agent were parallel. Fig. 3 shows the relaxation and antagonism of adrenaline caused by 1·4 μg/ml of dichloroisoprenaline without antagonism of aminophylline.

Isoprenaline was antagonised more than noradrenaline.

Dichloroadrenaline. This was qualitatively similar to dichloroisoprenaline but less potent.

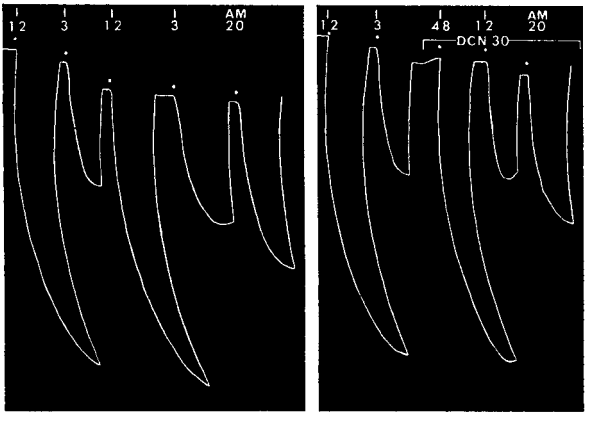

FIG. 4. Dichloronoradrenaline (DCN) antagonises isoprenaline(I) without relaxing the tracheal chain. Aminophylline (AM) is not antagonised. Note the very similar behaviour of the two members of this paired preparation—the left is used as a control to measure any spontaneous changes in drug sensitivity which may occur. A side writing point was used in making this record. Concentrations are in ng/ml for isoprenaline and in μg/ml for aminophylline and dichloronoradrenaline.

Dichloronoradrenaline was less potent still but had the advantage that a concentration could be found (about 40 μg/ml) which antagonised the catecholamines without causing a relaxation. Fig. 4 shows the antagonism of isoprenaline by dichloronoradrenaline without antagonism of aminophylline.

Propranolol in concentrations of 0·0025 to 20 μg/ml caused a dose dependent antagonism of the catecholamines without changing the tone. Catecholamine log concentration: effect curves were parallel before and after propranolol antagonism. This antagonism increased in size, if the propranolol was maintained in the bath, over several hours. It was thus impossible to perform experiments at a true equilibrium. Fig. 5 shows the effect on the adrenaline log concentration: effect curve of increasing concentrations of propranolol added 30 min before each cumulative noradrenaline challenge. Fig. 6 shows the results of six similar experiments with noradrenaline plotted after Arunlakshana & Schild (1959)—logarithm (noradrenaline dose ratio — 1) against negative

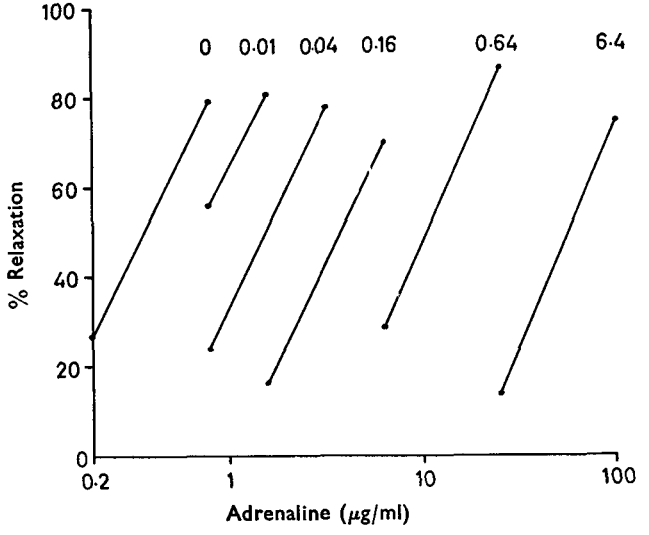

FIG. 5. Log concentration: effect lines for adrenaline obtained on the same preparation. The effect of increasing concentrations of propranolol (in μg/ml) added 30 min beforehand is shown. Propranolol causes a concentration-dependent parallel shift to the right of the adrenaline log concentration: effect line over a 640-fold range of increasing concentration.

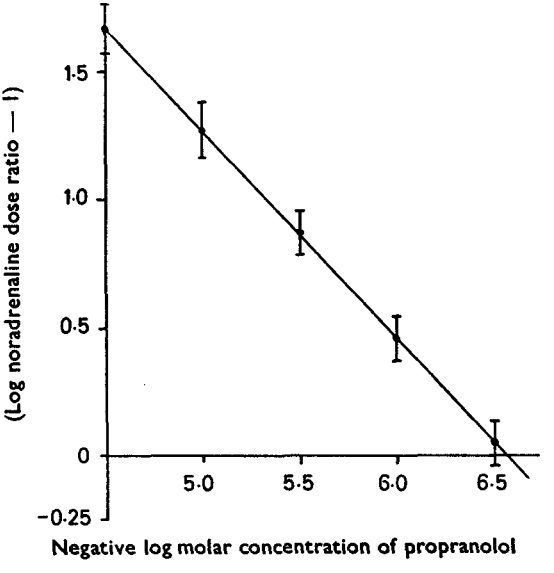

FIG. 6. Plot of log (noradrenaline dose ratio minus one) against negative log molar concentration of propranolol. Each point (with its standard error) is a mean derived from six experiments similar to that illustrated in Fig. 5 but using noradrenaline as agonist. Note that the points lie on a straight line over a 100-fold range of antagonist concentration. $pA_2 = 6{\cdot}56$; $pA_2 - pA_{10} = 1{\cdot}16$.

logarithm of molar concentration of propranolol. The mean pA_2 with standard error was 6·56 ± 0·21 and the mean $pA_2 - pA_{10}$ with standard error was 1·16 ± 0·05.

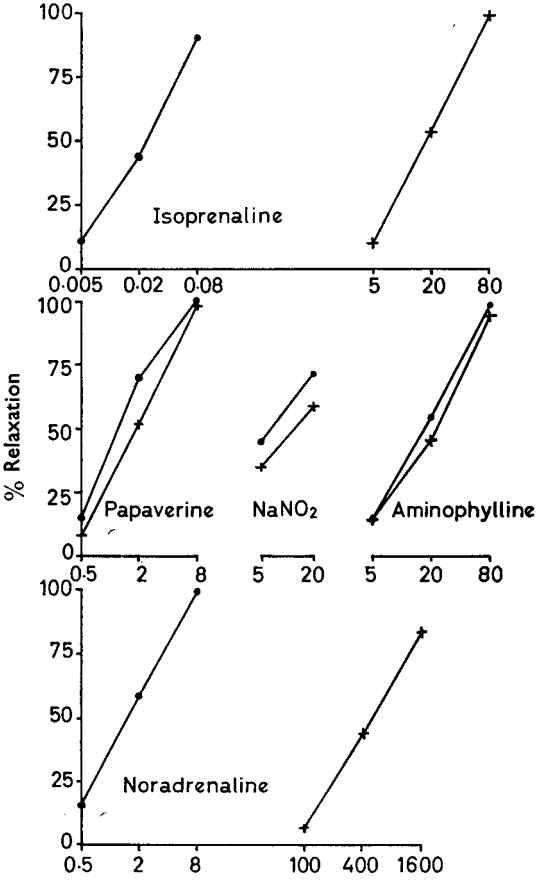

FIG. 7. Specificity of propranolol. Log concentration: effect curves obtained with five agonists on each member of the same paired preparation. One member (●———●) was bathed in normal Krebs solution, the other (+———+) was continuously exposed to propranolol, 20 μg/ml, the highest concentration used. Papaverine, sodium nitrite and aminophylline are insignificantly affected; noradrenaline is blocked by a factor of 320, isoprenaline by a factor of 780. All concentrations are expressed in μg/ml on the same logarithmic scale.

Both isoprenaline and adrenaline were blocked much more than noradrenaline by the same concentration of propranolol. This difference emerged from 3 types of experiment. (1) A concentration of propranolol could be found (about 12·5 ng/ml) which would significantly antagonise isoprenaline without antagonising noradrenaline. (2) When tested on the same preparation any one concentration of propranolol always antagonized isoprenaline more than noradrenaline (see for example

Fig. 7). (3) Single pA_2 values determined as in Figs 5 and 6 were larger for isoprenaline and adrenaline than for noradrenaline.

Fig. 7 shows the specificity of propranolol. One member of a pair of tracheal chains was continuously exposed to the β-blocking agent in very high concentration and cumulative log concentration:effect curves for sodium nitrite, aminophylline, isoprenaline, noradrenaline, and papaverine were determined on each. Only the catecholamines are antagonised and isoprenaline more than noradrenaline.

Attempts to wash the propranolol from the tissues were only partially successful even though continued for many hours.

Pronethalol was qualitatively very similar to propranolol. It was less potent by a factor (with range of standard error) of 18·6 (11·4 to 30·5). Its reported (Black & Stephenson, 1962) sympathomimetic action was only occasionally observed and is certainly much less of a problem than with dichloroisoprenaline.

Five determinations of the pA_2 of pronethalol against noradrenaline gave a mean value, with standard error, of $5{\cdot}29 \pm 0{\cdot}07$. The $pA_2 - pA_{10}$ was $1{\cdot}03 \pm 0{\cdot}07$.

Discussion

The time cycle of 40 min necessary for the tracheal chain preparation when drug potency is assessed by the sequential method is inordinately long compared with that applicable to most α-receptor containing tissues. It is effectively halved by the use of the paired preparation, since the two members forming the pair behave identically (Foster, 1960); even so, the amount of information obtainable in one day is limited. The use of the cumulative method reduces the effective time cycle still further by requiring only one 25-min wash period after every two or three drug additions. That the method is valid is indicated by the close similarity of log concentration:effect curves produced by it to those produced by the sequential method.

Analysis of the nature of the adrenergic receptors in any tissue is based on two fundamental experiments: the relative potency of catecholamines (Ahlquist, 1948) and the identity of specific blocking agents.

The decending order of potency of the catecholamines on the trachea is isoprenaline, adrenaline and noradrenaline. This is highly suggestive of interaction with β-receptors and this suggestion is strengthened by the sizes of their potency differences.

Even after very large concentrations of β-blocking agents (e.g. 20 μg/ml of propranolol) noradrenaline has never caused a contraction of the trachea such as described by Castro de la Mata & others (1962) on dog bronchioles *in vivo*.

Piperoxan, thymoxamine and the dihydrogenated ergot alkaloids have no significant actions on the trachea in concentrations many times larger than those which cause antagonism of noradrenaline on recognised α-receptor containing isolated tissues (Leitch, Liebig & Haley, 1954; Paterson, 1965). For instance, on the guinea-pig isolated vas deferens,

appreciable antagonism to noradrenaline (pA_2) was seen with 0·08 μg/ml, 0·008 μg/ml and 0·004 μg/ml of piperoxan, thymoxamine and dihydroergotamine respectively (Leach, 1956; Birmingham & Szolcsányi, 1965). Phentolamine and phenoxybenzamine, again in concentrations much greater than those necessary for α-blockade, produce a potentiation not only of noradrenaline but also of isoprenaline; this potentiating action is thus almost certainly unrelated to α-receptor blockade and has been observed on a variety of other tissues equipped with β-receptors. Holzbauer & Vogt (1955) found that phenoxybenzamine, 0·2 μg/ml, potentiated adrenaline and isoprenaline on the rat isolated uterus. Huković (1959) observed an increase in the effect of adrenergic nerve stimulation on the isolated rabbit atria with phenoxybenzamine, 25 μg/ml. Benfey & Greeff (1961) used isolated guinea-pig atria and showed that both phentolamine, 17 μg/ml, and phenoxybenzamine, 25 μg/ml, potentiated noradrenaline.

Direct evidence has recently become available which attributes this potentiating action of phenoxybenzamine to inhibition of noradrenaline uptake by the storage mechanism. Hertting, Axelrod & Whitby (1961) found that phenoxybenzamine markedly reduced the uptake of intravenous ^{3}H-noradrenaline into the heart, spleen and adrenal of the cat but phentolamine did not do this. Farrant, Harvey & Pennefather (1964) have shown that phenoxybenzamine blocks the uptake of noradrenaline into the stores of the cat kidney (but not the uterus) and rat heart, spleen and uterus. Iversen (1965b), working with the rat isolated perfused heart, found phenoxybenzamine, 10 μg/ml, to reduce the uptake of ^{3}H-noradrenaline by 92% and phentolamine, 10 μg/ml, to reduce it by 66%.

The present results were that phenoxybenzamine and phentolamine potentiated isoprenaline quite markedly, though less than noradrenaline. The uptake of noradrenaline into stores is greater than that of adrenaline (Iversen & Whitby, 1962; Iversen, 1965a). Isoprenaline has been ignored until recently. Evidence is appearing that there are two distinct uptake mechanisms. Uptake I operates at low catecholamine concentrations, has a greater affinity for noradrenaline than adrenaline and very little for isoprenaline; it is blocked by cocaine and desipramine (Iversen, 1963, 1965a). Uptake II operates at high catecholamine concentrations and is blocked by metanephrine (Iversen, 1965c); it does accumulate isoprenaline (Callingham, 1965). On this basis it would seem reasonable to suppose that phenoxybenzamine and phentolamine block uptake II in the guinea-pig trachea, since both potentiate isoprenaline. This point will be investigated further.

Arunlakshana & Schild (1959) have stated that "If two agonists act on the same receptors they can be expected to be antagonised by the same antagonist, and, if the antagonism is competitive, they can be expected to be antagonised by the same concentration of antagonist and to produce with it the same pA_x or dose ratio". While this is theoretically true if the competitive antagonist has only this action, quantitative work has demonstrated that various α-blocking agents antagonise noradrenaline

more than adrenaline on the guinea-pig isolated vas deferens (Leach, 1956).

A similar difference in the degree of antagonism to noradrenaline, adrenaline and isoprenaline is here reported for dichloroisoprenaline, pronethalol and propranolol and the question arises whether a different receptor is involved for each agonist. Dichloroisoprenaline and pronethalol are other agents known to block the uptake of noradrenaline into tissue stores. Muscholl (1961) found that dichloroisoprenaline completely blocked the uptake of noradrenaline into the rat heart and spleen. Farrant & others (1964) found it to block the uptake of noradrenaline into the cat uterus (but not the kidney); Iversen (1965b) has shown that dichloroisoprenaline, 2 μg/ml, and pronethalol, 5 μg/ml, reduced the uptake of ^{3}H-noradrenaline into the rat isolated perfused heart by 51% and 36% respectively. It is suggested that propranolol will be found to share this property and that this is the basis for the differential antagonism of β-blocking agents towards the catecholamines. It seems likely that the catecholamines are all blocked to the same extent at the receptor level but that at the same time noradrenaline is potentiated: this potentiation is "hidden" but serves to reduce the degree of antagonism experimentally observed.

The present work confirms the potency difference of about ten noted by Black, Crowther, Shanks & Dornhorst (1964) between propranolol and pronethalol and extends their basic pharmacology by demonstrating the competitive nature of their antagonism. The evidence for this may be summarised.

1. Over a 100-fold range of concentration propranolol or pronethalol causes a progressive parallel rightward shift of the log concentration: effect curve of each catecholamine. The maximum relaxation is not reduced.

2. Over the same range of concentration for propranolol or pronethalol there is a linear relation between the dose ratio of agonist minus one and the concentration of antagonist. The mean $pA_2 - pA_{10}$ values of $1{\cdot}16 \pm 0{\cdot}05$ and $1{\cdot}03 \pm 0{\cdot}07$ were in acceptable agreement with the theoretical value of 0·95 for competitive antagonism.

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The work on the relative potency of catecholamines, and the actions of their dichloro-analogues, was performed in the Pharmacology Department, King's College, London, and formed part of a thesis which has been accepted for the degree of Ph.D. in the University of London.

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