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Actions of phenylephrine on β-adrenoceptors in guinea-pig trachea

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1. Phenylephrine produced relaxation of the isolated guinea-pig tracheal chain preparation, its potency being 1/5 that of noradrenaline on normal tissues.

2. The potentiation of phenylephrine by cocaine (10^{-5}M) was only slight. Thus on cocaine-treated tissues phenylephrine was 1/45 as potent as noradrenaline.

3. The dose-response lines to phenylephrine were shifted in a parallel manner by propranolol 10^{-8}M and 10^{-7}M, suggesting that the relaxations were mediated through β-adrenoceptors.

4. Phenylephrine had a lower intrinsic activity than the catecholamines and produced multiphasic dose-response lines at the higher doses used in the presence of propranolol (10^{-6}M). These observations have been explained by the evidence obtained that phenylephrine is a partial agonist with β-adrenoceptor blocking activity.

5. From experiments using α-adrenoceptor blocking drugs, it has been concluded that stimulation of α-adrenoceptors has little influence on the β-adrenoceptor relaxation to phenylephrine on the guinea-pig tracheal chain preparation.

The present study of phenylephrine, a drug which is considered to be predominantly an α-adrenoceptor stimulant (Ahlquist & Levy, 1959), was undertaken to estimate its potency on the β-adrenoceptors of guinea-pig trachea. The influence which both loss of phenylephrine into adrenergic nerve terminals and stimulation of α-adrenoceptors might have on its potency has been considered. The affinity of phenylephrine for uptake into adrenergic nerves has been assessed from experiments using cocaine. Although Foster (1966) could find no evidence for the existence of α-adrenoceptors in the guinea-pig trachea, other workers believe that they might be present and when stimulated result in contraction (Chahl & O'Donnell, 1967 ; Takagi, Osada, Takayanagi & Taga, 1967 ; Everitt & Cairncross, 1969). The effects of α-adrenoceptor blocking drugs as well as of propranolol on the response of guinea-pig trachea to phenylephrine are therefore described.

A preliminary report of the results was presented to the eleventh meeting of the Australian Physiological and Pharmacological Society (Chahl & O'Donnell, 1968).

Methods

Isolated guinea-pig tracheal chain preparation

Relaxations of tracheal chain preparations from adult female guinea-pigs, weighing between 500 and 600 g, were recorded as described by Chahl & O'Donnell (1967) using a Statham 10B strain gauge modified to record isotonically. Preparations containing four tracheal rings were used and in some experiments the cartilaginous section of the ring was cut. The preparations were suspended in Krebs bicarbonate solution containing ascorbic acid 200 μg/ml. Cumulative dose-response curves to phenylephrine were obtained using the method of Van Rossum (1963). Since phenylephrine did not always produce the same maximum response of the tissue as the catecholamines, a maximum response of the tissue to adrenaline, noradrenaline or isoprenaline was obtained at the end of each cumulative dose-response curve. The effect of propranolol on responses of the tissue was tested after contact for 1 hr with the trachea, and of cocaine after contact for 0·5 hr. Cocaine and/or propranolol were replaced in the bath immediately after each washout.

Cocaine and propranolol experiments

The following experimental designs were used:

(1) To compare the effect of cocaine (10^{-5}M) on phenylephrine and isoprenaline, seven experiments, each with two preparations from the same animal (paired preparations), were set up. On one preparation, normal dose-response curves to phenylephrine were repeated until no change in sensitivity of the tissue was observed. A further dose-response curve in the presence of cocaine was then obtained. A similar procedure using isoprenaline was carried out on the other preparation.

(2) To compare the degree of block of phenylephrine by propranolol with that of isoprenaline, five paired preparations were set up. On one preparation a normal dose-response line to phenylephrine was obtained. Dose-response lines to phenylephrine were then obtained in the presence of 10^{-8}, 10^{-7} and 10^{-6}M propranolol. A similar procedure using isoprenaline was carried out on the other preparation. Results for phenylephrine from three other experiments of a similar design have been included.

In these two sets of experiments the dose-response lines obtained were drawn by eye and individual experiments analysed. In addition, to obtain the mean log ED50s (where ED50 is the dose (molar concentration) producing 50% maximum relaxation) and their standard deviations for phenylephrine on normal and on cocaine-treated tissues, the results from the first and second series of experiments were combined with those obtained from other preliminary experiments and the mean log ED50s calculated and compared by the methods described by Chahl & O'Donnell (1967). All mean values in the text are given together with the standard deviation.

Experiments using α-adrenoceptor blocking drugs

The effects of tolazoline (0·01 mg/ml.) left in the bath for 3 min or phenoxybenzamine (10^{-5}M) for 0·5 hr were observed on the responses (contractions) to adrenaline or phenylephrine in the presence of 10^{-6}M or 6×10^{-6}M propranolol which had been in contact with the tissue for 1 hr previously.

The effect of phenoxybenzamine (10^{-5}M) left in the bath for at least 0·5 hr was also examined on the cumulative relaxant dose-response lines to phenylephrine in the presence and absence of propranolol (10^{-8}M or 10^{-6}M). At the completion of all dose-response lines to phenylephrine, a maximum response to isoprenaline was obtained. All lines were plotted by eye.

Phenylephrine as a partial agonist

The details of these experiments are described in the text of the results.

Drugs

Drugs used were: cocaine hydrochloride (D.H.A.), (±)-isoprenaline sulphate (B-W), phentolamine methanesulphonate (Rogitine, Ciba), phenoxybenzamine hydrochloride (S.K.F.), (−)-phenylephrine hydrochloride (Winthrop Labs.), piperoxan hydrochloride (M&B), (±)-propranolol hydrochloride (I.C.I.), tolazoline hydrochloride (Priscol, Ciba). All doses of drugs refer to final concentration of drug in the bath fluid. All drugs used were pure solids except for phentolamine and tolazoline which were provided in ampoules and hence doses of these drugs are expressed in mg/ml. bath fluid.

Results

Maximum relaxation of tissues to phenylephrine

The maximum relaxation of the tracheal chain preparation to phenylephrine was not always the same as that to the catecholamines. The maximum relaxation to phenylephrine of forty-six different tissues ranged from 70 to 100% of the catecholamine maximum. Seven dose-response lines had maxima between 70 and 90%, thirty-two were between 90 and 99%. Seven lines had the same maximum as the catecholamines. The maxima of the dose-response lines to phenylephrine, repeatedly obtained on a particular preparation, did not always bear exactly the same relationship to the maximum of the catecholamine response throughout that experiment.

There was no significant difference between the mean log ED50 for phenylephrine, calculated from the forty-six dose-response lines, whether plotted as a percentage of the catecholamine maximum ($-5{\cdot}46 \pm 0{\cdot}24$) or as a percentage of the phenylephrine maximum ($-5{\cdot}51 \pm 0{\cdot}22$). Theoretically, it is not possible to compare the slopes of dose-response lines with different maximum responses. However, because most of the phenylephrine lines had a maximum of 90% or more of the catecholamine maximum, the slopes of the phenylephrine lines (plotted as log dose-% phenylephrine maximum) and the catecholamine log dose-response lines were not usually significantly different. Thus the phenylephrine results presented were obtained from dose-response lines plotted as a percentage of the phenylephrine maxima.

The tracheal chain preparations from some animals exhibited rhythmic activity which made quantitative experiments difficult. The reason for this is not clear but it appears to be seasonal, the tissues being more rhythmic during the winter months of May-September. Rhythmic activity could usually be reduced if the cartilaginous section of each ring was cut. The mean log ED50 for phenylephrine

obtained from preparations where the cartilage was cut was not significantly different from the mean log ED50 from preparations where the cartilage was not cut (both on normal and on cocaine-treated tissues). Thus the results from both kinds of preparation have been pooled in the results discussed.

Effect of cocaine on phenylephrine

The mean log ED50 of phenylephrine on forty-six normal tissues ($-5{\cdot}51 \pm 0{\cdot}22$) was significantly different ($t = 3{\cdot}59$, $P < 0{\cdot}001$) from the mean log ED50 of phenylephrine on twenty-five cocaine-treated tissues ($-5{\cdot}73 \pm 0{\cdot}28$). However, the degree of potentiation of phenylephrine by cocaine (0·22 log units) was only small compared with that previously observed for adrenaline and noradrenaline (Chahl & O'Donnell, 1967). It was also not clear from previous work (Chahl & O'Donnell, 1967, 1968; Foster, 1967) whether cocaine produces a significant potentiation of isoprenaline. Seven experiments were carried out to compare the effect of cocaine (10^{-5}M) on phenylephrine and isoprenaline (design (1) in **Methods**). The shift (in log units) of the log ED50 produced by cocaine was then measured from each individual experiment, using lines drawn by eye. There was considerable variation in the results from different tissues, but there was usually some potentiation of both phenylephrine and isoprenaline in the presence of cocaine. The mean potentiation of phenylephrine in the seven experiments was $0{\cdot}20 \pm 0{\cdot}14$ and of isoprenaline was $0{\cdot}13 \pm 0{\cdot}08$. Potentiation usually occurred independently of whether there were changes in tissue sensitivity between the dose-response curves obtained before adding cocaine.

The potency of phenylephrine on untreated tracheal preparations was 1/5 that of noradrenaline. Because the effect of cocaine on phenylephrine was very small compared with its effect on noradrenaline, phenylephrine was only 1/45 as potent as noradrenaline on cocaine-treated tissues.

Effect of propranolol on phenylephrine

Propranolol (10^{-6}M) has previously been used to antagonize the relaxation of the tracheal chain produced by adrenaline, noradrenaline, isoprenaline, orciprenaline and protokylol (Chahl & O'Donnell, 1967, 1968). This dose of propranolol was therefore used initially to examine whether phenylephrine produced tracheal relaxation by stimulating β-adrenoceptors in the tissue. When 10^{-6}M propranolol was in the bath, the relaxation to phenylephrine did not begin at the dose-range to be

TABLE 1. *Degree of block by propranolol*

Propranolol (M)	Degree of block (log units)	
	Isoprenaline	Phenylephrine
10^{-8}	$0{\cdot}70 \pm 0{\cdot}05$ (5)	$0{\cdot}72 \pm 0{\cdot}11$ (8)
10^{-7}	$1{\cdot}54 \pm 0{\cdot}17$ (4)	$1{\cdot}74 \pm 0{\cdot}13$ (8)
10^{-6}	$2{\cdot}30 \pm 0{\cdot}14$ (5)	approx. 3·2 (7)

Expressed as the mean ± standard deviation of the shift in the log ED50 caused by propranolol, measured from individual experiments. The number of experiments is in parentheses.

expected if the block of phenylephrine by this dose were similar to that of the previously tested drugs. Also, when relaxation did occur, a dose-response line could not always be completed because a plateau in the response, or even a contraction, occurred as the dose of phenylephrine was increased. A secondary relaxation phase frequently appeared if the dose of phenylephrine was further increased (Fig. 1). In

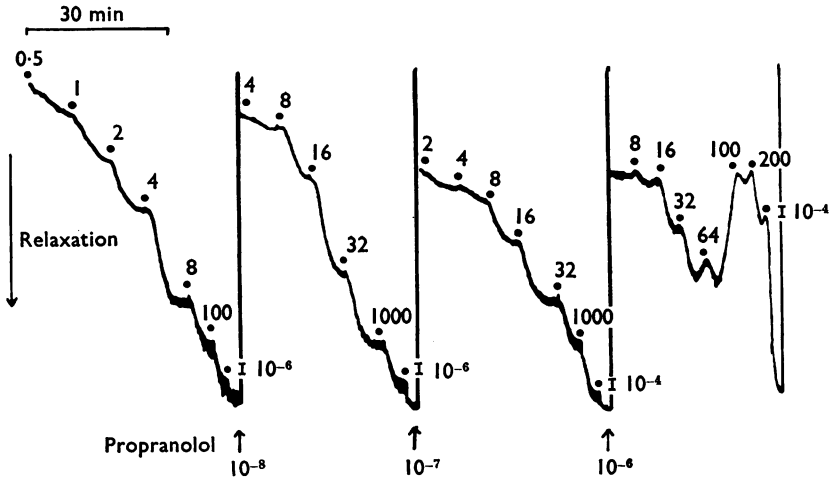

FIG. 1. Relaxations of guinea-pig tracheal chain to phenylephrine. Cumulative dose-response lines with no propranolol in the bath and with propranolol 10^{-8}M, 10^{-7}M and 10^{-6}M each in the bath for 1 hr. Concentrations of isoprenaline (I) used to produce maximum relaxation are indicated. All doses are given as molar concentrations in the bath fluid.

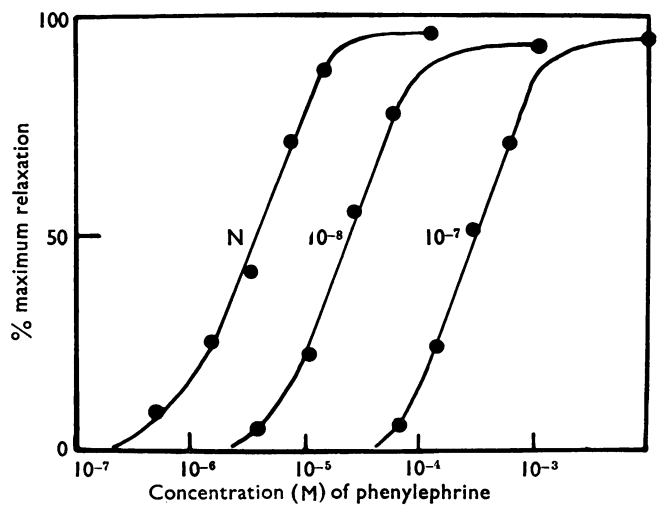

FIG. 2. Experiment illustrated in Fig. 1 plotted as molar concentration of phenylephrine on a logarithmic scale against response expressed as a percentage of the maximum relaxation to isoprenaline (to show that the maximum relaxation to phenylephrine is less than that to isoprenaline). In the presence of propranolol 10^{-8} and 10^{-7}M the phenylephrine line is parallel to the normal line (N).

the presence of propranolol (10^{-7} and 10^{-8}M) lower doses of phenylephrine were needed to produce relaxation and dose-response lines parallel to the normal phenylephrine line could be obtained (Fig. 2). This suggests a competition between phenylephrine and propranolol for β-adrenoceptors. Table 1 shows the results from experiments designed to compare the effect of three doses of propranolol (10^{-8}, 10^{-7}, 10^{-6}M) on phenylephrine and isoprenaline (design (2) in **Methods**). Isoprenaline was chosen for comparison because it is less affected by uptake into adrenergic nerve terminals than either noradrenaline or adrenaline. The degrees of block (log units) by propranolol were measured in each experiment from lines drawn by eye. The degrees of block of isoprenaline and phenylephrine were similar at the two lower doses of propranolol (10^{-8}, 10^{-7}M). Because of the unusual dose-response curves to phenylephrine obtained in the presence of 10^{-6}M propranolol, the degree of block could only be approximated. Nevertheless phenylephrine appeared to be blocked more than isoprenaline.

Effect of α-adrenoceptor blocking drugs

Takagi *et al.* (1967) obtained contractions of the trachea by phenylephrine in the presence of pronethalol which were blocked by tolazoline 0·01 mg/ml. We were able to observe small contractions to adrenaline, in the presence of 10^{-6}M propranolol, occurring between doses of 10^{-7} and 6×10^{-6}M. These contractions were blocked by tolazoline 0·01 mg/ml. In the presence of 6×10^{-6}M propranolol, small contractions to phenylephrine were observed in two out of six experiments. They occurred in the dose range 5×10^{-5}M to 10^{-4}M. They were not blocked by tolazoline (0·01 mg/ml.) but were blocked by 10^{-5}M phenoxybenzamine.

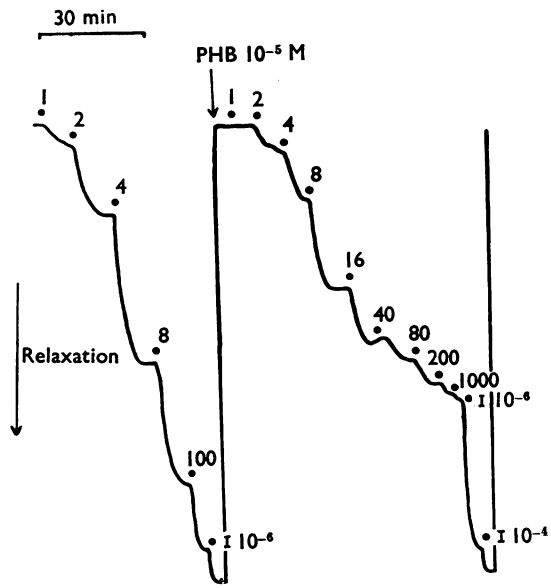

FIG. 3. Cumulative dose-response lines to phenylephrine obtained before and after treatment of the tissue with 10^{-5}M phenoxybenzamine (PHB) for 45 min. In the presence of phenoxybenzamine higher doses of phenylephrine are required to produce relaxations and the maximum relaxation to phenylephrine is reduced. All doses are given as molar concentrations in the bath fluid.

In five experiments in which 10^{-5}M phenoxybenzamine was in the bath fluid, phenylephrine produced a maximum relaxation which was less than that of the control line (Fig. 3). In no experiment did phenoxybenzamine (*a*) potentiate the relaxation to phenylephrine, (*b*) affect the position of the line to phenylephrine in the presence of propranolol (10^{-8}M) or cause the maximum response to phenylephrine to be the same as that of a catecholamine or (*c*) prevent multiphasic dose-response lines in the presence of propranolol (10^{-6}M).

Phenylephrine as a partial agonist

Some experiments carried out using single dose additions of phenylephrine suggested that phenylephrine might be a partial agonist with a dual action to block β-adrenoceptors as well as stimulate them. It was found that a single dose of phenylephrine (10^{-2}M) produced a smaller relaxation than did a 100 fold lower dose (10^{-4}M). Isoprenaline (10^{-6}M), which produced a maximal relaxation of the tissue in the presence of 10^{-4}M phenylephrine, did not do so in the presence of 10^{-2}M phenylephrine. Thus the higher dose of phenylephrine antagonized the relaxation to isoprenaline.

Ariens, Simonis & Van Rossum (1964) describe theoretical curves for the combination of a compound of high intrinsic activity with one of lower intrinsic activity (a partial agonist). We therefore studied the responses of the trachea to cumulative doses of phenylephrine in the presence of constant doses of isoprenaline. The maximum relaxation to phenylephrine in our previous experiments was nearly always between 70 and 100% of the catecholamine maximum. On two preparations used during this part of the study, however, phenylephrine had a lower intrinsic activity than usual which made it easier to examine the partial agonist effects of

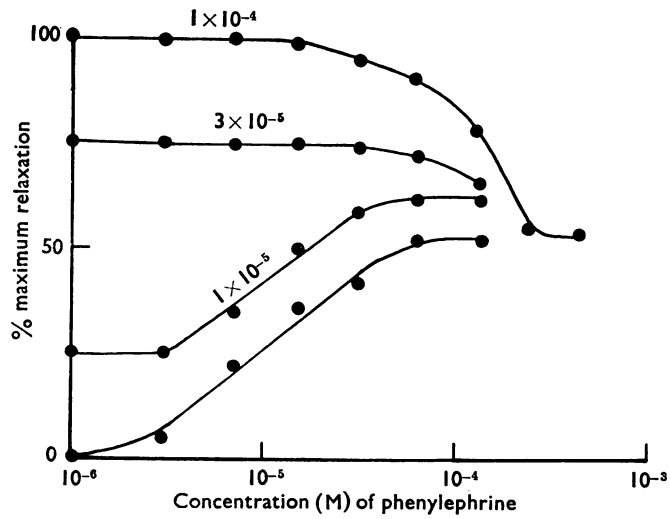

FIG. 4. Dose-response lines to phenylephrine with no isoprenaline in the bath (unlabelled curve) and with 1×10^{-5}, 3×10^{-5} and 1×10^{-4}M isoprenaline present in the bath. The lines are plotted as concentration of phenylephrine (M) on a logarithmic scale, against response expressed as a percentage of the maximal relaxation to isoprenaline. This experiment was selected to illustrate the partial agonist effect of phenylephrine since the maximum response of the tissue to phenylephrine was only 50% of that to isoprenaline. A maximum as low as 50% occurred only infrequently.

phenylephrine. The results of one of these experiments are illustrated in Fig. 4. These results seem to agree with the theoretical curves described by Ariens *et al.* (1964) and would support the concept that phenylephrine behaves as a partial agonist on this tissue.

Three experiments were also carried out in an attempt to mimic the responses which might be obtained on adding increasing doses of a partial agonist to the trachea. Propranolol was included in a solution of isoprenaline so that, as increasing doses of isoprenaline were added to the tissue to produce relaxation, increasing doses of antagonist were simultaneously added. Multiphasic dose-response lines were obtained similar in nature to those obtained to phenylephrine in the presence of 10^{-6}M propranolol.

Discussion

Phenylephrine was found to produce a relaxant effect on the isolated guinea-pig tracheal chain preparation, but it was less potent than adrenaline or noradrenaline. Phenylephrine was frequently slightly potentiated by cocaine which suggested that it might have a low affinity for uptake into adrenergic nerves. Iversen (1965) found phenylephrine to have slight affinity for the uptake$_1$ of rat heart. The significance of the effect of cocaine on phenylephrine was made less clear in view of the conflicting opinions on the effect of cocaine on isoprenaline. Because the sensitivity of tracheal preparations frequently increases slightly with time, the potentiation of isoprenaline by cocaine which was previously observed (Chahl & O'Donnell, 1967) might not have been significantly greater than that which would occur merely due to changes in tissue sensitivity (Chahl & O'Donnell, 1968). However, in this work, where paired experiments were done in which control lines to both phenylephrine and isoprenaline were obtained until there was no change in tissue sensitivity, both phenylephrine and isoprenaline were potentiated to a small extent on some tissues. This might depend on the density of the adrenergic innervation. It is also possible that cocaine potentiates sympathomimetic amines by more than one mechanism. A major specific effect on uptake into nerve terminals might account for most of its potentiating action on adrenaline and noradrenaline but some direct sensitizing effect of cocaine—for example, on the receptor (Reiffenstein, 1968)—might become apparent with drugs which are not significantly taken up by nerve terminals.

Phenylephrine relaxations were blocked by propranolol and the parallel shift in the log dose-response lines indicated that relaxation of the trachea by phenylephrine was mediated through β-adrenoceptors. However, the inability to complete dose-response lines to phenylephrine in the presence of 10^{-6}M propranolol (the dose used in previous studies, Chahl & O'Donnell, 1967, 1968) suggested some further actions of phenylephrine. Since phenylephrine had only a weak relaxant effect on the trachea, quite high doses were required to overcome the block produced by 10^{-6}M propranolol. At these high doses plateaux or contractile phases in the response occurred. If the dose of phenylephrine was greatly increased a secondary relaxation phase often occurred.

The maximum relaxation of the tissue to phenylephrine was nearly always less than that to the catecholamines both on normal preparations and in the presence of propranolol. Values for the maximum relaxation of less than 100% with respect to that produced by catecholamine would suggest that phenylephrine had a smaller intrinsic activity (Ariens *et al.*, 1964). Two possible explanations for this were con-

sidered. First, that phenylephrine might stimulate other receptors to produce an antagonism of the β-adrenoceptor relaxation. Second, that it might also block β-adrenoceptors as the dose of phenylephrine and the time it had been in contact with the tissue were increased. The most likely receptors to be stimulated by phenylephrine to produce a contraction of guinea-pig trachea are α-adrenoceptors (Takagi *et al.*, 1967). We were able to confirm the findings of these workers, that, with propranolol in the bath to mask relaxation effects, adrenaline contractions could be observed, which were blocked by 3 min contact of the tissue with tolazoline 0·01 mg/ml. However, contractions to phenylephrine in the presence of propranolol were far less readily observed. In the two experiments where contractions of the tissue did occur, they were in the approximate dose range reported by Takagi *et al.* (1967). However, these contractions were not reliably blocked by the dose of tolazoline used by these workers. Takagi and his co-workers seem to have used pronethalol in most of their experiments and since pronethalol has more β-mimetic properties than propranolol (Black, Duncan & Shanks, 1965), the tissue might have less tone in the presence of pronethalol and thus contract more readily.

Piperoxan, phentolamine and higher doses of tolazoline were found to have too much effect on the resting tone of the tissue, so we examined the possibility of using phenoxybenzamine. The use of a non-equilibrium competitive blocking drug was felt to be advantageous in that any block of α-receptors achieved would not be reversed as the dose of agonist was increased. Phenoxybenzamine (10^{-5}M) prevented the contractions of the tissue to both adrenaline and phenylephrine observed in the presence of propranolol (6×10^{-6}M). This dose was initially chosen because it was in the range of doses used on this tissue by both Foster (1966) and Furchgott (1967).

Phenoxybenzamine did not potentiate phenylephrine relaxations. It was also not possible to obtain a maximum relaxation to phenylephrine in the presence of phenoxybenzamine which was the same as the catecholamine maximum, either in the presence or absence of propranolol (10^{-8}M). If α-adrenoceptor stimulation occurs in the dose range observed by us and quoted by Takagi *et al.* (1967), the control dose-response relationship and that in the presence of 10^{-8}M propranolol would be the only ones affected. It was therefore concluded that α-adrenoceptor stimulation by phenylephrine is probably a negligible factor in the response of guinea-pig trachea to this drug.

It was considered more likely that on the isolated guinea-pig trachea the wide range of values for the maximum relaxation observed for phenylephrine might be due to the action of phenylephrine as a partial agonist on β-receptors. Thus at some stage in the dose-response relationship phenylephrine might not only stimulate β-adrenoceptors causing relaxation but also become a β-adrenoceptor antagonist. The exact dose of phenylephrine at which this would be observed would almost certainly depend on the sensitivity of the tissue and the time of contact of phenylephrine with the tissue. For those tissues on which β-receptor blocking doses of phenylephrine were reached, some depression of the value of the maximum relaxation might be expected. In the few experiments where phenylephrine and a catecholamine both relaxed the tissue maximally, it would appear that antagonistic doses of phenylephrine for that tissue had not been reached. The higher the dose of phenylephrine added to the tissue the more significant would be its blocking properties. Thus this might be the reason that, in

the presence of propranolol (10^{-6}M), relaxations to phenylephrine were inhibited more than expected, and that plateaux or contractions (see Fig. 1) occurred.

The following observations indicated that the partial agonist properties of phenylephrine could explain our observed effects: (1) Single doses of phenylephrine above approximately 10^{-3}M produced less relaxation than lower doses of phenylephrine. (2) Large doses of phenylephrine antagonized relaxations to isoprenaline. (3) When a solution containing both isoprenaline and propranolol was added to the tissue such that, as agonist activity was increased so was antagonist activity, tracheal responses of a similar multiphasic pattern to those observed with high doses of phenylephrine were produced. (4) In experiments where cumulative doses of phenylephrine were combined with constant doses of isoprenaline, plots of log dose-per cent maximum relaxation showed similarity to the theoretical curves described by Ariens *et al.* (1964) for combination of a compound of high intrinsic activity with one of lower intrinsic activity (a partial agonist).

In experiments with 10^{-5}M phenoxybenzamine, it was noticed that the maximum relaxation to phenylephrine was less than that of the control line obtained before addition of phenoxybenzamine (see Fig. 3). This decrease in the intrinsic activity of phenylephrine was more marked in those experiments where attempts were made to use 10^{-4}M phenoxybenzamine. This observation could suggest that phenoxybenzamine itself acts as a β-adrenoceptor blocking drug. It is more likely that phenoxybenzamine produces inhibition at the β-adrenoceptor site by combining with a different (or allosteric) site. This combination might induce a conformational alteration (allosteric transition) in the β-receptor protein such that the properties of the active site are modified. This mechanism would be similar to that postulated for regulatory proteins by Monod, Changeux & Jacob (1963).

In conclusion, phenylephrine relaxes the isolated guinea-pig tracheal chain via β-adrenoceptor stimulation but it is probably a partial agonist. Thus it is capable of blocking its own effects on β-receptors particularly at high doses, such as are used in the presence of propranolol (10^{-6}M). This is thought to account for the multiphasic responses observed in this study as well as for the wide variation in intrinsic activity values for phenylephrine. Stimulation of α-adrenoceptors by phenylephrine is considered to have little influence on β-adrenoceptor relaxations observed on this preparation although use of the presently available α-adrenoceptor blocking drugs does not enable conclusive evidence to be obtained. The relaxations which occurred with excessively high doses of phenylephrine (10^{-2}M) can only be considered at present to be non-specific.

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REFERENCES

AHLQUIST, R. P. & LEVY, B. (1959). Adrenergic receptive mechanism of canine ileum. *J. Pharmac. exp. Ther.*, **127**, 146–149.

ARIENS, E. J., SIMONIS, A. M. & VAN ROSSUM, J. M. (1964). *Molecular Pharmacology: The Mode of Action of Biologically Active Compounds*, vol. 1, pp. 138 and 171. New York: Academic Press.

BLACK, J. W., DUNCAN, W. A. M. & SHANKS, R. G. (1965). Comparison of some properties of pronethalol and propranolol. *Br. J. Pharmac. Chemother.*, **25**, 577–591.

CHAHL, L. A. & O'DONNELL, S. R. (1967). The interaction of cocaine and propranolol with catecholamines on guinea pig trachea. *Eur. J. Pharmac.*, **2**, 77–82.

CHAHL, L. A. & O'DONNELL, S. R. (1968). The actions of orciprenaline and protokylol on guinea pig trachea. *Br. J. Pharmac. Chemother.*, **33**, 552–559.

CHAHL, L. A. & O'DONNELL, S. R. (1968). Action of phenylephrine on β-receptors. *Aust. J. exp. Biol. med. Sci.*, **46**, 42–43*P*.

EVERITT, B. J. & CAIRNCROSS, K. D. (1969). Adrenergic receptors in the guinea pig trachea. *J. Pharm. Pharmac.*, **21**,, 97–102.

FOSTER, R. W. (1966). The nature of the adrenergic receptors of the trachea of the guinea pig. *J. Pharm. Pharmac.*, **18**, 1–12.

FOSTER, R. W. (1967). The potentiation of the responses to noradrenaline and isoprenaline of the guinea pig isolated tracheal chain preparation by desipramine, cocaine, phentolamine, phenoxybenzamine, guanethidine, metanephrine and cooling. *Br. J. Pharmac Chemother.*, **31**, 466–482.

FURCHGOTT, R. F. (1967). The pharmacological differentiation of adrenergic receptors. *Ann. N.Y. Acad. Sci.*, **139**, 553–570.

IVERSEN, L. L. (1965). The inhibition of noradrenaline uptake by drugs. *Adv. Drug Res.*, **2**, 1–46.

MONOD, J., CHANGEUX, J-P. & JACOB, F. (1963). Allosteric proteins and cellular control systems. *J. mol. Biol.*, **6**, 306–329.

REIFFENSTEIN, R. J. (1968). Effects of cocaine on the rate of contraction to noradrenaline in the cat spleen strip: mode of action of cocaine. *Br. J. Pharmac., Chemother.*, **32**, 591–597.

TAKAGI, K., OSADA, E., TAKAYANAGI, I. & TAGA, F. (1967). Adrenergic receptors on some organs. *Archs int. pharmacodyn. Thér.*, **168**, 212–219.

VAN ROSSUM, J. M. (1963). Cumulative dose-response curves. II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Archs int. pharmacodyn. Thér.*, **143**, 299–330.

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