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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

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Macmillan (1959) first suggested that cocaine might potentiate the effect of noradrenaline *in vitro* by blocking its uptake into tissue stores. A dynamic situation is implied by this suggestion in which the uptake process acts as a route of loss of relatively large amounts of noradrenaline from the immediate environment of the receptors (the "biophase" of Furchgott, 1955). Reduction of this loss allows the biophase concentration of noradrenaline to rise towards the concentration in the bath fluid to an extent which is determined by the initial uptake of noradrenaline and the degree of uptake blockade. The extent of this rise determines the amount of potentiation observed.

Since that time much has been learned about the occurrence of such uptake processes, their properties and their susceptibility to inhibition. The hypothesis that there is a causal relationship between inhibition of uptake and potentiation of noradrenaline by drugs has become generally accepted (for a recent review see Iversen, 1967; for a dissenting view see Maxwell, Wastila & Eckhardt, 1966). The evidence supporting this hypothesis is extensive but largely circumstantial and will remain so until a consistent quantitative correlation between the amount of potentiation and the degree of inhibition of uptake is demonstrated or disproved.

It was in the hope of placing this hypothesis on a quantitative footing that the present studies were started. This paper deals with the degree of potentiation produced by various procedures.

METHODS

In those experiments employing amphetamine or tyramine the guinea-pig isolated paired tracheal chain preparation (Foster, 1960) was used. In other experiments strict pairing was unnecessary; for these the trachea of 4 guinea-pigs (of either sex and any strain) weighing less than 550 g (or of 3 weighing more) were cut into a total of 21 rings to provide three preparations of 7 rings. These were mounted in 5 ml. tissue baths so that three experiments could be performed simultaneously. The baths were maintained at 37.5° C. Washing was by displacement of the bath fluid with double its volume of fresh Krebs solution. The load applied to the tissue was 200 mg; it was removed during drug-washout.

Krebs solution was bubbled with 95% oxygen and 5% carbon dioxide and had the following composition in m-mole/l.: NaCl 118, KCl 4.75, KH_2PO_4 1.19, $CaCl_2.6H_2O$ 2.55, $MgSO_4.7H_2O$ 1.20, $NaHCO_3$ 25, and glucose 5.56.

Potentiation experiments

The conduct of each experiment was essentially as described by Foster (1966). A maximum relaxation of the tissue was obtained using a large concentration of either noradrenaline, isoprenaline or aminophylline. When the drug had been washed off, a cumulative log. concentration: effect line to an agonist was obtained using two concentrations separated by a multiple of four and selected to produce relaxations of about 30 and 80% of the maximum. After washing to recovery the time was noted and the Krebs solution bathing the tissue was changed to one containing a known concentration of a potentiating drug: this solution was used for the rest of the experiment. The potency of the agonist was determined repeatedly, noting the time of each determination, until it no longer changed. While its potency was increasing rapidly the tissue was challenged with only single concentrations of the agonist, being selected to provide relaxations within the limits (about 30 to 80%) set by the control log. concentration: effect line. When a steady state approached, two point log. concentration: effect lines were again obtained. Finally a maximum relaxation was repeated.

At least five preparations were used to measure the activity of each concentration of potentiating drug.

Whenever an agonist or pyrogallol was applied to the tissue, ascorbic acid, 5.7×10^{-4} M, was added to the tissue bath 5 min beforehand to delay autoxidation: this concentration had no effect on the tone of the muscle.

All concentrations are expressed in mole/l. and apply to the Krebs solution in contact with the tissue.

The apparent ^{14}C-sorbitol space

^{14}C-sorbitol (100 nc/ml., 1.56×10^{-5} M) was applied to the preparation for times varying between 2.5 and 40 min. The tissue was rapidly removed from the bath, firmly blotted and weighed. It was then immersed in liquid nitrogen, removed and crushed in a chilled hammer-mill. The pellet was added to 10 ml. 0.4 N perchloric acid and shaken for 15 min. After centrifugation at 2860 *g* for 15 min, 0.5 ml. supernatant was added to 18 ml. phosphor and the total radioactivity present measured by liquid scintillation counting. 0.5 ml. bath fluid was also added to 18 ml. phosphor and counted. Quenching by 0.5 ml. 0.4 N perchloric acid extract of trachea did not significantly differ from that by 0.5 ml. Krebs solution.

The efficiency of this extraction procedure was assessed by incubating ^{14}C-sorbitol with the pellet for 15 min before the addition of perchloric acid: it was 94.1 ± 2.4% (mean $\pm$ standard error). The results were not corrected for this recovery.

The phosphor had the following composition: 8 ml. absolute alcohol and 10 ml. toluene (A.R.) containing 30 mg 5,5-diphenyloxazole and 3 mg 1,4-di-(2-(5-phenyloxazolyl))-benzene. All samples were counted for sufficient time to collect at least 10,000 counts.

Drugs used

Aminophylline (theophylline ethylene diamine dihydrate), (+)-amphetamine sulphate, (+)-ascorbic acid, (−)-cocaine hydrochloride, desipramine hydrochloride, guanethidine sulphate, harmine hydrochloride dihydrate, isocarboxazide, (−)-isoprenaline bitartrate dihydrate, (±)-metanephrine hydrochloride, (−)-noradrenaline bitartrate monohydrate, phenoxybenzamine hydrochloride, phentolamine hydrochloride, pyrogallol, sodium thiosulphate pentahydrate, ^{14}C-sorbitol and tyramine hydrochloride.

Ascorbic acid, pyrogallol and phentolamine were made up freshly in distilled water immediately before us. Stock solutions of other drugs were prepared; catecholamines were made up in N/10 hydrochloric acid, phenoxybenzamine and isocarboxazide in absolute alcohol and other drugs in distilled water. These stock solutions were stored at below 4° C and all dilutions from them were made in Krebs solution immediately before use.

Statistical methods

Usually parametric statistical methods (standard error and Student's *t* test) were used. For experiments using amphetamine and tyramine, a nonparametric method according to Siegel (1956) was used—the Wilcoxon matched-pairs signed-ranks test.

RESULTS

The amount of potentiation of noradrenaline effects: dependence on time

Figures 1 to 4 show the time courses of the development of potentiation of noradrenaline induced by the continuous presence in the tissue bath of various concentrations of cocaine, desipramine, guanethidine and phenoxybenzamine. The maximum potentiation produced is about 46-fold and this contrasts with a potentiation of 1.35-fold which developed over 240 min in control preparations in the absence of a potentiating drug (Fig. 2).

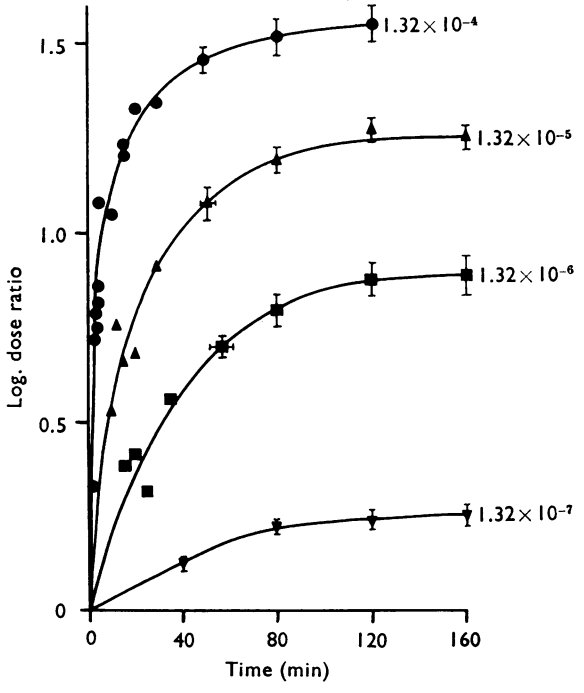

Fig. 1. Time courses for the development of potentiation of the action of noradrenaline at four concentrations (M) of cocaine. Potentiation was measured as the log. dose ratio, and plotted against time in min. Points to the left of 40 min were each derived from a single experiment. Points to the right are means with standard errors. Most of these were derived by interpolation at 40 min intervals from the time courses of individual experiments in which experimentally determined points were joined by straight lines. Where the slope was changing rapidly this method underestimated the log. dose ratio; therefore near the inflection of the curve all experimentally determined points were grouped—thus producing means (±S.E.) in the direction of both axes.

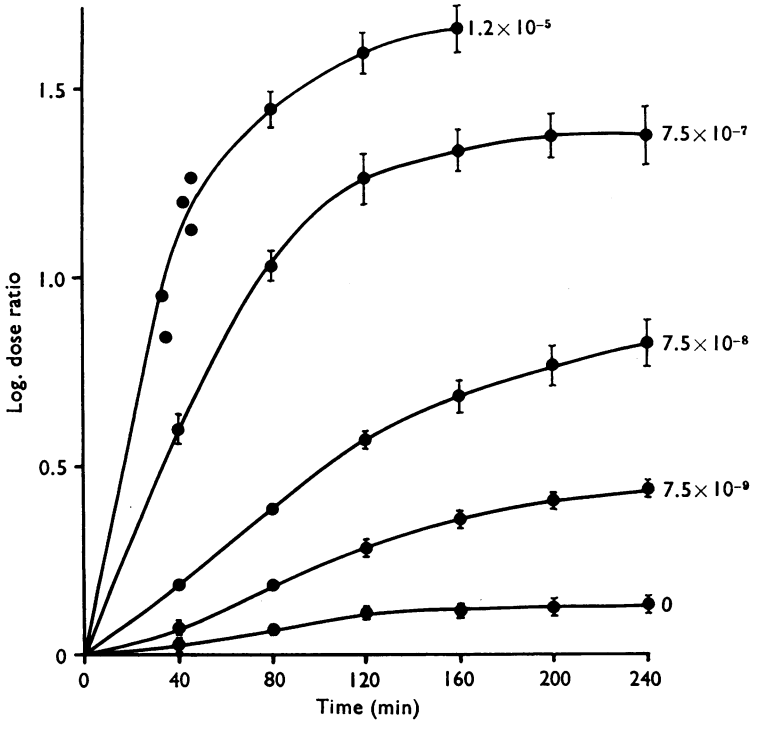

Fig. 2. Time courses for the development of potentiation of the action of noradrenaline by four concentrations (M) of desipramine. Five points on the upper curve were each derived from a single experiment. Other points are means, with standard errors, derived from the time courses of individual experiments by interpolation at 40 min intervals. The control curve (0) shows the change in sensitivity to noradrenaline over 240 min in preparations not treated with a potentiating drug.

The highest concentrations of cocaine, desipramine and phenoxybenzamine shown produced maximal potentiation for each drug. A further ten-fold increase in the concentrations of cocaine or desipramine, or a four-fold increase in that of phenoxybenzamine, resulted in a more rapid development of potentiation initially but a lesser degree of potentiation finally. The maximum potentiation produced by phenoxybenzamine was significantly ($P \leqslant 0.01$) larger than that produced by cocaine. Desipramine's maximum did not differ significantly ($P \leqslant 0.2$) from that of either of the other two drugs.

For each drug, a steady state was approached more rapidly with increasing concentrations of the drug. With cocaine the potentiation developed rapidly, with desipramine and guanethidine more slowly and with phenoxybenzamine very slowly.

None of these curves had an exponential shape, so the half-times listed in Table 1 cannot easily be transformed into rate constants.

Phentolamine and metanephrine acted more quickly than desipramine ; the latter seemed to potentiate as rapidly as did cocaine. Cooling potentiated very rapidly.

TABLE 1

EXPERIMENTAL HALF-TIMES FOR THE DEVELOPMENT OF POTENTIATION OF RESPONSES TO NORADRENALINE OF THE GUINEA PIG TRACHEAL CHAIN BY THE HIGHER CONCENTRATIONS OF COCAINE, DESIPRAMINE, GUANETHIDINE AND PHENOXYBENZAMINE

Drug	Concentration (M)	Half time (±S.E.) (min)
Cocaine	$1{\cdot}32\times10^{-6}$	approx. 25
	$1{\cdot}32\times10^{-5}$	approx. 12
	$1{\cdot}32\times10^{-4}$	approx. 3
Desipramine	$7{\cdot}5\times10^{-8}$	85·0±4·0
	$7{\cdot}5\times10^{-7}$	48·5±2·0
	$1{\cdot}2\times10^{-5}$	approx. 29
Guanethidine	$2{\cdot}53\times10^{-6}$	70·3±5·2
	$1{\cdot}01\times10^{-5}$	51·8±5·0
Phenoxybenzamine	$8{\cdot}2\times10^{-7}$	97·0±2·5
	$3{\cdot}3\times10^{-6}$	82·5±7·3

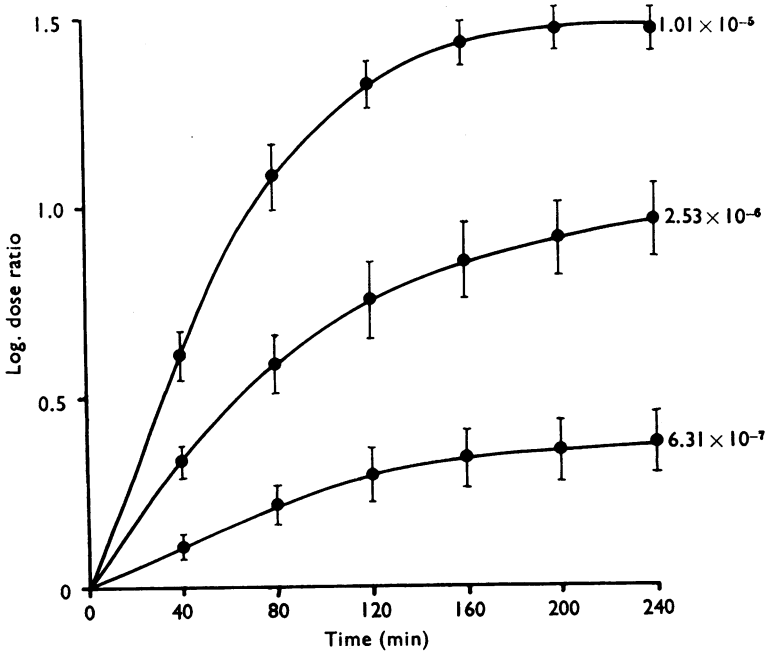

Fig. 3. Time courses for the development of potentiation of the action of noradrenaline at three concentrations (M) of guanethidine. All the points are means, with standard errors, derived from the time courses of individual experiments by interpolation at 40 min intervals.

The effect of thiosulphate on potentiation by phenoxybenzamine

Figure 4 also shows the results of three single experiments in which sodium thiosulphate was mixed with phenoxybenzamine, 3.3×10^{-6} M, in the molar proportions 10, 100 and 1000 to 1. Only the largest amount of thiosulphate markedly affected the potentiation of noradrenaline by phenoxybenzamine—mimicking the effect of reducing the concentration of phenoxybenzamine 12.5-fold. It did not affect the tone of the tracheal muscle.

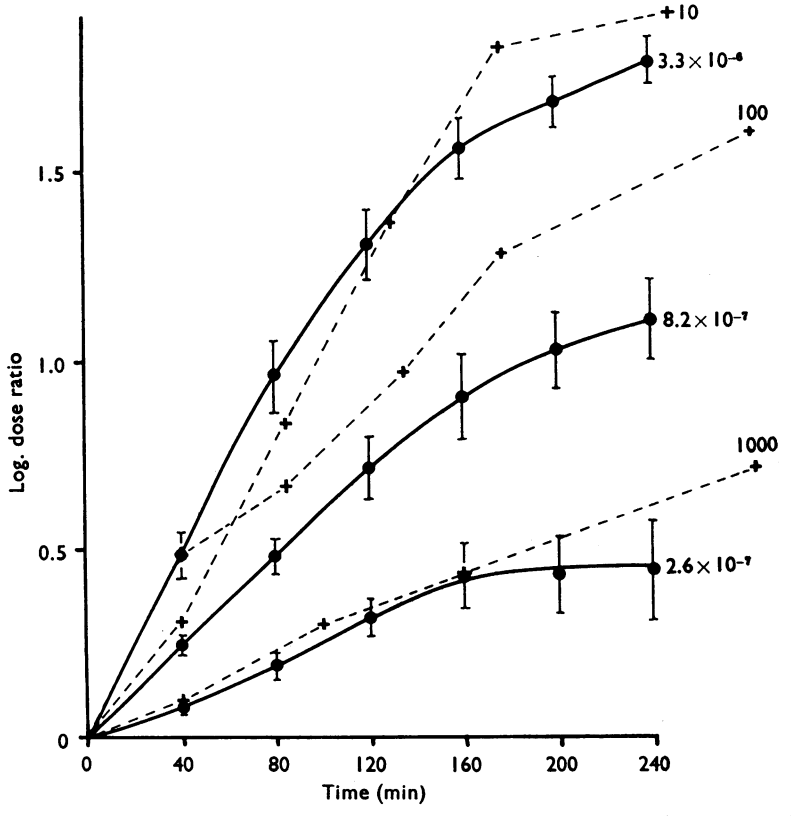

Fig. 4. Time courses for the development of potentiation of the action of noradrenaline at three concentrations (M) of phenoxybenzamine (●—●). The three other lines (+- - -+) show the results of single experiments using phenoxybenzamine, 3.3×10^{-6} M, mixed with sodium thiosulphate in 10, 100 and 1,000 times the equimolar amount.

The apparent ^{14}C-sorbitol space : dependence on time

Figure 5 shows the apparent ^{14}C-sorbitol space of the preparation at times ranging from 2.5 to 40 min after addition of ^{14}C-sorbitol (M.W. = 182) to the tissue bath to produce a concentration of 1.56×10^{-5} M. The curve produced was not exponential in shape; the experimental half time was about 2.7 min.

The time course of a noradrenaline relaxation

Figure 5 also shows the time course of the relaxation of the preparation induced by a concentration of $8.88 \pm 1.78 \times 10^{-6}$ M noradrenaline. The concentration selected was one which gave a relaxation of 60 to 80% of the maximum possible relaxation. The experimental half time was 3.7 ± 0.25 min.

The degree of potentiation of noradrenaline effects at a steady state : dependence on concentration

Potentiation at a steady state for each concentration of potentiating drug was measured as the log. dose ratio attained when this had stopped increasing with time.

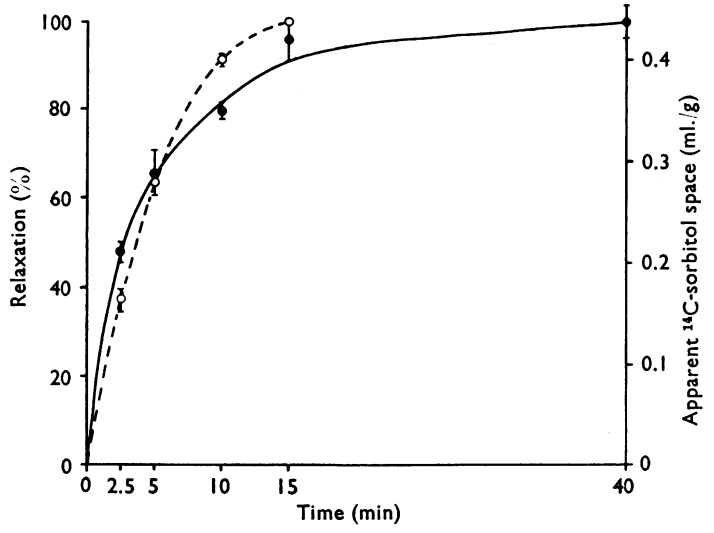

Fig. 5. Time courses of the relaxation induced by noradrenaline and of the expansion of the apparent ^{14}C-sorbitol space in the guinea-pig isolated tracheal chain preparation. A concentration of noradrenaline ($8.88 \pm 1.78 \times 10^{-6}$ M) which caused 60–80% of the maximum possible relaxation was used. The relaxations recorded at 2.5, 5 and 10 min were expressed as a proportion (%) of that recorded at 15 min. The apparent ^{14}C-sorbitol space was measured in ml./g blotted wet weight, and plotted against time of contact with 1.56×10^{-5} M sorbitol in min. The experimental points are means with standard errors and are joined by the best-fitting smooth curves drawn by eye.

Log. concentration: effect curves for potentiation at a steady state are plotted in Fig. 6 for desipramine, cocaine and phentolamine, and in Fig. 7 for phenoxybenzamine, guanethidine and metanephrine.

Desipramine, cocaine and phenoxybenzamine had no effect, by themselves, on the tone of the tracheal muscle; therefore their curves could be plotted in full. In contrast, phentolamine, guanethidine and metanephrine each caused a concentration-dependent relaxation of the tracheal muscle which limited the range of concentrations whose potentiating actions could be explored. Therefore the curves for each of these three drugs were only plotted up to the concentration where this became a problem.

The slopes of these log. concentrations: effect curves (Table 2) were read off, over the central segment (log. dose ratio = 0.55 to 1.05) and compared using Student's t test. The slopes for desipramine and cocaine were shallow and did not differ from each other significantly ($P \simeq 0.45$). The slopes for phenoxybenzamine, guanethidine and metanephrine also did not differ from each other but were significantly steeper. That for phentolamine did not differ significantly from any of the other five.

The amount of potentiation of isoprenaline at a steady state: dependence on concentration

These data are also plotted in Figs. 6 and 7. Desipramine and cocaine did not potentiate isoprenaline at any concentration shown—a log. dose ratio is therefore given

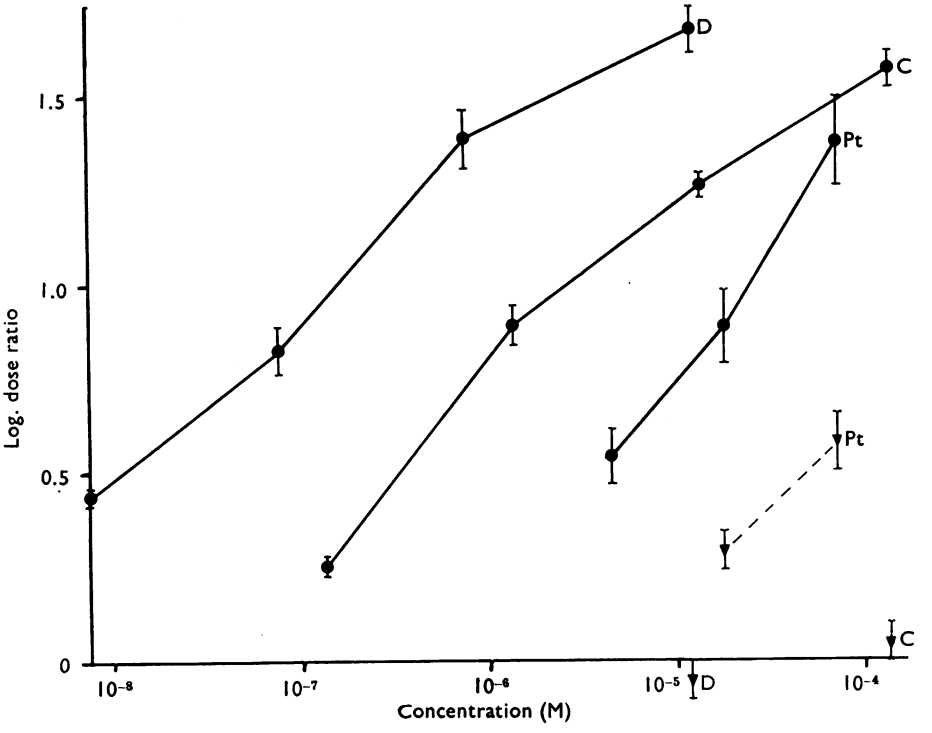

Fig. 6. Log. concentration: effect curves for potentiation (log. dose ratio with standard error) of responses to noradrenaline (●——●) and isoprenaline (▼- - -▼) by desipramine (D), cocaine (C) and phentolamine (Pt) at a steady state.

TABLE 2

SLOPES OF LOG. CONCENTRATION: EFFECT CURVES FOR POTENTIATION OF THE EFFECTS OF NORADRENALINE

The slope was measured over the central segment (log. dose ratio=0·55 to 1·05) and was expressed in units of change in log. dose ratio per 10-fold change in concentration of potentiating drug.

	Slope	
Drug	Mean	± Standard error
Desipramine (D)	0·442	0·071
Cocaine (C)	0·513	0·062
Phentolamine (Pt)	0·629	0·170
Metanephrine (M)	0·909	0·136
Guanethidine (G)	0·935	0·129
Phenoxybenzamine (Pb)	1·316	0·463

Comparison by Student's t test between all the pairs of means using $P=0{\cdot}05$ as the criterion of significance revealed: D=C<M=G=Pb. Pt was not different from any of the other five.

only for the highest concentration of each. Phentolamine and guanethidine caused relatively small potentiations of the effect of isoprenaline. Phenoxybenzamine potentiated it markedly and metanephrine caused at least as much potentiation of isoprenaline as it did of noradrenaline.

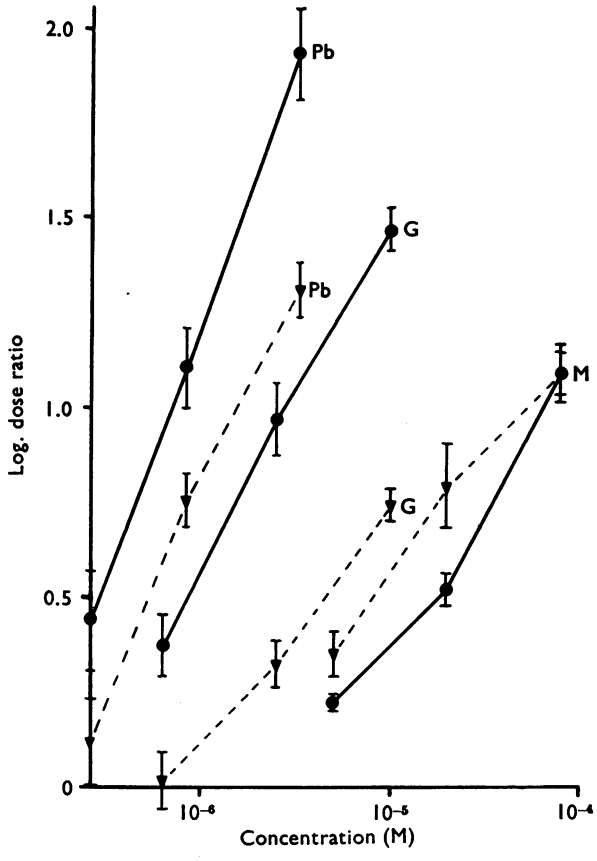

Fig. 7. Log. concentration:effect curves for potentiation (log. dose ratio with standard error) of responses to noradrenaline (●——●) and isoprenaline (▼- - -▼) by phenoxybenzamine (Pb), guanethidine (G) and metanephrine (M) at a steady state.

The potentiation of isoprenaline relative to that of noradrenaline was measured as the horizontal distance between the two log. concentration:effect curves at a constant level of potentiation (log. dose ratio = 0.55). The mean figures were compared using Student's *t* test. A spectrum of activity was found with four distinguishable groups (Table 3) : desipramine and cocaine, phentolamine and guanethidine, phenoxybenzamine, and metanephrine. There seemed to be a rough correlation, within these six drugs, between the slope of the log. concentration:effect curve for noradrenaline potentiation and the potentiation of isoprenaline relative to that of noradrenaline (means of Tables 2 and 3).

The amount of potentiation of noradrenaline and isoprenaline produced by cooling

Figure 8 shows that lowering the temperature of the preparation below 37.5° caused a temperature-dependent equal potentiation of the effects of noradrenaline and isoprenaline. Aminophylline was included as a control; the results suggest that cooling does not affect the potency of the catecholamines in a non-specific way.

TABLE 3

POTENTIATION OF THE RESPONSE TO ISOPRENALINE RELATIVE TO THAT TO NORADRENALINE

The relative potentiation was measured as the horizontal distance between the log. concentration: effect curves for potentiation of the action of the two catecholamines at the level, log. dose ratio=0·55. A positive figure indicates that the response to isoprenaline is potentiated less than that to noradrenaline.

Drug	Relative potentiation	
	Mean	±Standard error
Desipramine (D)	>2·93	—
Cocaine (C)	>2·54	—
Phentolamine (Pt)	1·14	0·214
Guanethidine (G)	0·75	0·118
Phenoxybenzamine (Pb)	0·255	0·119
Metanephrine (M)	−0·350	0·130

Comparison by Student's t test between all the pairs of means using $P=0·05$ as the criterion of significance revealed: D or C>Pt=G>Pb>M.

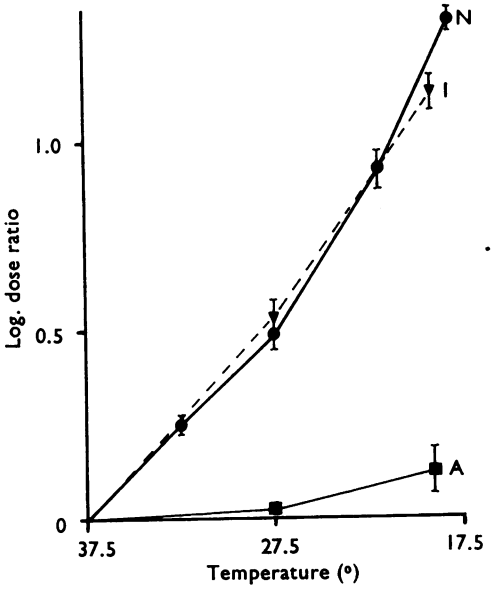

Fig. 8. Change in potency of noradrenaline (N), isoprenaline (I) and aminophylline (A) induced by cooling. Potentiation was measured as log. dose ratio and plotted against temperature in ° C.

Comparison of the slopes of noradrenaline and isoprenaline log. concentration: effect curves

In 40 preparations log. concentration: effect curves for noradrenaline and isoprenaline were obtained early in the experiment. The order in which the two catecholamines were applied was randomized. The slopes of the log. concentration: effect curves were derived over the four-fold concentration increment which caused relaxations of approximately 32% and 79% of the maximum possible relaxation. Each of these sets of slopes showed a normal distribution so that they could be compared using Student's t test. The mean slope of the log. concentration: effect curve for noradrenaline was 82.7±2.7% relaxation

per ten-fold increase in concentration while that for isoprenaline was 73.7 ± 1.8. These means were significantly different ($0.005 < P < 0.01$).

The change with time in the slope of the log. concentration : effect curve for noradrenaline

During a long-continued experiment the slope of the log. concentration : effect curve for noradrenaline declined linearly with time from the initial value given in the previous paragraph to one of $70.7 \pm 2.65\%$ relaxation per ten-fold increase in concentration at 240 min. This change in slope was significant ($0.005 < P < 0.01$).

The effect of potentiating procedures on the slope of the log. concentration : effect curve for noradrenaline

This slope was also measured at a steady state (about 240 min from the beginning of the experiment) with the highest concentration of each of the potentiating drugs used and with the greatest degree of cooling. In all cases, except that of metanephrine, the slope was not significantly different from controls at the same time ; with metanephrine it was shallower. Thus desipramine, cocaine, phentolamine, phenoxybenzamine, guanethidine and cooling each induce a parallel leftward shift of the log. concentration : effect curve for noradrenaline.

The effects of some inhibitors of catecholamine-metabolizing enzymes on the actions of noradrenaline, tyramine and amphetamine

Pyrogallol, 4×10^{-5} . M, failed to potentiate the actions of either noradrenaline ($0.1 < P < 0.15$) or tyramine ($P = 0.125$).

Harmine, 4×10^{-7} M, did not potentiate the action of noradrenaline ($0.15 < P < 0.2$) but potentiated that of tyramine (log. dose ratio $= 1.10$, $P \simeq 0.001$): it also caused a potentiation of the action of amphetamine ($P = 0.0125$).

Isocarboxazide, 3.2×10^{-6} M, influenced responses to noradrenaline, tyramine and amphetamine in exactly the same way as did harmine.

In these concentrations none of these drugs affected the tone of the tracheal muscle, though they did cause relaxation in higher concentrations.

Summation experiments

Both cocaine, 1.32×10^{-4} M, and cooling to 22.5° C failed to cause a significant further potentiation of the response to noradrenaline when applied during a steady state with the highest concentration of phenoxybenzamine. Cocaine, in the same concentration, also failed to cause a significant further potentiation when applied during a steady state with the highest concentration of desipramine.

DISCUSSION

Stafford (1963) found cocaine to be quick acting as a noradrenaline potentiator on the rabbit isolated atria while phenoxybenzamine was slow. Hrdina & Garattini (1966) using the isolated renal artery of the rat and Ursillo & Jacobson (1965) using the vas deferens found cocaine to be quick acting while desipramine was slow. In the present

experiments cocaine, metanephrine and cooling formed a group of rapidly active potentiating procedures, while desipramine, guanethidine and phenoxybenzamine were slow. This grouping of the procedures does not correlate with that found by examining either the slopes of their log. concentration: effect curves or their relative potentiation of isoprenaline. The different time courses may reflect differences in the site of action of the potentiating drugs: it is possible that a rapid effect may be associated with an extracellular site of action and a slow effect with an intracellular one. If the drug causes potentiation while in the act of penetrating, one would expect a more rapid action.

Dutta, Marks & Willman (1966) have examined the distribution of ^{3}H-cocaine in dogs and found no specific organ accumulation and a low tissue/plasma ratio. Such observations are consistent with an extracellular site of action and contrast with the suggestion that guanethidine has to accumulate within cells in order to exert its effects (Carlsson & Waldeck, 1965 ; Brodie, Chang & Costa, 1965). Schanker & Morrison (1965) examined the uptake of guanethidine by rat heart slices and found, for a concentration of 2 μg/ml. (1.01×10^{-5} M) equilibration in 2 to 3 hr and a half time of 48 min—values close to those for potentiation of noradrenaline by guanethidine in the present study. Giachetti & Shore (1966) have identified a concentrating mechanism for m-octopamine in heart slices which they locate intracellularly and which is more sensitive to guanethidine (5×10^{-6} M) than the concentrating mechanism for metaraminol, which they locate at the cell membrane.

Phenoxybenzamine may be slow acting for other reasons. Harvey & Nickerson (1953) attributed the slow development of α-blockade by dibenamine to the slow formation of ethyleneiminium ions in aqueous solution. Thiosulphate reacts quickly and in equimolar amounts with ethyleneiminium ions. In the present experiments phenoxybenzamine-potentiation was only markedly inhibited by thiosulphate when the latter was present at 1,000-times the equimolar concentration. It seems that ethyleneiminium ions formed in the bath fluid are not responsible for the potentiation ; perhaps they are formed intracellularly (out of reach of the thiosulphate) or perhaps the result merely reflects the known (Harvey & Nickerson, 1953) ability of thiosulphate to react slowly with the parent β-haloalkylamine.

The maximum obtainable potentiation of noradrenaline on the guinea-pig isolated tracheal chain preparation is 46-fold ; that is, cocaine, desipramine and phenoxybenzamine each cause a parallel leftward shift of the log. concentration: effect curve for noradrenaline so that a 50% relaxation is obtained after the drug with 1/46th of the concentration previously required. Even if there is a quantitative correlation between the degree of noradrenaline potentiation and the inhibition of its uptake, the possibility to produce large potentiation need not imply that there is a large uptake of noradrenaline. It is only necessary that the rate of uptake should be high *relative to* the rate of entry of exogenous noradrenaline into the biophase. There is some indirect evidence that the rate of penetration of exogenous noradrenaline into the biophase of the trachea is slow.

Foster (1964) showed that transmural stimulation of the guinea-pig trachea in the presence of atropine caused a relaxation which was fully developed in 30 sec: this elongation of the tracheal muscle was similar in size to that which took 15 min to develop in each tracheal ring by addition of exogenous noradrenaline in the present experiments (0·8 mm). Thus this slowness of the tracheal muscle to relax in response

to exogenous noradrenaline does not result from slowness of the contractile proteins to respond to relaxant stimuli. If noradrenaline penetration is similar to that of ^{14}C-sorbitol, it may be relevant that ^{14}C-sorbitol penetrated remarkably slowly. The tracheal ^{14}C-sorbitol space was only about 19% equilibrated after 1 min and 95% equilibration was achieved only after 15 min (it is realized that the preparation is heterogeneous and that the rate of equilibration of the muscle biophase may be very different from that of the whole preparation).

Thus it may be that the uptake of noradrenaline in the trachea is slow and, if the activity of the uptake process is related to the density of adrenergic innervation, the fluorescence microscopy of the trachea by Hollands & Vanov (1965) is relevant in showing only a low density of adrenergic fibres.

Desipramine and cocaine were similar to each other in having log. concentrations: effect curves of shallow slope. In contrast phenoxybenzamine, guanethidine and metanephrine had steep slopes. On the rabbit aorta, Maxwell *et al.* (1966) similarly found a steeper slope for guanethidine than for cocaine when these drugs acted as potentiating drugs. Since they had similar slopes as inhibitors of noradrenaline binding the authors concluded that a correlation does not exist between inhibition of uptake and potentiation. Instead they advanced the hypothesis that potentiation involves a change in the properties of adrenergic receptors. However, they measured potentiation as the percentage increase in response to a standard concentration of noradrenaline rather than as log. dose ratio. Measured in the latter way the response of the rabbit aorta has a very limited capacity for potentiation—only two-fold (Furchgott, Kirpekar, Rieker & Schwab, 1963). This limited maximum makes the rabbit aorta an unsuitable preparation for seeking such a correlation, because small differences in potentiation could easily be lost in the variability between tissues. It has a further disadvantage in being equipped with α-receptors; several of the drugs one would like to use in examining the correlation between amount of potentiation and degree of inhibition of uptake are α-blocking agents—for example, desipramine, methylphenidate, phenoxybenzamine and phentolamine. The α-blocking activity of desipramine caused considerable interference in the potentiation experiments of Ursillo & Jacobson (1965) on the rat vas deferens. It is hoped that the large available potentiation and the lack of detectable α-receptors (Foster, 1966) in the guinea-pig isolated tracheal chain, combined with the use of a battery of potentiating procedures, will make this a more suitable tissue in which to seek a clear-cut differentiation between the hypotheses for the origin of potentiation.

There is evidence that all of the six potentiating drugs which I have used inhibit noradrenaline and/or adrenaline uptake *in vitro* (Burn & Burn, 1961; Iversen, 1963, 1965a, b, c; Anden, Corrodi, Ettles, Gustafsson & Persson, 1964; Burgen & Iversen, 1965; Cervoni, Kirpekar & Schwab, 1966; Green & Miller, 1966; Maxwell *et al.*, 1966; and West, Bhagat, Dhalla & Shein, 1966). In Fig. 9 I have plotted the published data of Iversen (1963, 1965b, c) and Burgen & Iversen (1965) on the inhibition of uptake$_1$ in the rat isolated heart by these six drugs for comparison with Figs. 6 and 7. The relative potencies of the drugs seem to correspond in the two experimental situations and it also seems as though metanephrine may have a steeper log. concentration: effect curve than desipramine or cocaine, though the significance of this cannot be assessed in the absence of measurements of error.

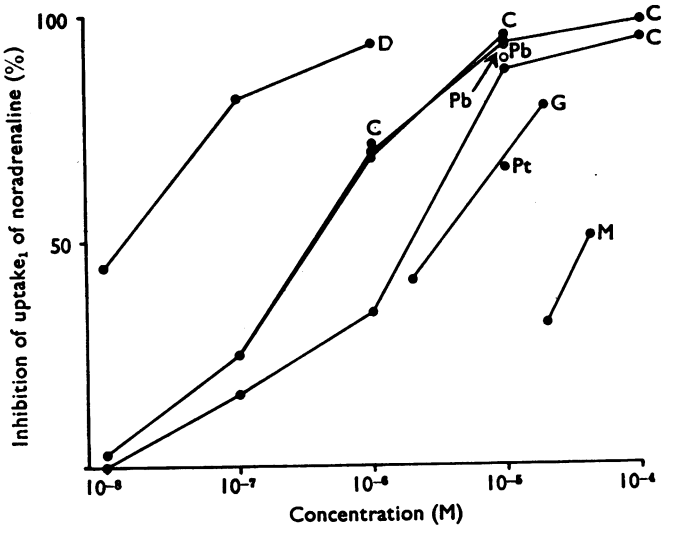

Fig. 9. Log. concentration: effect curves for the inhibition of uptake$_1$ of noradrenaline by desipramine (D), cocaine (C), phentolamine (Pt), phenoxybenzamine (Pb), guanethidine (G) and metanephrine (M), culled from the publications of Iversen (1963, 1965b, c) and Burgen & Iversen (1965).

There are few reports suggesting a different mechanism of action for desipramine and cocaine on the one hand and phenoxybenzamine, guanethidine and metanephrine on the other. The experiments of Giachetti & Shore (1966) on the effects of guanethidine on uptake of metaraminol and m-octopamine have been referred to already. Eisenfeld, Krakoff, Iversen & Axelrod (1967) have shown that phentolamine and phenoxybenzamine have a significant effect, when superimposed on inhibition of uptake by cocaine, which they interpret as prevention of access of noradrenaline to metabolizing enzymes. Day & Stockbridge (1964) have examined the effects of some of these drugs on the uptake of various amines by mast cells and find quite a different pattern of effects for cocaine on the one hand and phenoxybenzamine and guanethidine on the other.

Foster (1963) has previously shown that cocaine potentiated the action of noradrenaline more than that of adrenaline and failed to potentiate that of isoprenaline, while guanethidine potentiated noradrenaline and adrenaline equally and caused a smaller but significant potentiation of isoprenaline. The present examination of the potentiation of isoprenaline relative to that of noradrenaline split the drugs into more groups than did the foregoing.

Other previous examinations of isoprenaline potentiation by these drugs have been negative. Stafford (1963) saw no potentiation of the effect of isoprenaline with either cocaine, guanethidine or phenoxybenzamine on rabbit isolated atria at 30–31° though phenoxybenzamine did induce a small potentiation of the action of isoprenaline on the rabbit duodenum at 35° C. Anden *et al.* (1964) also failed to see potentiation of isoprenaline by either cocaine or phenoxybenzamine on the rabbit isolated heart. They also failed to detect an uptake of isoprenaline but it should be noted that they employed a 5 min washout after perfusion of the isoprenaline—a procedure which Callingham & Burgen

(1966) have shown would clear a large fraction of any isoprenaline which had been taken up.

Cooling potentiates the action of both agonists equally and clearly but does not affect that of aminophylline. This suggests that the process(es) affected by cooling may be the same as that affected by metanephrine, phenoxybenzamine and guanethidine. Paton (1966) and Green & Miller (1966) have shown cooling to reduce the uptake of noradrenaline and adrenaline. Day & Stockbridge (1964) and Giachetti & Shore (1966) have likened this action of cooling more to that of cocaine than to that of guanethidine.

The demonstration of significantly different slopes for the log. concentration: effect lines of noradrenaline and isoprenaline is unusual. It has considerable theoretical interest whichever interpretation is placed upon it. It may represent differences in the interaction of the two drugs with a single population of β-receptors ; if so it implies that isoprenaline, in spite of its higher affinity, has a lower efficacy than noradrenaline (Stephenson, 1956). It may indicate that the receptor population is not homogeneous in its interaction with these two drugs: or it may represent distortion of the slope of the noradrenaline line (and possibly of both) by differences in the biophase handling of the two drugs—for example, low concentrations of noradrenaline may be taken up more avidly than either high concentrations of noradrenaline or any concentration of isoprenaline. Foster (1966) has discussed the related problem of different degrees of antagonism of noradrenaline and isoprenaline by the same concentration of β-blocking agent.

Foster (1963) has previously shown that iproniazid, 20 μg/ml., potentiated the action of tyramine without affecting that of noradrenaline and that catechol, 10 μg/ml., failed to potentiate noradrenaline. The present results with harmine, isocarboxazide and pyrogallol confirm and extend these findings. Clearly, enzymatic breakdown of exogenous noradrenaline in the trachea does not lower its biophase concentration sufficiently for inhibition of this breakdown to produce 46-fold potentiation. Eisenfeld *et al.* (1967) have shown phentolamine and phenoxybenzamine to be inactive against either monoamine oxidase or catechol O-methyl transferase *in vitro*.

The potentiation of amphetamine by monoamine oxidase inhibitors *in vitro* confirms the results of Smith (1966) and suggests that, although monoamine oxidase does not cause significant destruction of exogenous noradrenaline in this preparation, it has important effects on endogenously released noradrenaline.

SUMMARY

1. The time courses of potentiation of the action of noradrenaline by various concentrations of cocaine, desipramine, guanethidine and phenoxybenzamine have been determined. Phentolamine and metanephrine were also examined.

2. Thiosulphate only reduced the action of phenoxybenzamine when present in great excess.

3. The amount of potentiation attained when a steady state was reached was measured, so that log. concentration: effect curves for this potentiating action could be drawn. The slopes of these curves showed cocaine and desipramine to act differently from phenoxybenzamine, guanethidine and metanephrine.

4. The log. concentration: effect curves for potentiation of the response to isoprenaline were also obtained. The potentiation of the action of isoprenaline relative to that of noradrenaline again showed cocaine and desipramine to act similarly, while the other four drugs fell into three groups—metanephrine being the most active.

5. Cooling caused an equal potentiation of the responses to noradrenaline and isoprenaline.

6. Log. concentration: effect curves for the relaxation of tracheal muscle induced by noradrenaline and isoprenaline had significantly different slopes.

7. Pyrogallol did not potentiate the effects of noradrenaline or tyramine.

8. Harmine and isocarboxazide did not affect the response to noradrenaline but did potentiate those to tyramine and amphetamine.

9. These results are discussed in relation to the hypotheses for the site and mechanism of action of agents potentiating *in vitro* the action of catcholamines.

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