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A CORRELATION BETWEEN INHIBITION OF THE UPTAKE OF ^3H FROM (±)-^3H-NORADRENALINE AND POTENTIATION OF THE RESPONSES TO (−)-NORADRENALINE IN THE GUINEA-PIG ISOLATED TRACHEA

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Foster (1967) has recently speculated that the guinea-pig tracheal chain might be a suitable isolated tissue in which to seek a correlation between inhibition of uptake and potentiation of the effects of noradrenaline. This was suggested because a variety of drugs caused a concentration and time-dependent potentiation up to a very large dose-ratio (about 46). Cooling also caused a graded potentiation.

The uptake of ^3H from (±)-^3H-noradrenaline has now been measured in the guinea-pig isolated trachea and the effects of desipramine, cocaine, phentolamine, (±)-metanephrine, guanethidine, phenoxybenzamine and cooling on this uptake assessed. The results of this assessment have been correlated with the previously reported potentiation of the action of (−)-noradrenaline caused by these procedures.

METHODS

The Krebs solution, drugs and guinea-pigs used were as described by Foster (1967).

Preparation of the trachea and method of incubation

A guinea-pig was stunned and exsanguinated. The whole trachea was removed and opened with scissors by an anterior midline cut. It was then divided into four or five equal fragments by transverse cuts. These fragments were strung loosely together on a short length of Terylene thread by piercing one cartilage plate of each. This thread was then tied into a loop which was attached to the end of a polyethylene bubbling tube transmitting 95% oxygen and 5% carbon dioxide from a manifold.

A rack with positions for twelve test tubes was mounted in a water bath at 37.5° C so that six tracheae could be handled at once. A test tube containing 10 ml. of Krebs solution was placed in this rack and the bubbling tube, and attached trachea, plunged into it. When it was necessary to change the bathing fluid, the bubbling tube with its attached trachea was lifted from the test tube and transferred to one containing fresh solution, which had been positioned 1 min before.

The interval between making the preparation and exposing it to a radioactive solution was never less than 45 min and the Krebs solution was changed at least every 30 min up to this time.

Exposure to radioactive solutions

The tissue was exposed to either ^{14}C-sorbitol or (±)-^{3}H-noradrenaline in 4 ml. of Krebs solution. This exposure usually lasted 15 min: when complete, 1 ml. of incubation medium was taken for radioassay.

The trachea was removed either immediately or after various degrees of washing. The tracheal fragments were rapidly but firmly blotted. They were slipped off their thread, weighed and placed in a mortar containing 2 ml. of 0.4 N perchloric acid and 1.5 g of acid-washed silver sand.

The tissue was ground with a pestle until no coarse fragments remained and transferred to a centrifuge tube. The pestle and mortar were rinsed with 3 ml. of 0.4 N perchloric acid and the washings transferred to the centrifuge tube. The tube was allowed to stand at room temperature for at least 15 min, shaken and centrifuged at 3,820 *g* for 10 min. One millilitre of clear supernatant was placed in a counting vial together with sufficient 5 N sodium hydroxide to neutralize the acid (otherwise severe quenching occurred).

Ten millilitres of phosphor was added to each counting vial. The phosphor had the composition described by Bray (1960) except that dimethyl-POPOP was substituted for POPOP (1,4-di-(2-(5-phenyloxazolyl))-benzene).

Samples were counted for 10 min or until 20,000 counts had accumulated (whichever was the shorter) using a liquid scintillation coincidence counter. Automatic external standardization was employed to correct for quenching. The mean ^{3}H-counting efficiency of this system using 1 ml. aqueous samples was 10%; it was a little higher for medium (Krebs) samples and a little lower for tissue extract (neutralized 0.4 N perchloric acid) samples.

Inhibition of cellular uptake of ^{3}H from (±)-^{3}H-noradrenaline by drugs

A stock of Krebs solution containing inhibitor was prepared and used throughout the experiment—thus the trachea was placed in this solution immediately after preparation for preincubation, exposure to (±)-^{3}H-noradrenaline and subsequent washing. Fresh Krebs stocks of phentolamine, metanephrine and phenoxybenzamine were prepared at hourly intervals—the other drugs were regarded as stable. Only in the case of desipramine, 1.2×10^{-5}M for 25 min, was this procedure varied—a short initial incubation in normal Krebs solution was used to wash blood and mucus from the trachea.

The time of contact with the inhibitor was measured from the moment of first exposure to the drug until the end of the 15 min incubation with (±)-^{3}H-noradrenaline.

The effect of one concentration of one drug on the uptake of ^{3}H from (±)-^{3}H-noradrenaline was measured on at least five tracheae and these five were distributed over two or three experimental runs. The effect of cooling was, however, measured on six tracheae simultaneously.

Drugs

(±)-^{3}H-Noradrenaline hydrochloride (1.9 c/mmole) was obtained from the Radiochemical Centre, Amersham. It was reported to have radiochemical purity greater than 96%. It was diluted with N-HCl to yield a stock solution of 400 μc/ml. This was further diluted with distilled water to produce a working stock such that 0.1 ml. added to 4 ml. of Krebs solution gave a final concentration of 90 nc/ml. and 4.7×10^{-8}M (±)-noradrenaline; 0.1 ml. ascorbic acid solution, 2.3×10^{-2}M, was added to produce a final concentration of 5.7×10^{-4}M.

^{14}C-Sorbitol was obtained from the Radiochemical Centre, Amersham. It was diluted with distilled water to yield a working stock such that 0.1 ml. added to 4 ml. Krebs solution gave a final concentration of 100 nc/ml. and 1.56×10^{-5}M sorbitol.

These stock solutions were stored at −10° C.

Other drugs were prepared as described previously (Foster, 1967).

Expression of results

Although only ^{3}H was actually measured, all results of uptake experiments are quoted in terms of (±)-^{3}H-noradrenaline.

The tissue to medium ratio was calculated as noradrenaline content of tissue (in pmole/g) divided by the concentration in medium (pmole/ml.). It therefore has dimensions of ml./g and is identical with the apparent noradrenaline space. The figures quoted in the text are in these dimensions.

Statistical methods

Normal parametric methods were used as well as, where appropriate, non-parametric methods according to Siegel (1956). All measures of variation of the means quoted are standard errors.

RESULTS

^{14}C-Sorbitol space

After exposure for 15 min to ^{14}C-sorbitol (100 nc/ml., 1.56×10^{-5}M), the mean ^{14}C-sorbitol space of six guinea-pig isolated tracheae was 0.41 ± 0.025 ml./g.

Total tissue (±)-^{3}H-noradrenaline uptake after 15 min exposure to 4.7×10^{-8}M

After exposure for 15 min to (±)-^{3}H-noradrenaline, the guinea-pig isolated trachea contained 121.18 ± 5.27 pmole/g of noradrenaline. One fraction of this, the noradrenaline assumed (on the basis of equality with the bath fluid concentration) to lie in the extracellular fluid space of 0.41 ml./g, namely 19.42 pmole/g, could be subtracted. Thus the amount of (±)-^{3}H-noradrenaline associated with the cellular and structural elements of the trachea was estimated at 101.76 ± 5.21 pmole/g.

Reduction of the apparent ^{14}C-sorbitol space by washing

Figure 1 shows the result of a pilot experiment in which six tracheae were exposed to ^{14}C-sorbitol (100 ng/ml., 1.56×10^{-5}M) for 15 min. One was removed for extraction and assay immediately while the remainder were immersed in one to five changes of 10 ml. Krebs solution—one change every 5 min. Five such 5 min 10 ml. washes seemed to remove most of the sorbitol, so this washing regime was tested more thoroughly. Nine tracheae treated in this way had an apparent ^{14}C-sorbitol space of 0.0052 ± 0.0011 ml./g. This is 1.27% of the mean space at the start of the washing regime. This regime was

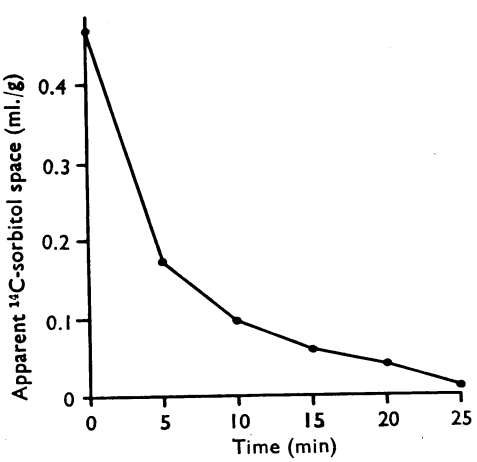

Fig. 1. Reduction of apparent ^{14}C-sorbitol space by washing. Each point represents one trachea. All six were exposed to ^{14}C-sorbitol for 15 min and then washed by immersion in 10 ml. of Krebs solution 0–5 times at 5 min intervals. The apparent ^{14}C-sorbitol space (ml./g) of each trachea is plotted against time (min) since its removal from ^{14}C-sorbitol.

selected for the purpose of removing free drug in the extracellular fluid space of the trachea. The 1.27% of such drug remaining was neglected.

Tissue uptake of (±)-^{3}H-noradrenaline after 15 min exposure to 4.7×10^{-8}M and 5 × 10 ml. washes at 5 min intervals

Tissue uptake measured in this way will be referred to as cellular uptake.

The cellular uptake of (±)-^{3}H-noradrenaline measured in twenty-nine tracheae was 71.75 ± 3.26 pmole/g. The individual values were normally distributed about this mean.

The mean tissue : medium ratio was 1.52.

The washing regime removed only 41% of the (±)-^{3}H-noradrenaline in contrast to 98.7% of the ^{14}C-sorbitol.

Dependence on time of cellular uptake of (±)-^{3}H-noradrenaline

Figure 2 shows that the cellular uptake of (±)-^{3}H-noradrenaline increased linearly with time up to 15 min.

Fifteen minutes was selected as the time for subsequent exposures to (±)-^{3}H-noradrenaline.

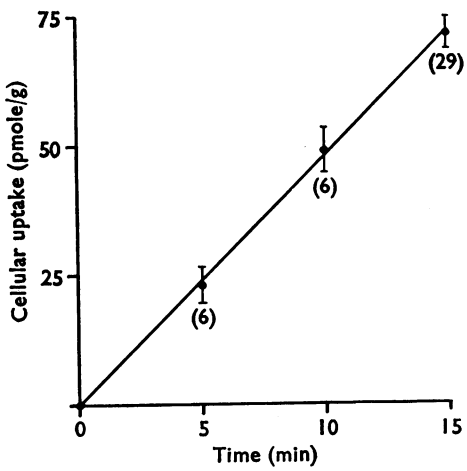

Fig. 2. Dependence of cellular uptake of (±)-^{3}H-noradrenaline on time of exposure. Cellular uptake (pmole/g) was measured as the ^{3}H content of the trachea after 5, 10 or 15 min exposure to 4.7×10^{-8}M (±)-^{3}H-noradrenaline followed by 5 × 10 ml. washes at 5 min intervals. Means and standard errors are plotted. Number of tracheae in each group is shown in brackets.

Cellular uptake of (±)-^{3}H-noradrenaline by the "cartilaginous" part of the trachea

Six tracheae were divided into two parts by two cuts made longitudinally on either side of the muscle. One part was thus composed of cartilage, connective tissue and mucosa while the other was smooth muscle and mucosa with a variable admixture of cartilage and connective tissue. The cellular uptake of (±)-^{3}H-noradrenaline by each part is shown in Table 1 along with a figure for the whole tracheae derived by summation.

The mean tissue : medium ratio rose to 2.2 by excluding some of the cartilage and connective tissue from the preparation.

Cellular uptake of (±)-^{3}H-noradrenaline in tracheae exposed to drugs and to cooling

The data on potentiation of the actions of (−)-noradrenaline (Foster, 1967) were used

TABLE 1
CELLULAR UPTAKE OF (±)-^{3}H-NORADRENALINE BY THE PARTS OF LONGITUDINALLY BISECTED TRACHEAE

Cellular uptake was measured as pmole of ^{3}H/g in each part of six tracheae. The uptake by each whole trachea was computed by summing the uptakes of its parts.

	Uptake (pmole/g)	±S.E.
"Muscle" part	104·37	12·85
"Cartilage" part	68·49	9·00
Whole trachea	80·99	9·18

to select the conditions for examination. All six drugs and cooling were examined at a concentration or temperature which would be expected to produce 50% of the mean maximum possible potentiation—namely log. dose ratio = 0.825: this concentration was left in contact with the tissue until equilibrium could be assumed. The drugs (and temperature) which caused near-maximal potentiation when given in adequate concentration—desipramine, cocaine, phenoxybenzamine and guanethidine—were examined at this concentration at equilibrium. For the slower acting of these—desipramine, phenoxybenzamine and guanethidine—the same concentration was also examined after a time of contact which would be expected to allow development of potentiation to log. dose ratio = 0.825. Cocaine and cooling were examined also at one intermediate point each.

Table 2 presents all the data on uptake of (±)-^{3}H-noradrenaline obtained in these various conditions, together with comparable information on potentiation of the effect of (−)-noradrenaline taken from the data of Foster (1967). In all cases but one, exposure to conditions which would be expected to increase the amount of potentiation reduced the mean uptake, though this reduction did not always achieve statistical significance. When each drug was considered separately there was a clear dependence of inhibition of uptake on concentration. Only in the case of cooling from 23° to 18° C did the mean uptake fail to fall. The other notable points where statistical significance was not achieved were the comparisons between a high concentration of each of desipramine, phenoxybenzamine and guanethidine for long and short times, though the mean uptakes did change in the right direction.

Correlation between inhibition of cellular uptake of (±)-^{3}H-noradrenaline and potentiation of the effect of (−)-noradrenaline

Figure 3 compares the effects of cocaine, guanethidine and cooling on potentiation of the effect of (−)-noradrenaline and on the cellular uptake of (±)-^{3}H-noradrenaline. The uptake scale has been fixed with reference to the potentiation (log. dose ratio) scale using the interrelationship defined by the best fitting straight line in Fig. 4. These three examples are representative in the sense that they show both how well and how badly the two sets of results of individual treatments agreed.

The mean slopes of the upper parts of the log. concentration: uptake lines of phenoxybenzamine and guanethidine were almost twice as steep as those of desipramine and cocaine though this difference was not statistically significant.

Figure 4 shows the mean cellular uptake of (±)-^{3}H-noradrenaline plotted against the mean potentiation of the effect of (−)-noradrenaline for all the different treatments used under equilibrium conditions. Points representing tissues treated with the same drug

TABLE 2

EFFECTS OF DRUGS AND COOLING ON POTENTIATION OF THE ACTION OF (−)-NORADRENALINE (MEASURED AS LOG. DOSE RATIO) AND CELLULAR UPTAKE OF (±)-^{3}H-NORADRENALINE (MEASURED AS pmole OF ^{3}H/g)

The data on potentiation were taken from Foster (1967). N=number of tracheae in each group. S= Student's t test was applied to the difference in mean cellular uptake between adjacent pairs of treatments in each group: *, $P<0{\cdot}05$; †, $0{\cdot}1>P>0{\cdot}05$; no symbol, $P>0{\cdot}1$.

	Concentration	Time	Potentiation		Cellular uptake			
Drug	(M)	(min)	L.D.R.	±S.E.	N	S	pm/g	±S.E.
Control 37·5° C	—	—	0	0	29		71·75	3·26
30° C	—	—	0·375	0·040	6	*	52·51	4·32
23° C	—	—	0·825	0·055	6	*	30·01	5·51
18° C	—	—	1·320	0·035	11		31·44	1·54
							Control	
Desipramine	$7{\cdot}50\times10^{-8}$	240	0·825	0·065	8	*	30·84	6·16
Desipramine	$1{\cdot}20\times10^{-5}$	160	1·665	0·065	6	*	11·78	3·61
Desipramine	$1{\cdot}20\times10^{-5}$	25	0·825	—	6		16·75	4·62
							Control	
Cocaine	$1{\cdot}06\times10^{-6}$	120	0·825	0·055	10	*	52·21	4·44
Cocaine	$1{\cdot}25\times10^{-5}$	120	1·250	0·035	5	*	22·44	5·86
Cocaine	$1{\cdot}32\times10^{-4}$	80	1·555	0·050	13	†	12·67	2·72
							Control	
Phentolamine	$1{\cdot}42\times10^{-5}$	160	0·825	0·100	6	*	43·75	5·27
							Control	
Metanephrine	$4{\cdot}32\times10^{-5}$	120	0·825	0·050	9	*	43·45	6·04
							Control	
Guanethidine	$1{\cdot}80\times10^{-6}$	240	0·825	0·100	7	*	21·79	4·97
Guanethidine	$1{\cdot}01\times10^{-5}$	200	1·470	0·060	6	*	9·59	3·08
Guanethidine	$1{\cdot}01\times10^{-5}$	60	0·825	0·080	6		19·36	7·04
							Control	
Phenoxybenzamine	$5{\cdot}28\times10^{-7}$	240	0·825	0·115	6	*	29·36	3·91
Phenoxybenzamine	$3{\cdot}30\times10^{-6}$	240	1·790	0·065	8	*	16·87	2·84
Phenoxybenzamine	$3{\cdot}30\times10^{-6}$	70	0·825	0·090	6	†	22·44	2·37

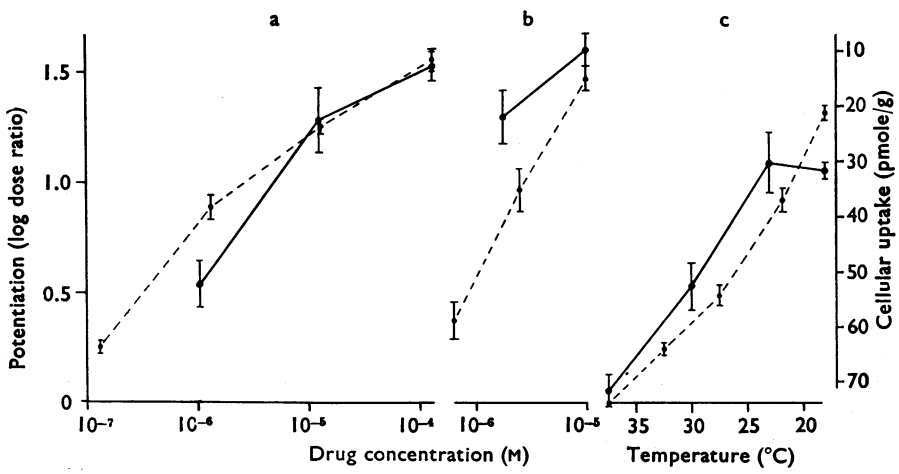

Fig. 3. Log. concentration : effect curves for (a) cocaine and (b) guanethidine. (c) Temperature : effect curves. Two effects are plotted in each panel: (●·····●), potentiation of the action of (−)-noradrenaline (measured as log. dose ratio) taken from Foster (1967); (●——●), cellular uptake of (±)-^{3}H-noradrenaline (measured as pmole of ^{3}H/g). Means and standard errors of at least six experiments are shown.

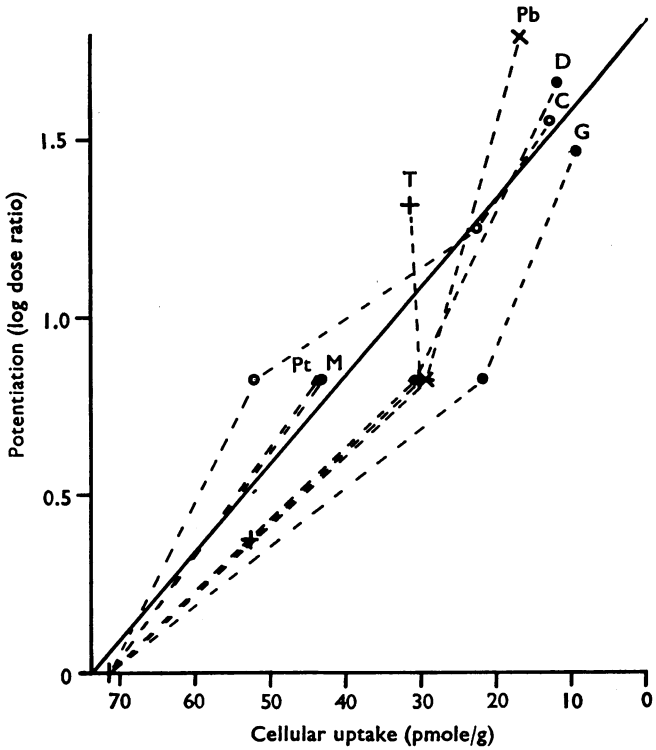

Fig. 4. Correlation between mean potentiation of the action of (−)-noradrenaline (measured as log. dose ratio) and mean cellular uptake of (±)-^{3}H-noradrenaline (measured as pmole of ^{3}H/g) under equilibrium conditions. Each point is the mean of at least five experiments. T=temperature, D=desipramine, C=cocaine, Pt=phentolamine, G=guanethidine, Pb=phenoxybenzamine, M=(±)-metanephrine. The best fitting straight line is included.

are joined by straight lines, as are points representing cooled tissues. The best fitting straight line was computed from all the points and is included. The relationship between potentiation and uptake represented by this line was used for the vertical scales in Fig. 3.

A test of correlation was applied to all these points. Because the plotted points are means on both axes, and because there is no reason to predict a linear relationship between them, a non-parametric test of correlation was applied—the Kendall rank correlation coefficient (Siegel, 1956). The correlation coefficient (τ) was 0.602 and the probability associated with this value was $P \leqslant 0.0009$. One may therefore conclude that the two variables—inhibition of cellular uptake of (±)-^{3}H-noradrenaline and potentiation of the effect of (−)-noradrenaline—are associated in the population from which these sample means were drawn.

DISCUSSION

The ^{14}C-sorbitol space, measured after 15 min contact, of 0.41 ± 0.025 ml./g for this preparation of guinea-pig trachea in the test tube agrees well with the value of 0.421 ± 0.022 ml./g found previously (Foster, 1967) for the tracheal chain preparation in the tissue bath (when it represented 95% equilibration). This was assumed to be the volume of extracellular distribution of free noradrenaline after the same time of contact, for the molecular weights are quite close (182 and 169). If the concentration of noradrenaline in the extracellular fluid space after 15 min contact equals that in the medium, then the amount of extracellular free noradrenaline can be calculated and subtracted from the total tissue content to yield the amount of noradrenaline associated with the cellular and structural elements of the trachea.

Of the 121.18 pmole/g (±)-^{3}H-noradrenaline present in the preparation after exposure to a concentration of 4.7×10^{-8}M for 15 min, 16% was calculated to be free drug in the extracellular fluid space. A further 25% was more closely associated with the cellular and structural elements but was cleared by five washes over 25 min. The remaining 59% was more firmly bound to the tissue in that it resisted the washing procedure ; this fraction of the total content will be referred to as the cellular uptake. It seemed most profitable to examine the effects of drugs on this cellular uptake, for Iversen (1963) employed a washing period after perfusion of the rat heart with (±)-^{3}H-noradrenaline and an apparent correspondence between the relative potencies of several drugs as inhibitors of uptake and as noradrenaline-potentiators in the guinea-pig isolated trachea has been reported (Foster, 1967).

It is interesting that Maxwell, Wastila & Eckhardt (1966) found a similar fraction of the total (±)-^{3}H-noradrenaline taken up by the rabbit isolated aortic strip to resist washing.

The ratio of the cellular content of (±)-^{3}H-noradrenaline to the concentration in the medium (tissue : medium ratio) was small—1.52. The isolated trachea did not generate a tissue : medium ratio of the order of that found by Iversen (1963) in the rat isolated heart. There are, however, several reports of ratios of about this size in tissue slices after 15 min contact (Dengler, Michaelson, Spiegel & Titus, 1962 ; Dengler, Wilson, Spiegel & Titus, 1962 ; Green & Miller, 1966). The data given by Maxwell *et al.* (1966) allow calculation of the ratio in the rabbit isolated aorta—it was 0.5 after contact for 10 min.

The rate of cellular uptake into the trachea was constant over the 15 min examined. This agrees with the experience of others using tissue slices or immersed (rather than perfused) isolated tissues (Dengler, Michaelson *et al.*, 1962 ; Dengler, Wilson *et al.*, 1962 ; Ross & Renyi, 1964 ; Green & Miller, 1966 ; Maxwell *et al.*, 1966). Maxwell *et al.* (1966) found that the rabbit isolated aorta bound approximately 25% per minute of the total diffusible radioactivity in the strip at a bath concentration of 2×10^{-8}M: the cellular uptake in the guinea-pig isolated trachea amounted to 24.6% per min of the free (±)-^{3}H-noradrenaline in the extracellular fluid, at a medium concentration of 4.7×10^{-8}M.

In an effort to see whether the cellular uptake of (±)-^{3}H-noradrenaline by the trachea was localized in the area of the smooth muscle it was measured in bisected tracheae. The " muscle " part had a significantly higher uptake than the " cartilaginous " part such that the ratio of mean tissue to medium ratio rose to 2.2 by excluding some of the cartilage and connective tissue from the preparation. If there are more adrenergic nerve endings in the smooth muscle than in the cartilage, it is surprising that the " cartilaginous " part took up so much noradrenaline. Hollands & Vanov (1965) found that the guinea-pig trachea " showed less specific fluorescence than all other tissues examined. Sparsely scattered nerve fibres exhibiting green fluorescence were found in the perivascular spaces." If the uptake of noradrenaline is quantitatively dependent on the density of adrenergic nerve terminals the low tissue : medium ratio could have been predicted (Foster, 1967). The relatively high uptake of the " cartilaginous " part may reflect the density of vasomotor nerves in this fragment, or it may represent an extraneuronal binding of noradrenaline. Avakian & Gillespie (1968), using quantitative fluorescence microscopy,

have shown an affinity of noradrenaline for collagen but this only began at much higher medium concentrations than those used here, and was easily removed by washing for 15 min.

The purpose of this study was to investigate the hypothesis that there is a correlation between the inhibition of uptake of (−)-noradrenaline and potentiation of its effect *in vitro*. The technique has been to correlate the inhibition of uptake of ^{3}H from a solution of (±)-^{3}H-noradrenaline with potentiation of the effect of (−)-noradrenaline. The assumption must be made that the uptake of (±)-^{3}H-noradrenaline is a fair model of that of (−)-noradrenaline but it is still only ^{3}H uptake that has been measured—not that of noradrenaline. It is further assumed that, although the ^{3}H may not be a label of noradrenaline once inside the cell (as a result of metabolism), it was at the moment of entry. The reason behind this supposition is that inhibitors of mono-amine oxidase or catechol-*O*-methyl transferase do not potentiate the action of exogenous noradrenaline on the guinea-pig isolated tracheal chain preparation (Foster, 1967). Therefore the activity of these enzymes extracellularly does not reduce the concentration of noradrenaline in equilibrium with the receptors nor, presumably, its access to the uptake mechanism. If the ^{3}H is no longer a label of noradrenaline once within the cell, then the metabolites which it now labels are likely to be more diffusible than noradrenaline and thus the present estimate of noradrenaline uptake in the trachea may be an underestimate. Dengler, Michaelson *et al.* (1962); Dengler, Wilson *et al.* (1962); Green & Miller (1966) and Maxwell *et al.* (1966), however, have all shown that the bulk of the radioactivity taken up by their preparations was in the form of noradrenaline at times of exposure to (±)-^{3}H-noradrenaline varying from 10 to 60 min.

Diffusion into the tissue probably only accounts for a small fraction of the cellular uptake observed in view of the latter's susceptibility to cooling and to drugs. It is relevant that Titus, Matussek, Spiegel & Brodie (1966), using slices of cat ventricle, showed that desipramine inhibits the uptake of (±)-^{3}H-noradrenaline from low medium concentrations but not from high ones. They concluded that desipramine does not prevent cellular entry of noradrenaline by diffusion but does prevent its entry by a carrier mechanism.

Both potentiation of the action and inhibition of the uptake of noradrenaline depend on drug concentration so it is scarcely surprising that there is a correlation between them for any one drug. The strength of the present evidence for a correlation between potentiation and inhibition of uptake of noradrenaline in general lies in the demonstration that the same relationship between them holds for six different drugs and for cooling. This is so despite the suggestion that the drugs probably do not all cause potentiation in the same way (Foster, 1967) and indicates that they do not all cause inhibition of uptake in the same way. The sampling error in the present study is too great to allow a precise statement of the relationship between the parameters studied. It is possible that these parameters may be related by a curve rather than a straight line. This would dictate a cautious approach to the attempt in Fig. 3 to plot log. concentration : effect curves for potentiation and inhibition of uptake on the same graph.

Maxwell *et al.* (1966) using a similar approach, and a tissue with similar characteristics, have denied such a correlation. They found that cocaine, guanethidine and methylphenidate each caused a concentration-dependent reduction in the binding of (±)-^{3}H-

noradrenaline by rabbit isolated aortic strip, and a concentration-dependent increase in the response to noradrenaline. They plotted the potentiation against the decrease in binding and found that the data for all three drugs clustered around a common line. They then set up a mathematical model of the relationship between inhibition of uptake and potentiation and showed that it did not generate curves which fitted their experimental data. They concluded that "it is unlikely that reduction in binding rate can be considered a sole cause of supersensitivity." It must remain a possibility, however, that the mathematical model failed to describe adequately the dynamics of noradrenaline penetration of the aortic strip.

West, Bhagat, Dhalla & Shein (1966) have reported that in atria from reserpinized animals, the rate of uptake of seven different sympathomimetics correlated with the supersensitivity to them induced by cocaine. The present approach to the problem is the reverse of this and it is gratifying that it produces the same end result. This result is consistent with a mass of circumstantial evidence (for a recent review see Iversen, 1967).

Schneider & Gillis (1966) found a depression of (±)-^{3}H-noradrenaline uptake of 11% in cat isolated atria and of 25% in cat ventricle slices when the temperature was reduced from 37° to 30° C (the depression was 25% in the present study). This reduction in uptake was associated with a potentiation of the chronotropic effect of noradrenaline in the atria. The action of isoprenaline was equally potentiated by the same treatment, just as it was in the guinea-pig isolated trachea (Foster, 1967). This raises the question —is there an uptake of isoprenaline which is inhibited by the drugs which potentiate the effects of isoprenaline ? This possibility is currently being investigated.

SUMMARY

1. The uptake of (±)-^{3}H-noradrenaline by the guinea-pig isolated trachea has been measured.

2. Fifty-nine per cent of the total (±)-^{3}H-noradrenaline taken up resisted a washing procedure which cleared the extracellular fluid space ; it was thus firmly bound (cellular uptake).

3. The smooth muscle had a higher cellular uptake than the cartilaginous part of the trachea.

4. Desipramine, cocaine, phentolamine, (±)-metanephrine, guanethidine, phenoxybenzamine and cooling caused concentration (and temperature) dependent reductions in the cellular uptake of noradrenaline.

5. The inhibition of uptake by these procedures correlated with the potentiation of the action of (−)-noradrenaline found previously ; the relationship between inhibition of uptake and potentiation seemed to be the same for all seven procedures.

6. This probably indicates a causal relationship between inhibition of uptake and potentiation of noradrenaline *in vitro*.

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