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Some evidence of the active uptake of noradrenaline in the guinea-pig isolated trachea

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Summary

1. Guinea-pig isolated trachea immersed in a low concentration (50 nM) of (−)-^{3}H-noradrenaline accumulates radioactive material against an apparent concentration gradient.

2. Compartmental analysis based on the decay curve of radioactive material content during washout and its comparison with that of ^{14}C-sorbitol shows that some extracellular noradrenaline is adsorbed. A considerable mass exists in a slowly exchanging compartment, i.e. is retained. This retention is inversely concentration dependent.

3. Over a 25-fold range of concentration the entry of either total radioactive material or noradrenaline followed Michaelis-Menten kinetics.

4. The uptake of radioactive material was sodium-dependent and ouabain-sensitive.

5. The uptake was susceptible to metabolic poisons which deprive the tissue of all available energy but it was not exclusively associated with either oxidative or glycolytic energy supply.

6. Although net uptake could not be distinguished from exchange with endogenous amine, other evidence has been obtained for an active noradrenaline transport system with properties similar to neuronal uptake.

Introduction

Foster (1968) found that when the guinea-pig isolated trachea was exposed to (±)-^{3}H-noradrenaline, radioactive material accumulated in the preparation. The amount retained after washing was reduced by various drugs (e.g. cocaine and desipramine) and by cooling. There was a strong correlation between this inhibition of uptake and the potentiation of the pharmacological action of (−)-noradrenaline by the same procedures (Foster, 1967). These findings were attributed to an active transport process for noradrenaline in the trachea like the neuronal or uptake$_1$ process of Iversen (1963).

This paper describes additional evidence for the existence of this transport process in the guinea-pig trachea. The uptake of radioactive material from (−)-^{3}H-noradrenaline solutions has the characteristics of uphill net transport by a mechanism which is saturable, sodium ion-dependent, ouabain-sensitive and susceptible to metabolic poisons. A preliminary account of this work has already appeared (Foster & O'Donnell, 1971).

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Methods

Preparation of the trachea

The method was similar to that of Foster (1968) but the laryngeal and bronchial halves of the trachea were often used separately, each cut into three equal fragments (mass c. 30 mg). Tissues were immersed in a volume of at least 4 ml medium per trachea so that uptake produced negligible reduction of the substrate concentration.

Media

The ionic composition of the solutions used is shown in Table 1. All the solutions were gassed with 95% O_2 and 5% CO_2 unless otherwise stated.

Washout curves

Data which allow compartmental analysis of the distribution of radioactive material in the trachea were collected. Twelve guinea-pigs were used. To ensure an adequate mass of tissue the 6 fragments of each whole trachea were exposed in one test tube. Krebs solution was the medium used. Four tissues were exposed to ^{14}C-sorbitol (16 μmol/l and 100 μCi/l) and four tissues were exposed to each of two concentrations of (−)-^{3}H-noradrenaline (50 and 6,400 nmol/l, both with 205 mCi/l); the medium contained ascorbic acid (0·6 mmol/l).

At the end of the 15 min incubation period 1 ml of the incubation medium was taken for radioassay. The tissue was briefly immersed in fresh Krebs solution to remove the surface film of radioactive material. It was then (t=0 min) placed in the first of 9 washing tubes each containing 10 ml of fresh Krebs solution; it was transferred to the next tube at 5, 10, 15, 20, 25, 30, 40, 50 and 60 minutes. One ml from each washing tube was taken for radioassay. The tissue was blotted and weighed and an extract was prepared in 5 ml chilled 0·4 N perchloric acid with an Ultra-Turrax homogenizer. The homogenate was centrifuged at 6,000 g for 10 min at 4° C. The clear supernatant was decanted and 1 ml taken for radioassay.

The radioactive content of the trachea at any time, t min, was derived by adding the amount of radioactive material in the tissue extract at 60 min to the cumulative amount found in the washings between t min and 60 minutes. The logarithm of the tissue to medium ratio of radioactive material was plotted against time. The washout curve so obtained was resolved into two exponential functions as described by Riggs (1963).

TABLE 1. *Composition of media used*

Media	Constituents of media (mM)								
	NaCl	$NaHCO_3$	NaH_2PO_4	KH_2PO_4	KCl	$MgSO_4$	$CaCl_2$	Glucose	Sucrose
Krebs solution	118	25	—	1·2	4·75	1·2	2·55	5·55	—
Low Na^+ solution (63 mEq/l)	38	25	—	1·2	4·75	1·2	2·55	5·55	150
Low Na^+ solution (25 mEq/l)	—	25	—	1·2	4·75	1·2	2·55	5·55	220
K^+-free solution	118	25	1·2	—	—	1·2	2·55	5·55	9·5
Glucose-free solution	118	25	—	1·2	4·75	1·2	2·55	—	—

Compartmental analysis was performed by Mr. S. B. Lucas of the Harris College of Further Education, Preston, to whom we are deeply indebted for this assistance. The method used an iterative computer programme. This found values of the clearances, volumes of distribution and initial concentration in the two-compartment model shown in Table 2, such that a model washout curve lying within one standard error of the observed mean washout curve was generated.

Estimation of the uptake-with-retention of radioactivity from (−)-^{3}H-noradrenaline

Half-tracheae were exposed to (−)-^{3}H-noradrenaline for 3·75, 7·5 or 15 minutes. The concentration of (−)-noradrenaline varied between 20 nM and 10,000 nM in the kinetic study and was 50 nM in the study of the effects of modified incubation media. The amount of radioactivity present was 82 μCi/l at the lowest concentration and 205 μCi/l at higher concentrations of noradrenaline. At the end of the incubation period 1 ml of the incubation medium was taken for radioassay. The tissue was transferred to the first of 6 washing tubes each containing 10 ml of fresh medium ; it was transferred to another tube every 5 minutes. After the tissue had been blotted and weighed an extract was prepared in 0·4 N perchloric acid as described in the previous section. One ml of the supernatant was taken for radioassay and the remainder stored at −10° C.

Separation of noradrenaline and its metabolites

Four extracts from similarly treated tracheae were pooled, 0·5 ml taken for radioassay and the remainder brought to pH 6 and subjected to ion exchange chromatography (Iversen, 1963) with a 20 mm by 6 mm diameter bed of Dowex 50W resin (X4, 200–400 mesh, in the Na^+ form). One ml of the aqueous effluent, containing the acidic metabolites, was taken for radioassay. Elution was performed with 11 ml of 1 N HCl. Preliminary experiments, detecting eluted noradrenaline both by the polarographic method of Merrills & Farrier (1967) and by fluorimetric assay of successive fractions, showed that all the noradrenaline was contained in the fraction eluting between 1 and 11 ml. This fraction was therefore collected and 0·5 ml taken for radioassay. The remainder was stored at −10° C.

Radioassay

The phosphor used was that of Bray (1960) slightly modified (Foster, 1969). Up to 1 ml of aqueous sample was added to 10 ml of the phosphor. When the sample was strongly acidic, sufficient 5 N NaOH to neutralize the acid was also added. Samples were counted for 20 min or until 20,000 counts had accumulated. Automatic external standardization was used to correct for quenching.

Fluorimetric assay of noradrenaline

Noradrenaline in the 10 ml of 1 N HCl eluate from each Dowex resin column was estimated by the trihydroxyindole method, essentially as described by Bertler, Carlsson & Rosengren (1958). A Farrand spectrophotofluorimeter was used ; the activating wavelength was 415 nm and fluorescence was read at 515 nm (uncorrected instrumental values). Both internal and external standards were employed.

In 21 experiments 88·2 ± 4·8% of the noradrenaline carried through the neu-

tralization, resin column and assay stages was recovered. Results have not been corrected for this recovery.

Radioactive solutions

(−)-Noradrenaline-7-^{3}H (4·1 Ci/mmol), radiochemical purity greater than 97%, was obtained from the Radiochemical Centre, Amersham. This material showed only one peak of radioactivity corresponding in position with that of noradrenaline after descending paper chromatography on Whatman cellulose phosphate cation exchange paper (P81) with ammonium acetate-acetic acid buffer (0·2 M, pH 6): isopropranolol (2:1, v:v) as developing solvent (Roberts, 1962). Substrate concentrations of 20 or 50 nM were achieved by adding this material to Krebs solution; 0·1 ml of a suitable concentration of nonradioactive (−)-noradrenaline was also added to obtain substrate concentrations higher than 50 nM.

^{14}C-Sorbitol (7 Ci/mol) uniformly labelled and of radiochemical purity greater than 99% was obtained from the Radiochemical Centre, Amersham.

Stock solutions were stored at 4° C.

Statistical analysis

The measure of variation of the mean quoted is the standard error unless otherwise stated. Student's *t* test was used to assess the significance of the difference between means.

Results

Comparison of laryngeal and bronchial halves of trachea

Many uptake experiments were carried out with only one-half of a trachea. Bias due to any systematic differences between laryngeal and bronchial halves was

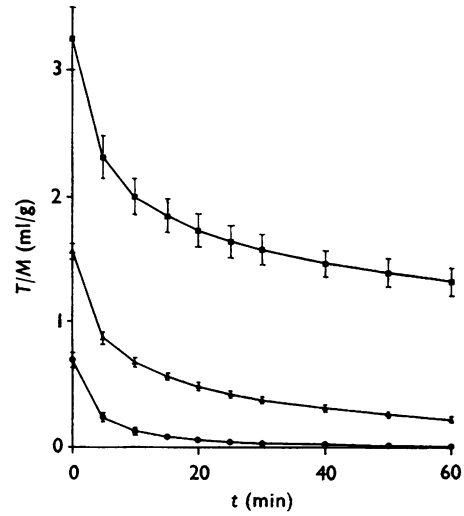


FIG. 1. Washout curves of tissue radioactive material. Each curve is obtained from four tracheae that were exposed for 15 min to ^{14}C-sorbitol 16 μM ●, (−)-^{3}H-noradrenaline, 6,400 nM ▲, or 50 nM ■. At t=0 they were removed, rinsed and the washout process started. The tissue concentration of radioactive material is expressed relative to that in the incubation medium as the tissue to medium ratio. Mean points with their standard errors are plotted.

eliminated prospectively by the use of balanced experimental designs. Such differences were sought retrospectively by comparing the T/M ratios of the two halves after uptake of radioactivity from (−)-^{3}H-noradrenaline at various times and at various concentrations. There was a trend, which did not reach statistical significance in any one group, for the laryngeal half to show a higher T/M than the bronchial half.

Washout curves

The results are shown in Fig. 1. The tissue to medium ratio achieved by exposure to the smaller concentration of noradrenaline exceeded that with the larger concentration, and greatly exceeded that with the reference compound ^{14}C-sorbitol. Some of the radioactive material found in the tissue washed out rather readily, and in the case of sorbitol almost completely, but with noradrenaline a substantial amount was retained in the tissue. The retention seems to be concentration dependent.

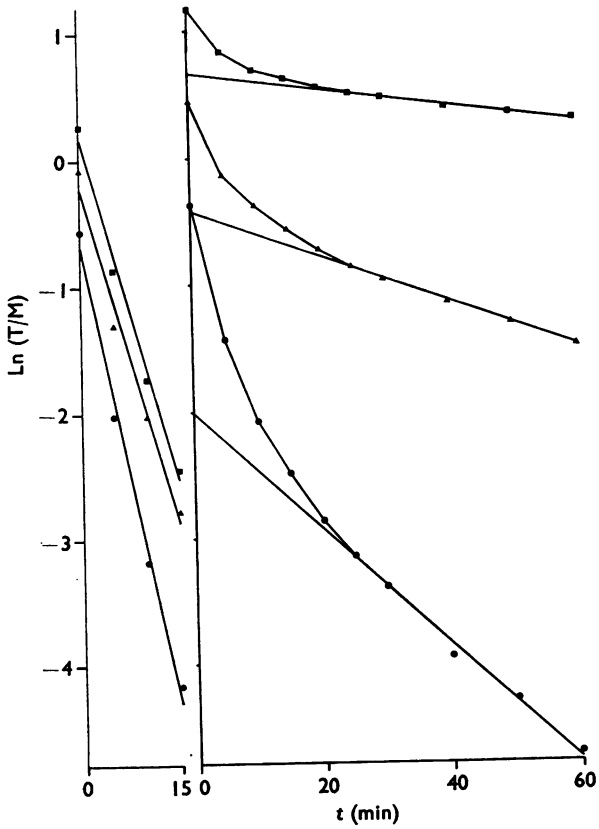


FIG. 2. Semilogarithmic transformation of the mean washout curves of Fig. 1, each resolved into two exponential components illustrated by straight lines. The tissue concentration of radioactive material is expressed relative to that in the incubation medium as the natural logarithm of the tissue to medium ratio. The data from 20 to 60 min were used to define the second exponential component. The difference between the tissue to medium ratios obtained by extrapolating this line back to $t=0$ min and the experimental mean values was used to define the first component, shown offset to the left for clarity.

Decay of tissue ^{14}C-sorbitol

The results are shown in Fig. 2 in the form of a semi-logarithmic plot of the declining tissue content of radioactivity with time of washing. This resolves into two linear components suggesting that two exponential functions describe the clearance of two compartments, a rapidly and a slowly exchanging space. After a 30 min washing period less than 5% of the radioactivity remains, all of it in the slowly exchanging compartment.

Decay of tissue (−)-^3H-noradrenaline

The results are shown in Fig. 2. Again each curve resolves into two linear components. The first component resembles that of sorbitol but the second component shows that noradrenaline is strongly retained, particularly at the lower concentration.

Compartmental analysis

Table 2 presents three double exponential equations which fit these mean washout curves. It also shows values of the clearances, volumes of distribution and initial concentration for the two compartment model.

Kinetic analysis of the accumulation of radioactivity in the tissue in contact with (−)-^3H-noradrenaline

In order to make a Michaelis–Menten analysis the initial rates of uptake are required. To estimate this the cellular uptake of radioactivity after a fixed incubation time was used. Preliminary experiments revealed a linear accumulation from a solution of 50 nM with time up to 15 minutes. However, when the substrate concentration was 6,400 nM the rate of uptake decreased with increasing time

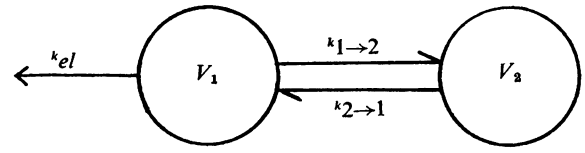
TABLE 2. *Separation of exponents*

$$y_t = Ae^{-\alpha t} + Be^{-\beta t}$$

	1*st exponential decay*			2*nd exponential decay*		
	A (ml/g)	α (min^{-1})	$t_{\frac{1}{2}}$ (min)	B (ml/g)	β (min^{-1})	$t_{\frac{1}{2}}$ (min)
Noradrenaline 50 nM	1·18	0·18	4	1·94	0·006	115
Noradrenaline 6,400 nM	0·79	0·17	4	0·65	0·018	38
Sorbitol	0·50	0·24	3	0·13	0·046	15

Compartmental analysis

	V_1 (ml/g)	k_{el} ((ml/g)/min)	V_2 (ml/g)	Relative conc. in comp. 2 at $t=0$	$k_{1\to2}$ ((ml/g)/min)	$k_{2\to1}$ ((ml/g)/min)
Noradrenaline 50 nM	1·45	0·17	0·30	5·35	0·006	0·001
Noradrenaline 6,400 nM	0·99	0·19	0·21	2·66	0·011	0·004
Sorbitol	0·60	0·17	0·41	0·25	0·005	0·021



k=clearance. el=elimination. V=volume.

over the 15 min examined. Thus incubation times of 3·75 and 7·5 min were chosen for the kinetic experiments. The dependence of uptake-with-retention on concentration of (−)-noradrenaline (over a 500-fold range) was examined at these two incubation times. The results were plotted in the form s/v against s (where s= noradrenaline concentration, v=initial rate of uptake). The mean experimental values were found to lie on a straight line up to s=500 nM where there was an inflection. Above this concentration (1,600 to 10,000 nM) the graph was horizontal (i.e. v increased proportionately with s).

Only the 25-fold concentration range, 20 to 500 nM is considered further. Since the lines derived from rates estimated at 3·75 and 7·5 min did not differ significantly in slope or intercept the results were combined for presentation in Fig. 3. The mean estimate of K_m obtained is 530 nM; the 95% confidence limits show that it is not less than 310 nM. V_{max} is estimated as 70 (pmol/min)/g.

The effect of substrate concentration on metabolism of (−)-^{3}H-noradrenaline

The radioactive material content of pooled extracts of tracheae which had been similarly treated (substrate concentration and time of exposure) was separated by ion exchange chromatography into noradrenaline and acidic metabolite fractions. The results are presented as the proportion (%) of the total radioactivity which is in each form. The data were plotted against log concentration of (−)-noradrenaline to which exposure had occurred and the results from different incubation times (3·75, 7·5 and 15 min) were analysed separately by linear regression. Since the lines obtained did not differ significantly in slope or intercept the data for each fraction were pooled (Fig. 4).

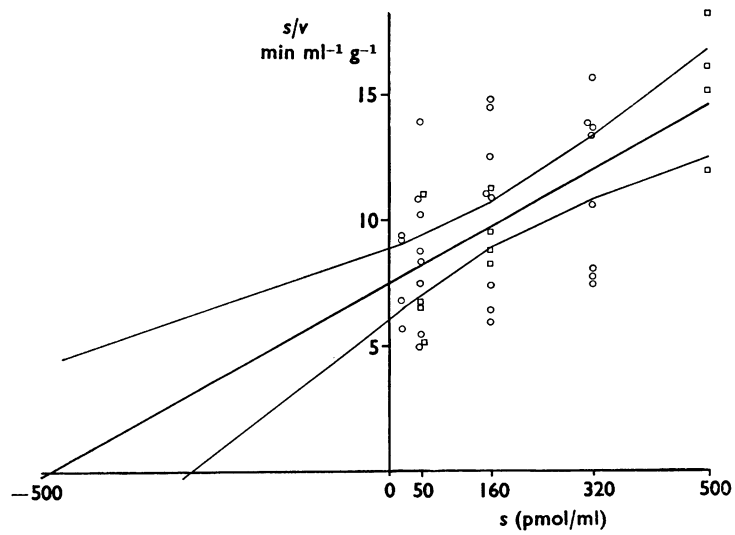


FIG. 3. A linear transformation of the Michaelis-Menten equation. s=substrate concentration, v=initial velocity of uptake. The initial velocity was estimated as the total radioactive material content of the trachea (exposed to substrate for 3·75 min ○ or 7·5 min □, and washed for 30 min) divided by the time of exposure and tracheal mass. Regression analysis is used to fit the mean line and its 95% confidence limits. K_m is found from the intercept on the abscissa and V_{max} from the slope.

The proportion of the total radioactivity retained, which is in the form of noradrenaline, declines as the substrate concentration increases. This slope, of −9·7% per tenfold increase in substrate concentration, is significant ($P=0{\cdot}005$). The acidic metabolites show a positive slope ($0{\cdot}1>P>0{\cdot}05$).

The endogenous noradrenaline content of the guinea-pig trachea

The noradrenaline content of each pooled tracheal extract was assayed fluorimetrically after isolation on the ion exchange resin. The mean noradrenaline content of the 15 groups of tracheae exposed to up to 320 nM was 2,770 ± 380 pmol/g. After subtraction of the amount of noradrenaline taken up during incubation (as shown by the tracer) and on the assumption that all is net uptake and not exchange the content was 2,730 ± 390 pmol/g.

The mean noradrenaline content of the 15 groups of tracheae exposed to more than 320 nM was 2,650 ± 300 pmol/g. After subtraction of the amount of noradrenaline taken up during incubation (as shown by the tracer) and on the assumption that all is net uptake and not exchange the content was 2,470 ± 290 pmol/g.

Dependence of uptake on ionic transport

Table 1 shows how alterations in sodium and potassium ion concentrations of the incubation medium were achieved with minimal disturbance of other ionic constituents and tonicity. Table 3 presents the results on the uptake-with-retention of radioacivity of these ionic changes. A progressive reduction in the sodium ion concentration had a progressive inhibitory effect on uptake. The omission of potassium ion reduced uptake, but not severely. Ouabain (100 μM) caused a

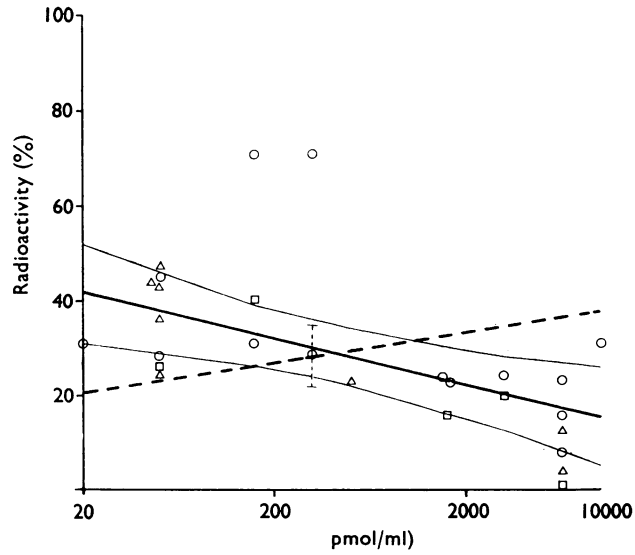


FIG. 4. Increase in substrate concentration increases the mean percentage of the total tissue radioactivity which is found in the form of acidic metabolites – – –, and reduces that in the form of noradrenaline ——. 95% confidence limits are shown for the whole noradrenaline mean line but only centrally on the acidic metabolite mean line. Plotted points express the percentage of total tracheal radioactive material which is in the form of noradrenaline after incubation for 3·75 min ○, 7·5 min, □, or 15 min △ followed by 30 min wash. Each point relates to the pooled extracts of 4 tracheae.

greater reduction in the concentration of radioactive material found in the tissue than any other treatment.

Dependence of uptake on energy metabolism

Table 3 shows that 2,4,dinitrophenol (1 mM), bubbling with 95% nitrogen/5% CO_2 or exclusion of glucose did not reduce the amount of radioactive material transported into and retained within the trachea. Only bubbling with 95% nitrogen/5% CO_2 plus exclusion of glucose, or addition of sodium fluoride (20 mM) severely inhibited the uptake.

Discussion

Foster (1968) described as 'cellular' uptake the amount of radioactive material that was taken up by a tracheal preparation and retained despite washing. The possibility that this uptake involved an active transfer process was discussed and the results presented here provide additional evidence for such a process.

The results of washout experiments showed a fast washout phase with similar $t_{1/2}$ (3 to 4 min) for sorbitol and both concentrations of noradrenaline. An interesting finding was that the volume of this rapidly-exchanging compartment was larger for noradrenaline than for sorbitol. This suggests that some of the noradrenaline here is not simply in free solution in the extracellular fluid but rather adsorbed and in 'instantaneous' exchange with the drug in extracellular fluid.

The nature of the second component of efflux of ^{14}C-sorbitol is not clear. Sorbitol is usually regarded as a satisfactory small molecular weight extracellular fluid marker, unable to enter tissue cells at least during limited incubations. Our findings of a slow phase of efflux of significant magnitude prompted a check on the radiochemical purity of the material but this was quite satisfactory (single peak of radioactivity after paper chromatography with either of two different solvent systems, Mireylees, personal communication). A small slowly exchangeable residuum has been found in guinea-pig taenia coli by Brading & Jones (1969).

TALBE 3. *Effect of modifications of the incubation medium on uptake-with-retention of radioactivity from (−)-^{3}H-noradrenaline*

Incubation medium	Tracheal uptake of radioactivity (pmol/g)	*n*	*P* *t* test 2-tailed
Krebs solution	65·0± 4·4	13	—
Low Na^+ solution (63 mEq/l)	54·0± 4·1	10	≏0·1
Low Na^+ solution (25 mEq/l)	30·0± 0·7	10	<0·001
K^+-free solution	47·5± 1·5	5	<0·005
Ouabain, 100 μM	9·6± 0·6	5	<0·001
2,4,dinitrophenol, 1 mM	72·4± 5·9	5	>0·1
Bubbled with 95% N_2/5% CO_2	85·4±13·9	4	>0·1
Glucose-free solution	60·4± 6·8	4	>0·1
Glucose-free solution bubbled with 95% N_2/5% CO_2	13·4± 1·0	4	<0·001
NaF, 20 mM	2·53± 3·7	5	<0·001

Most of these conditions were maintained for 1 h before addition of (−)-^{3}H-noradrenaline. Glucose-free conditions were maintained for 3 h and bubbling with 95% N_2/5% CO_2 lasted 30 minutes. (−)-^{3}H-noradrenaline (50 nM) was in contact with the tissue for 15 min and the extracellular fluid was then cleared by 6 washes over 30 minutes. The preincubation conditions were maintained during the exposure to radioactivity and washing. *n*=number of tracheal preparations.

Also Morgan, Henderson, Regen & Park (1961) described a double exponential washout curve from the perfused rat heart.

The sorbitol was included as a control of the behaviour of noradrenaline. With noradrenaline the notable features are a much larger relative mass in the slowly exchanging compartment, a much longer half time of decay and some evidence that both of these are concentration dependent. The more rapid loss of the retained material when uptake had been from the higher concentration (6,400 nM) may be a reflection of loss from an intraneuronal cytoplasmic site or from an extraneuronal site, in either case associated with increased metabolism of the noradrenaline. If the extraneuronal uptake mechanism is implicated here the efflux is surprisingly slow for Iversen (1967) found that material accumulated by uptake$_2$ washed out very readily, though this finding may not be capable of strict application to the trachea in which there is some retention of isoprenaline (Foster, 1969).

The relationship between initial velocity of uptake (v) and substrate concentration (s) over the 25-fold range 20–500 nM, showed that the process by which the retained noradrenaline had entered the tissue obeyed saturation kinetics of the Michaelis–Menten type. The K_m (530 nM) for the uptake is acceptably close to that for the neuronal accumulation of noradrenaline of about 270 nM in the rat heart (Iversen, 1967), embryo chick heart (Ignarro & Shideman, 1968), rat synaptosomes (Coyle & Snyder, 1969) and rat brain slices (Hendley, Taylor & Snyder, 1970). The low V_{max} may reflect a smaller area of noradrenergic nerve terminal membrane per gramme of tissue in trachea than heart (or fewer carriers per unit area) or at least a smaller area reached by the substrate in the time allowed. Some evidence for the latter possibility is that the noradrenaline content of the tissue is lower than in the heart but certainly not in the ratio 1:20 of their V_{max}. However, the experiments with different incubation times should have given different results if the penetration time were limiting the uptake. Fluorescence histochemical studies on guinea-pig trachea and heart support the idea that there is a much smaller area of innervation per gramme of tissue in trachea than heart (O'Donnell & Saar, unpublished observations).

From 500 to 10,000 nM the plot of s/v against s yielded a horizontal line, i.e., v appeared to increase with the concentration of noradrenaline. Even higher concentrations would have to be explored to distinguish diffusional entry from a second saturable process having very high K_m and V_{max}. Some evidence that noradrenaline can bind to various extraneuronal tissue structures of guinea-pig trachea after exposure to high concentrations of exogenous amine (50,000 nM) has been obtained with fluorescence histochemistry (O'Donnell & Saar, unpublished observations). Some of this accumulated noradrenaline is quite firmly retained and may not be completely removed after 30 min of washing in Krebs solution at 37° C.

The use of total radioactive material content in the estimation of initial velocity of uptake (v) may be questioned on the grounds that it is noradrenaline's uptake which we wish to measure. We feel that at the moment of uptake the radioactive material subsequently assayed was in the form of noradrenaline; however once taken up considerable metabolism does occur. Some time (33·75 to 45 min) after the beginning of an exposure to a low concentration of noradrenaline only about 40% of the radioactive material in the trachea is noradrenaline. Most authors have found that a large fraction of the retained radioactive material in such experiments is noradrenaline though Bhagat, Bovell & Robinson (1967) found only 33%

in guinea-pig atria and Maitre & Krahenbuhl (1971) found 50% in the rat uterus. As the substrate concentration increased the formation of metabolites increased in the same way as reported by Lightman & Iversen (1969).

We have repeated the plot of s/v against s, using as our estimate of v the amount of labelled noradrenaline found in the trachea after a 3·75 or 7·5 min exposure to noradrenaline and 30 min of washing in Krebs solution divided by the time of exposure and tissue mass. This still generated a straight line over the 25-fold range of substrate concentration 20 to 500 nM ; the V_{max} was reduced to (15 pmol/min)/g, the K_m found was 270 nM. Thus the main conclusions drawn from this experiment, the saturation type kinetics and a K_m similar to that for the neuronal uptake of noradrenaline, are unaffected by the use of either total radioactivity or only labelled noradrenaline in presenting the results.

The concentration of noradrenaline found in the guinea-pig isolated trachea by fluorimetric assay (2,730 $\pm$ 390 pmol/g) is considerably greater than the substrate concentrations from which uptake occurs by a saturable mechanism (50 to 320 pmol/ml). Therefore this uptake occurs against an apparent concentration gradient. As the substrate concentration increases the tissue to medium ratio and the percentage of total radioactive content which is noradrenaline both decline. Thus the possible increase in noradrenaline content was small and a greater content of noradrenaline could not be demonstrated in trachea which had been exposed to large concentrations of substrate. Therefore we could not distinguish between a net uptake of exogenous noradrenaline and an exchange with endogenous noradrenaline on this basis.

The neuronal uptake process for noradrenaline shows as characteristics of an active transport process (Iversen, 1970) a rate which is severely reduced if sodium ions are removed from the external medium ; a requirement for a low concentration of potassium ion in order to maintain normal rates of uptake ; inhibition by ouabain and by metabolic poisons. Our tracheal uptake process shows these properties. Inhibition of oxidative metabolism of the tissue by anoxia (bubbling with nitrogen) or by preincubation with 10 mM dinitrophenol did not have any significant effect on the uptake. Neither did the inhibition of glycolytic metabolism (removal of glucose from the Krebs solution). However, if both metabolic processes were inhibited simultaneously, e.g. a combination of anoxia and glucose deprivation, there was a marked inhibition of uptake. These findings are very similar to those described for uptake of noradrenaline into the guinea-pig left atrium (Wakade & Furchgott, 1968) and would be consistent with the uptake occurring by an energy-dependent process.

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