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THE INHIBITION OF NORADRENALINE UPTAKE BY SYMPATHOMIMETIC AMINES IN THE RAT ISOLATED HEART

BY

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In the rat isolated heart, noradrenaline can be accumulated by two distinct processes. The first process (Uptake$_1$) is half saturated at a (±)-noradrenaline concentration of 0.11 μg/ml. and continues to operate at external noradrenaline concentrations up to 1 μg/ml. (Iversen, 1963). The second process comes into play at slightly higher concentrations and becomes half saturated at 42.6 μg/ml. (Iversen, 1965b). Both of these processes act also upon adrenaline, which competes for uptake when noradrenaline is also present (Iversen, 1965a, b). It seems probable, therefore, that other sympathomimetic amines would have an affinity for the systems operative in accumulation, and indeed, there is some evidence in the literature that amphetamine, tyramine and ephedrine inhibit noradrenaline uptake (Dengler, Spiegel & Titus, 1961; Axelrod & Tomchick, 1960).

This paper is concerned with the measurement of the affinity of sympathomimetic amines for the uptake system as measured by inhibition of noradrenaline accumulation. Needless to say, the demonstration that a substance inhibits noradrenaline uptake does not prove that it is also transported by the system; this would require a direct measurement of the accumulation of the substance in the tissue. A preliminary account of some of these results has already been published (Iversen, 1964).

METHODS

Inhibition of Uptake$_1$

In control experiments, hearts were perfused with a medium containing (±)-[^{14}C]noradrenaline (Nichem Inc., Bethesda, Maryland, U.S.A.) at a concentration of 10 ng/ml. (1.3 mμc/ml.). Inhibition was studied by adding the substance to this medium in various concentrations. All perfusions were for 10 min and were followed by a 2-min perfusion with a medium free of noradrenaline and inhibitor to remove extracellular noradrenaline (Iversen, 1963). The uptake of [^{14}C]noradrenaline was assessed by liquid scintillation counting of heart extracts purified by the simplified ion-exchange procedure previously described (Iversen, 1963). Previous studies had established that only negligible amounts of radioactive normetanephrine accumulated in the heart under these conditions, so that it was not necessary to use the more complicated ion-exchange chromatographic procedure employed in previous studies. Control uptakes were measured frequently and were highly consistent. After 10 min the uptake of [^{14}C]noradrenaline was 0.187±0.016 μg/g (mean and standard deviation of thirty perfusions).

Inhibition of Uptake$_2$

Rat hearts were perfused with a medium containing (±)-[^{3}H]noradrenaline at a concentration of 5 μg/ml• (45 mμc/ml.). Drugs were added to this medium as before. All perfusions were for 2 min and were not followed by a wash-out perfusion; the results were corrected for the presence of extracellular [^{3}H]noradrenaline assuming an extracellular water space of 325 μl./g wet weight (Iversen, 1965b). Noradrenaline uptake was measured by liquid scintillation counting of heart extracts purified by the simplified ion-exchange procedure as described above. The uptake of [^{3}H]noradrenaline measured in this way was practically identical to the net uptake of noradrenaline measured by fluorimetric analysis. The uptake was approximately linear in control hearts perfused for 1 or 2 min at this perfusion concentration, and it has been assumed that the uptake of [^{3}H]noradrenaline in the first 2 min was proportional to the initial rate of noradrenaline uptake. The uptake of [^{3}H]noradrenaline in control hearts was 3.21±0.23 μg/g (mean and standard error of twenty perfusions).

Determination of ID50

From two to four concentrations of each drug were tested on groups of four hearts, and the mean percentage inhibition of noradrenaline uptake in each group was plotted against the molar concentration of added drug on probability/log paper. In this way a linear relationship was obtained from which it was possible to determine the ID50 (the concentration of drug which caused half inhibition of noradrenaline uptake). For both Uptake$_1$ and Uptake$_2$ the ID50 values were further converted into arbitrary measures of affinity for the uptake site by using the reciprocal of ID50 expressed on a scale on which phenethylamine was taken as a reference molecule and was assigned a value of 100 in each case. These values were referred to as Relative Affinities.

$$\text{Relative Affinity} = \frac{\text{ID50 of phenethylamine}}{\text{ID50 of drug}} \times 100$$

It should be emphasized that these experiments should be regarded only as screening tests; the use of the term " affinity " is not intended to imply any knowledge of the kinetics of inhibition.

Drugs

These were: (−)-adrenaline (L. Light); (±)-amphetamine sulphate (L. Light); (+)-amphetamine (dexamphetamine) sulphate (*Smith Kline & French); (−)-amphetamine (levamfetamine) sulphate (*Smith Kline & French); (±)-cyclopentamine hydrochloride (*Eli Lilly); (−)-3,4-dihydroxyphenylalanine (dopa, *F. Hoffman-La Roche); 3,4-dihydroxyphenylethylamine (dopamine hydrochloride, L. Light); (±)-α-methyldopamine solution (*Dr M. J. Rand, School of Pharmacy, London); 6-hydroxydopamine (Merck, Sharpe & Dohme, from *Dr M. Vogt, A.R.C. Institute for Animal Physiology, Babraham, Cambridge); (−)-ephedrine hydrochloride (*Burroughs Wellcome); *N*-methyldopamine hydrochloride (*Wellcome Research Laboratories); (±)-α-ethylnoradrenaline (*Sterling Winthrop Research Institute); hordenine sulphate (L. Light); (±)-hydroxyamphetamine hydrobromide (*Smith Kline & French); (±)-isoprenaline sulphate (*Burroughs Wellcome); mephentermine sulphate (*John Wyeth); (±)-metanephrine hydrochloride (California Corporation for Biochemical Research); (−)-metaraminol bitartrate (*Merck, Sharpe & Dohme); (+)-methylamphetamine sulphate (*Burroughs Wellcome); (±)-methoxamine hydrochloride (*Burroughs Wellcome); 2-(naphth-2-yl)ethylamine hydrochloride (*I.C.I.); (±)-methoxyphenamine hydrochloride (*Upjohn); mescaline sulphate (L. Light); (±)-*N*-ethylnoradrenaline hydrochloride, (±)-*N*-butylnoradrenaline hydrochloride, (±)-*N*-isobutylnoradrenaline hydrochloride and (±)-*N*-propylnoradrenaline sulphate (*Boehringer); noradrenalone bitartrate (Aldrich Chemical Co.); (±)-nordefrin (corbadrine) hydrochloride (*Dr Crawford, Edinburgh University); (−)-nordefrin solution (*Dr M. J. Rand, School of Pharmacy, London); (±)-normetanephrine hydrochloride (California Corporation for Biochemical Research); (±)-buphenine (nylidrin) hydrochloride (*Smith & Nephew); (±)-octopamine hydrochloride (L. Light); (±)-oxedrine (synephrine) tartrate (L. Light); (±)-phenylethanolamine (Aldrich Chemical Co.); phenethylamine hydrochloride (L. Light); 2-(*p*-methoxyphenyl)ethylamine (L. Light), 2-(3, 4-dimethoxyphenyl)ethylamine (L. Light); (−)-phenylephrine hydrochloride (*Boots Pure Drug Co.); (±)-phenylpropanolamine hydrochloride (*Merck,

Sharpe & Dohme); phenelzine (*Warner-Chilcott Laboratories); (±)-propylhexedrine (*Smith Kline & French); (±)-prenylamine gluconate (*Hoechst); tuaminoheptane sulphate (*Eli Lilly); tranylcypromine (*Smith Kline & French); tyramine hydrochloride (B.D.H.); metatyramine hydrochloride (*Burroughs Wellcome, New York); and 5-hydroxytryptamine creatinine sulphate (B.D.H.). Asterisks indicate organizations and individuals who generously donated drugs, and we gratefully acknowledge these gifts.

RESULTS

Uptake$_1$

The validity of using uptake after a fixed time for measuring the inhibition of uptake depends on equilibrium being rapidly established. In the results shown in Fig. 1 this has been tested in the case of tyramine by measurements of the uptake of [^{14}C]noradrenaline after 5 and 10 min. It can be seen that linearity was maintained, so that inhibition measured after 5 min was the same as after 10 min, the inhibition appears to reach equilibrium very rapidly. Similar measurements were made with some other drugs and the same result was obtained. It appears, therefore, that measurements after 10 min are quantitatively reliable, and only these measurements have been made for the majority of the substances listed in Table 1.

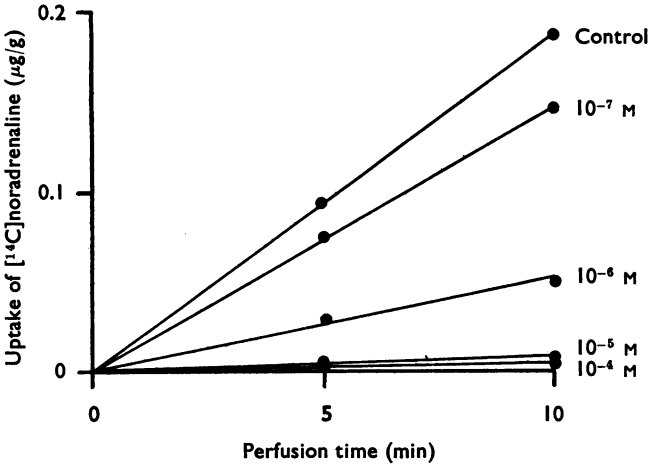


Fig. 1. The uptake of [^{14}C]noradrenaline in the isolated rat heart after perfusion with a medium containing 10 ng/ml. (±)-[^{14}C]noradrenaline and various concentrations (values on right) of added tyramine. Each point is the mean value for four hearts.

The ID50 for each amine was determined as described above. The probit plot method gave satisfactory linear plots in all experiments; some typical results are shown in Fig. 2. In general, the results from the different amines yielded probit plots of very similar slope, though there were some exceptions to this rule, particularly for amines with very low affinities for the uptake site in which the slope was much less (for example, normetanephrine in Fig. 2). The results for all the amines tested are listed in Table 1, and in Tables 2 to 7 the compounds have been grouped to illustrate the effects of various structural features of the molecules on their affinity for the uptake process.

TABLE 1

INHIBITION OF NORADRENALINE UPTAKE BY SYMPATHOMIMETIC AMINES IN THE RAT ISOLATED HEART

* Affinities determined by direct analysis of uptake kinetics (Iversen, 1963, 1965a). In this and subsequent tables the ID50 is the drug concentration producing 50% inhibition of noradrenaline uptake; affinities are relative to phenethylamine=100

Drug	ID50 (M)	Relative affinity
(−)-Metaraminol	$7{\cdot}6\times10^{-8}$	1,440
Dopamine	$1{\cdot}7\times10^{-7}$	650
(±)-α-Methyldopamine	$1{\cdot}8\times10^{-7}$	610
(+)-Amphetamine	$1{\cdot}8\times10^{-7}$	610
(±)-Hydroxyamphetamine	$1{\cdot}8\times10^{-7}$	610
(−)-Nordefrin	$2{\cdot}0\times10^{-7}$	550
(−)-Noradrenaline*	$2{\cdot}7\times10^{-7}$	407
(±)-Nordefrin	$4{\cdot}2\times10^{-7}$	260
Tyramine	$4{\cdot}5\times10^{-7}$	245
(±)-Amphetamine	$4{\cdot}6\times10^{-7}$	240
Metatyramine	$5{\cdot}1\times10^{-7}$	215
(+)-Methylamphetamine	$6{\cdot}7\times10^{-7}$	165
(±)-Noradrenaline*	$6{\cdot}7\times10^{-7}$	165
(±)-Prenylamine	$7{\cdot}4\times10^{-7}$	149
N-Methyldopamine	$7{\cdot}6\times10^{-7}$	145
(+)-Buphenine	$8{\cdot}5\times10^{-7}$	130
(±)-Propylhexedrine	$8{\cdot}5\times10^{-7}$	130
(−)-Adrenaline	$1{\cdot}0\times10^{-6}$	110
Mephentermine	$1{\cdot}0\times10^{-6}$	110
PHENETHYLAMINE	$1{\cdot}1\times10^{-6}$	100
(±)-Octopamine	$1{\cdot}3\times10^{-6}$	85
Tranylcypromine	$1{\cdot}3\times10^{-6}$	85
(+)-Noradrenaline*	$1{\cdot}4\times10^{-6}$	78
(±)-Cyclopentamine	$1{\cdot}4\times10^{-6}$	78
(±)-Adrenaline*	$1{\cdot}4\times10^{-6}$	78
Noradrenalone	$1{\cdot}5\times10^{-6}$	73
2-(Naphth-2-yl)ethylamine	$1{\cdot}5\times10^{-6}$	73
(±)-Phenylpropanolamine	$2{\cdot}0\times10^{-6}$	55
(±)-3,4-Dichloroisoprenaline	$2{\cdot}0\times10^{-6}$	55
(−)-Ephedrine	$2{\cdot}2\times10^{-6}$	50
Hordenine	$2{\cdot}5\times10^{-6}$	45
(±)-α-Ethylnoradrenaline	$3{\cdot}2\times10^{-6}$	34
(−)-Amphetamine	$3{\cdot}7\times10^{-6}$	30
Phenelzine	$3{\cdot}8\times10^{-6}$	29
(±)-Phenylethanolamine	$4{\cdot}8\times10^{-6}$	23
(−)-Phenylephrine	$5{\cdot}6\times10^{-6}$	20
Tuaminoheptane	$5{\cdot}6\times10^{-6}$	20
(±)-*N*-Ethylnoradrenaline	$9{\cdot}2\times10^{-6}$	12
2-(*p*-Methoxyphenyl)ethylamine	$1{\cdot}0\times10^{-5}$	11
(±)-Methoxyphenamine	$1{\cdot}1\times10^{-5}$	10
(±)-Oxedrine	$1{\cdot}2\times10^{-5}$	9
5-Hydroxytryptamine	$2{\cdot}0\times10^{-5}$	5·5
(±)-Isoprenaline	$2{\cdot}5\times10^{-5}$	4·5
(±)-*N*-Butylnoradrenaline	$3{\cdot}5\times10^{-5}$	3·2
(±)-*N*-Isobutylnoradrenaline	$4{\cdot}0\times10^{-5}$	2·6
(±)-Metanephrine	$4{\cdot}3\times10^{-5}$	2·6
(−)-Dopa	$6{\cdot}0\times10^{-5}$	1·2
(±)-Normetanephrine	$2{\cdot}0\times10^{-4}$	0·55
6-Hydroxydopamine	$>2{\cdot}0\times10^{-4}$	<0·55
2-(3,4-Dimethoxyphenyl)ethylamine	$2{\cdot}0\times10^{-4}$	0·55
(±)-Methoxamine	$1{\cdot}0\times10^{-3}$	0·11
Mescaline	$1{\cdot}5\times10^{-2}$	0·007

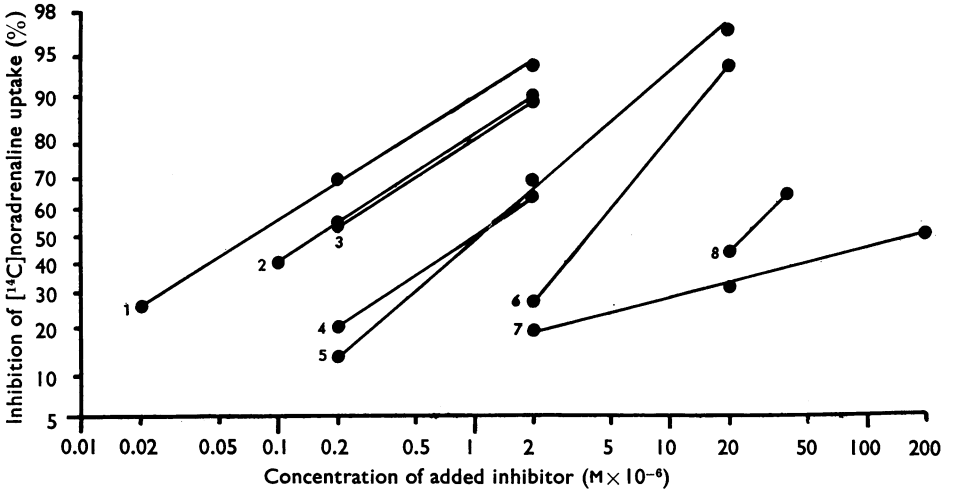


Fig. 2. The inhibition of [^{14}C]noradrenaline uptake at a concentration of 10 ng/ml. (±)-[^{14}C]noradrenaline by various sympathomimetic amines added simultaneously to the perfusion medium. Percentage inhibition of noradrenaline uptake (ordinate, probability scale) is plotted against log concentration of added inhibitor. Each point is the mean for a group of four hearts. 1=(−)-Metaraminol, 2=dopamine, 3=(+)-amphetamine, 4=(−)-adrenaline, 5=phenethylamine, 6=(−)-amphetamine, 7=(±)-normetanephrine and 8=(±)-isoprenaline.

Effects of β-hydroxylation (*Table* 2). In all cases the introduction of a β-hydroxyl group reduced the affinity compared with the parent molecule. This effect was relatively small when the configuration around the β-carbon atom corresponded to that in (−)-noradrenaline, and on average reduced affinity to 53% of that of the parent molecule. This effect was greater with (+)-noradrenaline, which had only 12% of the affinity of dopamine; a similarly reduced affinity is present with (+)-adrenaline (Iversen, 1965a). It is this low affinity of the (+)-enantiomer that accounts for most of the reduction of affinity seen with the racemic mixtures. It appears that the difference of affinity between (+)- and (−)-noradrenaline is not due to an increased affinity of the (−)-enantiomer compared with dopamine, but rather to a smaller affinity-reducing effect of the (−)-configuration at the β-carbon atom compared with the (+)-configuration.

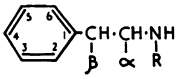
Effects of α-methylation (*Table* 3). The effect of α-methylation of non-phenolic or monophenolic amines is most clearly seen in comparing (+)-amphetamine with phenethylamine—the affinity is increased sixfold. On the other hand, (−)-amphetamine has only 30% of the affinity of phenethylamine. The reduced affinity produced by an α-methyl group in the (−)-configuration can also be seen in the comparison between (+)-methylamphetamine and the αα-dimethyl compound, mephentermine. The influence of the α-methyl group is attenuated in the catechol amines, as seen in the comparison of nordefin and noradrenaline, and of α-methyldopamine and dopamine. In α-ethylnoradrenaline, the α-substituent is an ethyl group and this lowers the affinity well below that of the unsubstituted molecule (noradrenaline).

Effects of phenolic hydroxyl groups (*Table* 4). The presence of phenolic hydroxyl groups enhanced the affinity for the uptake site. Phenolic groups in either the *meta-* or *para-*

TABLE 2

EFFECTS OF β-HYDROXYLATION ON INHIBITION OF UPTAKE$_1$ BY SYMPATHOMIMETIC AMINES

Substituents

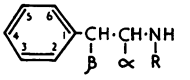


Drug	4	3	β	α	R	ID50 (M)	Relative affinity
Phenethylamine	H	H	H	H	H	$1{\cdot}1\times10^{-6}$	100
(±)-Phenylethanolamine	H	H	OH	H	H	$4{\cdot}8\times10^{-6}$	23
Dopamine	OH	OH	H	H	H	$1{\cdot}7\times10^{-7}$	650
(−)-Noradrenaline	OH	OH	OH	H	H	$2{\cdot}7\times10^{-7}$	407
(±)-Noradrenaline	OH	OH	OH	H	H	$6{\cdot}7\times10^{-7}$	164
(+)-Noradrenaline	OH	OH	OH	H	H	$1{\cdot}4\times10^{-6}$	79
N-*Methyldopamine*	OH	OH	H	H	CH_3	$7{\cdot}6\times10^{-7}$	145
(±)-Adrenaline	OH	OH	OH	H	CH_3	$1{\cdot}4\times10^{-6}$	78
(−)-Adrenaline	OH	OH	OH	H	CH_3	$1{\cdot}0\times10^{-6}$	110
(±)-*Amphetamine*	H	H	H	CH_3	H	$4{\cdot}6\times10^{-7}$	240
(±)-Phenylpropanolamine	H	H	OH	CH_3	H	$2{\cdot}0\times10^{-6}$	55
(+)-*Methylamphetamine*	H	H	H	CH_3	CH_3	$6{\cdot}7\times10^{-7}$	165
(−)-Ephedrine	H	H	OH	CH_3	CH_3	$2{\cdot}2\times10^{-6}$	50
Tyramine	OH	H	H	H	H	$4{\cdot}5\times10^{-7}$	245
(±)-Octopamine	OH	H	OH	H	H	$1{\cdot}3\times10^{-6}$	85
(±)-α-*Methyldopamine*	OH	OH	H	CH_3	H	$1{\cdot}8\times10^{-7}$	610
(±)-Nordefrin	OH	OH	OH	CH_3	H	$4{\cdot}3\times10^{-7}$	256

TABLE 3

EFFECTS OF α-METHYLATION ON INHIBITION OF UPTAKE$_1$ BY SYMPATHOMIMETIC AMINES

Substituents



Drug	4	3	β	α	R	ID50 (M)	Relative affinity
Phenethylamine	H	H	H	H	H	$1{\cdot}1\times10^{-6}$	100
(+)-Amphetamine	H	H	H	CH_3	H	$1{\cdot}8\times10^{-7}$	610
(±)-Amphetamine	H	H	H	CH_3	H	$4{\cdot}6\times10^{-7}$	240
(−)-Amphetamine	H	H	H	CH_3	H	$3{\cdot}7\times10^{-6}$	30
Tyramine	OH	H	H	H	H	$4{\cdot}5\times10^{-7}$	245
(±)-Hydroxyamphetamine	OH	H	H	CH_3	H	$1{\cdot}8\times10^{-7}$	610
Dopamine	OH	OH	H	H	H	$1{\cdot}7\times10^{-7}$	650
(±)-α-Methyldopamine	OH	OH	H	CH_3	H	$1{\cdot}8\times10^{-7}$	610
(−)-*Noradrenaline*	OH	OH	OH	H	H	$2{\cdot}7\times10^{-7}$	407
(−)-Nordefrin	OH	OH	OH	CH_3	H	$2{\cdot}0\times10^{-7}$	550
(±)-*Noradrenaline*	OH	OH	OH	H	H	$6{\cdot}7\times10^{-7}$	164
(±)-Nordefrin	OH	OH	OH	CH_3	H	$4{\cdot}3\times10^{-7}$	256
(±)-α-Ethylnoradrenaline	OH	OH	OH	C_2H_5	H	$3{\cdot}2\times10^{-6}$	34
(+)-*Methylamphetamine*	H	H	H	CH_3	CH_3	$6{\cdot}7\times10^{-7}$	165
Mephentermine	H	H	H	$(CH_3)_2$	CH_3	$1{\cdot}0\times10^{-6}$	110
(±)-*Phenylethanolamine*	H	H	OH	H	H	$4{\cdot}8\times10^{-6}$	23
(±)-Phenylpropanolamine	H	H	OH	CH_3	H	$2{\cdot}0\times10^{-6}$	55

TABLE 4

EFFECTS OF PHENOLIC HYDROXYL GROUPS ON INHIBITION OF UPTAKE$_1$ BY SYMPATHOMIMETIC AMINES

Drug	Substituents 4	3	β	α	R	Other	ID50 (M)	Relative affinity
Phenethylamine	H	H	H	H	H		$1{\cdot}1\times10^{-6}$	100
Tyramine	OH	H	H	H	H		$4{\cdot}5\times10^{-7}$	245
Metatyramine	H	OH	H	H	H		$5{\cdot}1\times10^{-7}$	215
Dopamine	OH	OH	H	H	H		$1{\cdot}7\times10^{-7}$	650
6-Hydroxydopamine	OH	OH	H	H	H	6-OH	$>2{\cdot}0\times10^{-4}$	$<0{\cdot}55$
(±)-*Phenylpropanolamine*	H	H	OH	CH_3	H		$2{\cdot}0\times10^{-6}$	55
(−)-Metaraminol	H	OH	OH	CH_3	H		$7{\cdot}6\times10^{-8}$	1,440
(−)-*Phenylephrine*	H	OH	OH	H	CH_3		$5{\cdot}6\times10^{-6}$	20
(−)-Adrenaline	OH	OH	OH	H	CH_3		$1{\cdot}0\times10^{-6}$	110
(±)-*Oxedrine*	OH	H	OH	H	CH_3		$1{\cdot}2\times10^{-5}$	9
(±)-Adrenaline	OH	OH	OH	H	CH_3		$1{\cdot}4\times10^{-6}$	78
(±)-*Amphetamine*	H	H	H	CH_3	H		$4{\cdot}6\times10^{-7}$	240
(±)-Hydroxyamphetamine	OH	H	H	CH_3	H		$1{\cdot}8\times10^{-7}$	610
(±)-α-Methyldopamine	OH	OH	H	CH_3	H		$1{\cdot}8\times10^{-7}$	610
(±)-*Phenylethanolamine*	H	H	OH	H	H		$4{\cdot}8\times10^{-6}$	23
(±)-Octopamine	OH	H	OH	H	H		$1{\cdot}3\times10^{-6}$	85
(±)-Noradrenaline	OH	OH	OH	H	H		$6{\cdot}7\times10^{-7}$	164

positions had the same effect; the presence of a single phenolic group in either position enhanced the affinity for the uptake site by a factor of two to three. The presence of a second phenolic group in catechol amines had a further additive effect in increasing affinity by a factor of two to three if compared with mono-phenolic compounds, or by a factor of approximately six if compared with non-phenolic compounds. 6-Hydroxydopamine had no effect on noradrenaline uptake at the highest concentration tested (2×10^{-5} M). The affinity is therefore less than 1% of that of phenethylamine. This very great reduction of affinity due to an additional *o*-hydroxyl group is interesting.

Effects of N-*substitutions* (*Table* 5). The trend towards decreasing affinity for uptake in *N*-substituted amines previously described for noradrenaline and adrenaline was confirmed when other *N*-substituted compounds were tested. In the presence of an *N*-methyl group, the affinity for the uptake site was depressed by a factor of about three. The presence of the two *N*-methyl substituents in hordenine depressed the affinity by a factor of 5.6, and thus had little more effect than the presence of a single methyl group. The presence of larger *N*-substituents produced an even greater depression of affinity; when compared with noradrenaline the affinity was depressed by a factor of 13.7 with *N*-ethylnoradrenaline, and by a factor of 55 with *N*-propylnoradrenaline. Further increases in the size of the *N*-substituent, or the presence of branching as in isoprenaline or *N*-isobutylnoradrenaline, had little further effect on the affinity. However, high activity was found in two amines with a large aralkyl *N*-substituent, namely prenylamine and buphenine. Indeed, the affinity of the latter is greater than that of the corresponding primary amine and much greater than that of the *N*-methyl derivative. The only other exception to the rule that *N*-substitution decreased affinity was metanephrine, which had a higher affinity than normetanephrine.

TABLE 5

EFFECTS OF *N*-SUBSTITUTION ON INHIBITION OF UPTAKE$_1$ BY SYMPATHOMIMETIC AMINES

Drug	Substituent 4	3	β	α	R	ID50 (M)	Relative affinity
(−)-*Noradrenaline*	OH	OH	OH	H	H	$2{\cdot}7\times10^{-7}$	407
(−)-Adrenaline	OH	OH	OH	H	CH_3	$1{\cdot}0\times10^{-6}$	110
(±)-*Noradrenaline*	OH	OH	OH	H	H	$6{\cdot}7\times10^{-7}$	164
(±)-Adrenaline	OH	OH	OH	H	CH_3	$1{\cdot}4\times10^{-6}$	78
(±)-*N*-Ethylnoradrenaline	OH	OH	OH	H	C_2H_5	$4{\cdot}2\times10^{-6}$	12
(±)-Isoprenaline	OH	OH	OH	H	$CH(CH_3)_2$	$2{\cdot}5\times10^{-5}$	4·5
(±)-*N*-Propylnoradrenaline	OH	OH	OH	H	C_3H_7	$3{\cdot}7\times10^{-5}$	3·0
(±)-*N*-Butylnoradrenaline	OH	OH	OH	H	C_4H_9	$3{\cdot}5\times10^{-5}$	3·2
(±)-*N*-Isobutylnoradrenaline	OH	OH	OH	H	$CH_2.CH(CH_3)_2$	$4{\cdot}0\times10^{-5}$	2·6
(+)-*Amphetamine*	H	H	H	CH_3	H	$1{\cdot}8\times10^{-7}$	610
(+)-Methylamphetamine	H	H	H	CH_3	CH_3	$6{\cdot}7\times10^{-7}$	165
(±)-Prenylamine	H	H	H	CH_3	$CH_2.CH_2.CH(C_6H_5)_2$	$7{\cdot}4\times10^{-7}$	149
Tyramine	OH	H	H	H	H	$4{\cdot}5\times10^{-7}$	245
Hordenine	OH	H	H	H	$(CH_3)_2$	$2{\cdot}5\times10^{-6}$	44
Dopamine	OH	OH	H	H	H	$1{\cdot}7\times10^{-7}$	650
N-Methyldopamine	OH	OH	H	H	CH_3	$7{\cdot}6\times10^{-7}$	145
(±)-*Phenylpropanolamine*	H	H	OH	CH_3	H	$2{\cdot}0\times10^{-6}$	55
(−)-Ephedrine	H	H	OH	CH_3	CH_3	$2{\cdot}2\times10^{-6}$	50
(±)-*Octopamine*	OH	H	OH	H	H	$1{\cdot}3\times10^{-6}$	85
(±)-Oxedrine	OH	H	OH	H	CH_3	$1{\cdot}2\times10^{-5}$	9
(±)-Buphenine	OH	H	OH	CH_3	$CH(CH_3).CH_2.CH_2.C_6H_5$	$7{\cdot}9\times10^{-7}$	140

Effects of O-*methylation* (*Table* 6). *O*-Methylation of phenolic hydroxyl groups led to very striking decreases in the affinity of such compounds for the uptake site. The presence of an *O*-methyl group in the *para*-position in 2-(*p*-methoxyphenyl)ethylamine (*p*-methoxytyramine) depressed the affinity for uptake by a factor of 22 when compared with tyramine, or by a factor of 9 when compared with phenethylamine. The presence of a *m*-methoxyl group in normetanephrine depressed the affinity by a factor of 298 if compared with noradrenaline or by a factor of 155 if compared with octopamine. The presence of two methoxyl groups further depressed the affinity; in 2-(3,4-dimethoxyphenyl)ethylamine (3,4-dimethoxydopamine) the affinity was depressed by a factor of 1,180 if compared with dopamine or by a factor of 183 if compared with phenethylamine. In methoxamine (2,5-dimethoxyphenylpropranolamine) the affinity was depressed by a factor of 454 if compared with phenylpropanolamine; in methoxyphenamine the affinity was depressed by a factor of 8 when compared with methylamphetamine. In mescaline the three methoxyl groups in positions 3, 4 and 5 depressed the affinity still further, so that this compound had an affinity for the uptake site 14,000 times less than that of phenethylamine. There seemed to be little difference between the effects of methoxyl groups in the *para*- or *ortho*-positions; *m*-methoxyl groups, however, produced a much greater depression of affinity for the uptake site.

Effects of other structural changes (*Table* 7). Surprisingly little alteration in affinity was found when the benzene ring was replaced either by five- or six-membered alicyclic rings,

TABLE 6

EFFECTS OF *O*-METHYLATION ON INHIBITION OF UPTAKE$_1$ BY SYMPATHOMIMETIC AMINES

Drug	Substituents 4	3	β	α	R	Other	ID50 (M)	Relative affinity
Phenethylamine	H	H	H	H	H		$1{\cdot}1\times10^{-6}$	100
2-(*p*-Methoxyphenyl)ethylamine	OCH_3	H	H	H	H		$1{\cdot}0\times10^{-5}$	11
2-(3,4-Dimethoxyphenyl)ethylamine	OCH_3	OCH_3	H	H	H		$2{\cdot}0\times10^{-4}$	0·55
Mescaline	OCH_3	OCH_3	H	H	H	5-OCH_3	$1{\cdot}5\times10^{-2}$	0·007
(±)-*Noradrenaline*	OH	OH	OH	H	H		$6{\cdot}7\times10^{-7}$	164
(±)-Normetanephrine	OH	OCH_3	OH	H	H		$2{\cdot}0\times10^{-4}$	0·55
(±)-*Adrenaline*	OH	OH	OH	H	CH_3		$1{\cdot}4\times10^{-6}$	78
(±)-Metanephrine	OH	OCH_3	OH	H	CH_3		$4{\cdot}3\times10^{-5}$	2·6
(±)-*Phenylpropanolamine*	H	H	OH	CH_3	H		$2{\cdot}0\times10^{-6}$	55
(±)-Methoxamine	H	H	OH	CH_3	H	2,5-$(OCH_3)_2$	$1{\cdot}0\times10^{-3}$	0·11
(+)-*Methylamphetamine*	H	H	H	CH_3	CH_3		$6{\cdot}7\times10^{-7}$	165
(±)-Methoxyphenamine	H	H	H	CH_3	CH_3	2-OCH_3	$1{\cdot}1\times10^{-5}$	10

TABLE 7

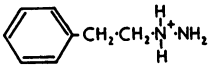
EFFECTS OF OTHER STRUCTURAL CHANGES ON INHIBITION OF UPTAKE$_1$ BY SYMPATHOMIMETIC AMINES

Drug	R	CH	CH	NH	ID50 (M)	Relative affinity
(+)-*Methamphetamine*	Phenyl	H	CH_3	CH_3	$6{\cdot}7\times10^{-7}$	165
(±)-Propylhexedrine	Cyclohexyl	H	CH_3	CH_3	$8{\cdot}5\times10^{-7}$	130
(±)-Cyclopentamine	Cyclopentyl	H	CH_3	CH_3	$1{\cdot}4\times10^{-6}$	78
Phenethylamine	Phenyl	H	H	H	$1{\cdot}1\times10^{-6}$	100
2-(Naphth-2-yl)ethylamine	Naphth-2-yl	H	H	H	$1{\cdot}5\times10^{-6}$	73
5-Hydroxytryptamine	5-Hydroxyindol-3-yl	H	H	H	$2{\cdot}0\times10^{-5}$	5·5
(±)-*Amphetamine*	Phenyl	H	CH_3	H	$4{\cdot}6\times10^{-7}$	240
Tuaminoheptane	Butyl	H	CH_3	H	$5{\cdot}6\times10^{-6}$	20
Dopamine	3,4-Dihydroxyphenyl	H	H	H	$1{\cdot}7\times10^{-7}$	650
(−)-Dopa	3,4-Dihydroxyphenyl	H	CO_2H	H	$9{\cdot}4\times10^{-5}$	1·2

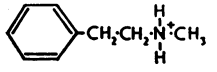
as in propylhexedrine and cyclopentamine, or by the bicyclic naphthalene ring; even the aliphatic amine tuaminoheptane had an affinity 20% of that of phenethylamine, but since it has an α-methyl group it must strictly be compared with (±)-amphetamine, which has an affinity twelve times that of tuaminoheptane. Substitution of chlorine atoms in positions 3 and 4 in dichloroisoprenaline increased the affinity twelve times compared with isoprenaline. Unfortunately, we were unable to obtain the corresponding dichloronoradrenaline, which might be expected to be very active. The 5-hydroxyindole analogue, 5-hydroxytryptamine, while markedly less active, nevertheless has quite appreciable affinity. This is interesting in view of the report by Owman (1964) that sympathetic fibres in the pineal gland store 5-hydroxytryptamine formed by the surrounding pinealocytes.

The effects of two changes in the side-chain are shown with phenelzine and tranylcypromine (Table 1). In phenelzine the terminal amino-group of phenethylamine is replaced

by a hydrazino-group. The activity is about the same as that to be expected for *N*-methylphenylethylamine. Since the terminal nitrogen atom of monosubstituted hydrazines is very feebly basic and the amino-group has a molecular volume only slightly smaller than that of a methyl group, this is not difficult to explain.



Phenelzine



N-Methylphenethylamine

In tranylcypromine [(±)-*trans*-2-phenylcyclopropylamine], the α-methyl group of amphetamine is covalently linked with the β-carbon atom to give a cyclopropane ring. The resulting compound is only one-seventh as active as (+)-amphetamine, but whether this is due to the altered bond angle imposed on the side-chain, or because the *trans*-isomer is not the optimal form, cannot be decided in the absence of results on *cis*-2-phenylcyclopropylamine.

Uptake$_2$

Many of the compounds tested on Uptake$_1$ have also been examined for their ability to inhibit noradrenaline uptake by the second uptake process (Uptake$_2$). The probit plots for some of these substances can be seen in Fig. 3 and the numerical values for their affinity, together with ratios of affinity for the two uptake systems, are shown in Table 8. It can be seen that the pattern of affinity is radically different from that for Uptake$_1$. The effect of *N*-alkylation is to increase affinity, adrenaline having 3.6 times that of noradrenaline; *N*-ethylnoradrenaline and *N*-isopropylnoradrenaline (isoprenaline) also have a greater affinity than noradrenaline although less than that of adrenaline. A smaller effect of

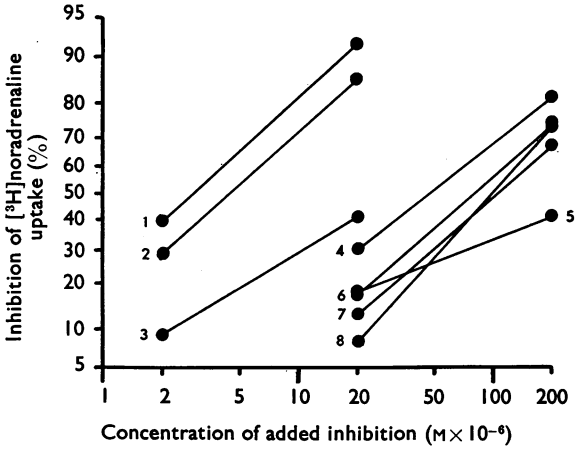


Fig. 3. The inhibition of [^{3}H]noradrenaline uptake at a concentration of 5 μg/ml. (±)-[^{3}H]noradrenaline by various sympathomimetic amines added simultaneously to the perfusion medium. Percentage inhibition of noradrenaline uptake (ordinate, probability scale) is plotted against the log concentration of added inhibitor. Each point is the mean value for a group of four hearts. 1=(±)-Metanephrine, 2=(±)-normetanephrine, 3=2-(3,4-dimethoxyphenyl)ethylamine, 4=hordenine, 5=dopamine, 6=phenethylamine, 7=(±)-amphetamine and 8=tyramine.

TABLE 8

INHIBITION OF UPTAKE$_2$ BY SYMPATHOMIMETIC AMINES

*Affinities determined by direct analysis of uptake kinetics (Iversen, 1965b)

Drug	ID50 for Uptake$_2$ (M)	Relative affinity for Uptake$_2$	Ratio $\frac{\text{ID50 Uptake}_2}{\text{ID50 Uptake}_1}$
(±)-Metanephrine	$2{\cdot}9\times10^{-6}$	2,585	0·068
(±)-Normetanephrine	$4{\cdot}2\times10^{-6}$	1,785	0·021
(±)-Buphenine	$7{\cdot}6\times10^{-6}$	985	9·1
(±)-Oxedrine	$1{\cdot}2\times10^{-5}$	625	1·0
2-(3,4-Dimethoxyphenyl)ethylamine	$3{\cdot}2\times10^{-5}$	234	0·16
Hordenine	$4{\cdot}6\times10^{-5}$	163	18·4
(±)-Adrenaline*	$5{\cdot}2\times10^{-5}$	144	37·1
PHENETHYLAMINE	$7{\cdot}5\times10^{-5}$	100	68·2
Metatyramine	$9{\cdot}5\times10^{-5}$	79	187
Tyramine	$1{\cdot}0\times10^{-4}$	75	223
(±)-Amphetamine	$1{\cdot}1\times10^{-4}$	68	239
Mescaline	$1{\cdot}2\times10^{-4}$	62	0·008
(±)-Isoprenaline	$1{\cdot}2\times10^{-4}$	62	4·8
(±)-*N*-Ethylnoradrenaline	$1{\cdot}8\times10^{-4}$	42	19·6
2-(*p*-Methoxyphenyl)ethylamine	$2{\cdot}0\times10^{-4}$	37	20·0
(±)-Noradrenaline*	$2{\cdot}5\times10^{-4}$	30	374
(±)-Methoxamine	$2{\cdot}7\times10^{-4}$	28	0·27
Dopamine	$4{\cdot}0\times10^{-4}$	19	2,350
(−)-Metaraminol	$>5{\cdot}0\times10^{-4}$	<15	>6,600

N-alkylation is seen with the *O*-methyl compounds, metanephrine and normetanephrine. A large *N*-substituent, as in buphenine, raises affinity again as it did with Uptake$_1$.

A phenolic hydroxyl group has a contrary effect to that on Uptake$_1$. Both tyramine and metatyramine are somewhat less active than phenethylamine, and dopamine is considerably less active still; similarly, adrenaline is much less active than oxedrine. It is particularly striking that metaraminol, which had the highest affinity of any compound for Uptake$_1$, should have such a poor affinity for Uptake$_2$; it is, in fact, a highly selective inhibitor of Uptake$_1$. Equally striking is the enhancing effect of *m*-*O*-methylation. Metanephrine and normetanephrine, which were very feeble inhibitors of Uptake$_1$, are the most active compounds so far encountered for inhibiting Uptake$_2$. That it is the *m*-methoxyl group that is important is emphasized by the relatively high activity of 2-(3,4-dimethoxyphenyl)ethylamine compared with that of 2-(*p*-methoxyphenyl)ethylamine. The latter is less active than either tyramine or phenethylamine. The introduction of two additional methoxyl groups in the *meta*-positions of 2-(*p*-methoxyphenyl)ethylamine, as in mescaline, was less effective, although this compound was the most selective inhibitor of Uptake$_2$. The 2,5-dimethoxy-compound methoxamine also had a relatively low affinity.

The effect of β-hydroxylation was slightly to increase affinity as judged by the slightly higher affinity of noradrenaline compared with dopamine; it is known that both stereoisomers of noradrenaline have a similar affinity for Uptake$_2$ (Iversen, 1965b), so that the configuration at the β-carbon atom does not seem to matter. An α-methyl group, however, slightly reduced activity, since amphetamine was less active than phenethylamine. The effect of both β-hydroxylation and α-methylation is, therefore, the opposite of that found in Uptake$_1$.

DISCUSSION

Structural specificity of inhibitors of Uptake$_1$

The results have shown that a wide range of sympathomimetic amines have an affinity for Uptake$_1$ of the same order as that of noradrenaline. A number of interesting structure-activity features have emerged from which it should be possible to build a preliminary picture of the uptake site.

The presence of a β-hydroxyl group reduced affinity, this effect being greater for the (+)-configuration than for the (−)-configuration. The absolute configuration of this group is known, the (−)-stereoisomer having an absolute L-configuration (see van Rossum, 1963). One possible explanation of this finding is that the β-carbon atom fits into a valley on the transport site which is constituted so that the extra bulk of the hydroxyl group prevents the β-carbon atom reaching its optimal rest position and that this interference with fit is more serious with the L- than with the D-configuration. An alternative explanation, that is perhaps equally plausible, is that the introduction of the β-hydroxyl group changes the proportion of conformational forms of the side-chain that are present. There are three probable conformations of the benzene ring and amino-groups attached to the α- and β-carbon atoms, the *trans*- and two *skew*-forms. Usually the *trans*-form in substituted ethanes is appreciably more stable than the *skew* forms. The presence of a β-hydroxyl group introduces the possibility of intramolecular hydrogen bonding in the *trans*-conformation, and in one of the *skew*-forms. Consequently, a β-hydroxyl group will increase the number of molecules in the *trans*-form. If we postulate that the conformation of the phenethylamine molecule having the highest affinity for the transport site is *skew*, the lower affinity of β-hydroxylated compounds could be explained. The greater affinity of the L-isomer might be due to the addition of a positive effect due to affinity of the hydroxyl group for the uptake site. This explanation has the merit of offering an explanation of the positive effect of the α-methyl group. This group tends to reduce the amount of *trans*-conformation considerably and would probably put *skew*-configurations in preponderance. However, the more conventional explanation of a Van der Waal's attraction of the methyl group for a non-polar residue in the transport site is equally plausible. The large differences between the affinity of the stereoisomers of amphetamine (twentyfold) once again point to a close fit of the ethylamine side-chain. It should be noted that α-methylation produced a smaller effect in the catechol amines than in non-phenolic or mono-phenolic compounds. This may be due to the phenolic groups forcing the ring into such a position that the side-chain is displaced away from its optimum rest position.

The importance of correct configuration at both the α- and β-carbon atoms makes the interpretation of the results difficult in compounds which have both carbon atoms substituted. In such compounds the absolute configurations at the α- and β-carbon atoms cannot be determined directly from the optical rotation of the compounds. However, in some instances the compounds have been analysed in more detail (van Rossum, 1963) and it has been established that in (−)-ephedrine and (−)-metaraminol the configurations are α=D, β=L. Unfortunately, the optical configuration of (±)-phenylpropanolamine is not known, so that comparisons were not possible when this substance was the reference molecule. In the other compounds the configuration at the α- and β-carbon atoms are those which would give optimal affinity for the uptake site.

A phenolic hydroxyl group in either position 3 or 4 increased affinity by a factor of two to three and the combined effects of disubstitution in positions 3 and 4 were exactly that expected from the additive effect of the two groups. This suggests that the intramolecular hydrogen bonding involving the two hydroxyl groups does not interfere with their binding capacity. The binding could be due to hydrogen-bond formation with suitable groups in the transport site, but the fact that chlorine atoms appear to be even more effective, although they are weaker hydrogen-bond acceptors and, of course, cannot act as hydrogen donors, suggests that hydrogen bonding is not the correct explanation. The increase in affinity produced by phenolic hydroxyl groups or chlorine atoms may be due either to Van der Waal's interaction or to the induction of a dipole in the site by the electronegative oxygen or chlorine atom. The very strong inhibitory effect of the *o*-hydroxyl group in 6-hydroxydopamine is very interesting and should be considered in association with the finding that methoxyphenamine, which has an *o*-methoxyl group, is quite active. The most probable explanation may lie either in the combination of groups in positions 3, 4 and 6 rendering fitting of the ring structure impossible or that the *o*-hydroxyl group forms a strong hydrogen bond with the amino-group to give a cyclic structure of inappropriate configuration.

The striking reduction in affinity produced by *O*-methylation, particularly in position 3, is very difficult to understand. In 3-*O*-methylated compounds resonance in the ring would force the methyl group to lie in the plane of the ring approximately mid-way between positions 2 and 3. A similar position would be taken by the 2-*O*-methyl group of methoxyphenamine, and yet the inhibitory effect in the latter case is less. Understanding of this problem will probably need detailed studies of the electronic structure of the catechol amines.

The minor effects of replacing the benzene ring by the alicyclic cyclohexene and cyclopentane rings suggest that the site is not sufficiently complementary to demand a planar ring, but that Van der Waal's or hydrophobic association occurs at this end of the molecule, and in their ability to form these bonds there is little to choose between benzene derivatives and the alicyclic compounds. The effect of the naphthalene ring, and of the aliphatic chain in 1-methylhexylamine (tuaminoheptane), reinforces the conclusion that the association at the ring end is relatively uncritical.

The effect of *N*-substitution is particularly interesting. Noradrenaline has optimal affinity which falls off rapidly with increasing chain length of the *N*-substituents, so that the *N*-propyl derivative has only 2% of the affinity of the primary amine. Yet an *N*-phenylpropyl group, as in prenylamine, causes virtually complete restoration of affinity; a similar effect is seen in buphenine, which has an *N*-phenylisopropyl group. It appears that a long-chain substituent or the nitrogen atom may interfere with the fitting of the ethylamine side-chain, but that this may be countered by a strong hydrophobic association formed by a terminal benzene ring; but since both substituents on the nitrogen atom have structural resemblances to the catechol amines it must remain uncertain which benzene ring becomes associated with the uptake site.

Structural specificity of inhibitors of Uptake$_2$

In almost every respect the structural requirements for inhibition of Uptake$_2$ differed from those for Uptake$_1$. *N*-Methylation increased affinity, as did β-hydroxylation, whereas

both a phenolic hydroxyl group and α-methylation decreased affinity. The effect of *N*-alkylation is interesting, both because of the interchange of relative affinities in the two uptake systems but also because, while an *N*-methyl group appears to be the optimum alkyl substituent, the affinity of isoprenaline is twice that of noradrenaline. Studies of the uptake of isoprenaline would clearly be of great interest and preliminary studies have shown that both isoprenaline and *N*-ethylnoradrenaline are accumulated by $Uptake_2$ at a rate comparable to that for noradrenaline. The most striking difference between the specificity of $Uptake_1$ and $Uptake_2$, however, is in the effect of *O*-methylation. This had a powerful depressant effect on the affinity for $Uptake_1$, especially when the methoxyl group is in the *meta*-position ; but for $Uptake_2$ this group greatly enhances affinity—for instance normetanephrine has an affinity sixty times that of noradrenaline. The positive effect of this group in $Uptake_2$ offers as great a pharmacological problem as the negative effect in $Uptake_1$. In this instance, too, it would be interesting to know whether the high affinity for the system is accompanied by a transport of normetanephrine and metanephrine.

The clear-cut differences in structural requirements for the two uptake processes certainly gives strong support to the conclusion (Iversen, 1965b) that $Uptake_1$ and $Uptake_2$ are distinct processes.

Some pharmacological implications of the present results

An important consequence of inhibiting the inactivation of noradrenaline by tissue uptake should be a potentiation of the effects of released noradrenaline on the effector tissue, and this will include a potentiation of the effects of noradrenaline introduced exogenously to the tissue, since the inactivation of exogenous noradrenaline is presumably by the same mechanism. In the present experiments it has been found that a large number of sympathomimetic amines are potent inhibitors of noradrenaline uptake. This finding has several implications; for instance, the ability of sympathomimetic amines such as tyramine to inhibit noradrenaline uptake may explain why the very small amounts of noradrenaline which are released by these compounds appear to have such potent actions on the effector tissue (Lindmar & Muscholl, 1961; Weiner, Draskóczy & Burack, 1962). Tyramine and other indirectly acting amines not only release noradrenaline but also potentiate the actions of the released noradrenaline by inhibiting the normal inactivation of noradrenaline by tissue uptake. The ability of tyramine and ephedrine to potentiate the actions of exogenous noradrenaline and adrenaline can also be explained on this basis (Gaddum & Kwiatkowski, 1938; Furchgott, Kirpekar, Rieker & Schwab, 1963). A second consequence of these results is the possibility that many of the amines which have a high affinity for the uptake site may also prove to be accumulated in the tissue. In the case of indirectly acting amines such an uptake into the tissue may be a necessary prelude to their subsequent displacement of noradrenaline from intracellular storage sites. However, for directly acting amines, uptake into the tissue may serve as an inactivation mechanism as it does for noradrenaline and adrenaline. The tissue uptake of such substances would therefore be expected to depress their direct pharmacological actions on the receptor tissue. In support of this hypothesis it is well known that uptake inhibitors such as cocaine potentiate the actions not only of noradrenaline but also of adrenaline and several other directly acting sympathomimetic amines, suggesting that these amines are taken up by the cocaine-sensitive noradrenaline uptake mechanism. Furthermore, cocaine and other uptake

inhibitors such as guanethidine and phenoxybenzamine potentiate the actions of noradrenaline to a greater extent than they potentiate the actions of adrenaline, and they have practically no effect on the actions of isoprenaline (Stafford, 1963). These findings are consistent with the present results, which indicated that noradrenaline has a higher affinity than adrenaline for the uptake mechanism, and that isoprenaline has only a very low affinity. Since tissue uptake was therefore most important for noradrenaline, less important for adrenaline and probably not important at all for isoprenaline, the effects of these amines are potentiated to various degrees by inhibiting tissue uptake. Since these experiments were completed Hertting (1964) has provided direct evidence that tissue uptake plays only a minor role in the inactivation of isoprenaline *in vivo*.

In this discussion it has been assumed that the inactivation mechanism involved in the removal of noradrenaline into tissues is equivalent to $Uptake_1$. However, as pointed out previously (Iversen, 1965b), the possibility cannot be ruled out that $Uptake_2$ may play some role in the inactivation of noradrenaline after its release from adrenergic nerve endings.

In the group of sympathomimetic amines tested as inhibitors of $Uptake_1$ there was no obvious correlation between affinity for the tissue uptake site and ability to displace noradrenaline from tissue stores. Many directly acting sympathomimetic amines had very high affinities for the uptake site, yet they do not appear to release noradrenaline from tissue stores. Among the indirectly acting amines tested there was no correlation between displacing activity and affinity for the uptake site. For instance, in the rat heart Potter & Axelrod (1963) found that tyramine and amphetamine were active in displacing endogenous noradrenaline, oxedrine was even more potent in this respect but phenethylamine was almost without displacing activity. These results do not correlate with the relative potencies of these amines as inhibitors of noradrenaline uptake; tyramine and amphetamine were only slightly more potent that phenethylamine, and oxedrine had the lowest affinity of the four amines for $Uptake_1$. The potent noradrenaline-displacing agent, metaraminol (Porter, Totaro & Stone, 1963), had a very high affinity for the uptake site, but, on the other hand, 6-hydroxydopamine, which is also reported to be a potent displacing agent (Porter *et al.*, 1963), had no measurable affinity for the uptake site.

It is also clear that neither the specificity of $Uptake_1$ nor that of $Uptake_2$ correlate with the known structure-activity relationships for either α- or β-receptors, or for any of the enzymes concerned in noradrenaline metabolism (monoamine oxidase or catechol-*O*-methyl transferase). The present results thus do not support the view that the uptake of catechol amines is explicable in terms of a binding at sympathetic α- or β-receptors (Kirpekar & Cervoni, 1963). Indeed, the diversity of specificity surrounding the systems with which the catechol amines interact emphasizes the difficulties in trying to understand structure-activity relationships at the molecular level and also the dangers of attributing pharmacological specificity to molecules when they are capable of interacting with tissue elements in so complex a manner.

SUMMARY

1. The affinities of a series of sympathomimetic amines for the sites of uptake of catechol amines in the rat heart were determined by measuring the potencies of these amines as inhibitors of the uptake of radioactively labelled noradrenaline in the isolated perfused organ.

2. Forty-eight amines were tested as inhibitors of the noradrenaline uptake process which operates at low perfusion concentrations of noradrenaline ($Uptake_1$). A wide range of derivatives of phenethylamine had affinities for $Uptake_1$ of the same order as that of noradrenaline. From these results it was possible to describe the structural specificity of $Uptake_1$. Among the derivatives of phenethylamine tested, β-hydroxylation, *N*-substitution or *O*-methylation decreased affinity for $Uptake_1$, but a phenolic hydroxyl group in position 3 or 4 and α-methylation increased affinity.

3. Seventeen of these amines were also tested as inhibitors of noradrenaline uptake by a second process which operates only at high perfusion concentrations of noradrenaline ($Uptake_2$). The results showed that the two uptake processes for catechol amines have quite different structural specificities. α-Methyl and phenolic hydroxyl groups decreased affinity for $Uptake_2$, whilst *N*-substitution, β-hydroxylation and particularly *m-O*-methylation increased affinity.

4. Some pharmacological implications of these results are discussed.

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