Inhibition of noradrenaline uptake by sympathomimetic amines

SIR,—The uptake of noradrenaline into tissues innervated by the sympathetic nervous system can be inhibited by a wide variety of drugs (Whitby, Hertting & Axelrod, 1960; Axelrod, Whitby & Hertting, 1961; Hertting, Axelrod & Whitby, 1961; Dengler, Spiegel & Titus, 1961; Axelrod, Hertting & Potter, 1962). Some of these inhibitors such as the sympathomimetic amines tyramine, amphetamine and ephedrine are closely related chemically to noradrenaline, and the present study was undertaken as a systematic and quantitative investigation of the inhibition of noradrenaline uptake by these and other chemically related amines to elucidate the structural specificity of the noradrenaline uptake site.

A simple test procedure has been designed for this purpose. Rat hearts were perfused by the Langendorff technique with a medium containing DL-β-^{14}C-noradrenaline (specific activity = 130 μc/mg) at a concentration of 10 ng/ml. The uptake of noradrenaline was measured by assaying the tissue content of ^{14}C-noradrenaline at the end of a 10 min perfusion. The methods used for measuring the uptake of radioactively-labelled noradrenaline in this preparation have been described in detail elsewhere (Iversen, 1963). Drugs were added

TABLE 1. INHIBITION OF NORADRENALINE UPTAKE BY SYMPATHOMIMETIC AMINES IN THE ISOLATED RAT HEART

Drug	ID50 (Drug concentration (M) producing 50% inhibition of noradrenaline uptake)	Relative affinity for uptake site (β-phenylethylamine = 100)
(−)-Metaraminol	$7{\cdot}6 \times 10^{-8}$	1440
Dopamine	$1{\cdot}7 \times 10^{-7}$	650
(±)-α-Methyldopamine	$1{\cdot}8 \times 10^{-7}$	610
(+)-Amphetamine	$1{\cdot}8 \times 10^{-7}$	610
(±)-Hydroxyamphetamine	$1{\cdot}8 \times 10^{-7}$	610
(−)-Nordefrin (Corbasil)	$2{\cdot}0 \times 10^{-7}$	550
(−)-Noradrenaline	$3{\cdot}3 \times 10^{-7}$	330
(±)-Nordefrin	$4{\cdot}2 \times 10^{-7}$	260
Tyramine	$4{\cdot}5 \times 10^{-7}$	245
(±)-Amphetamine	$4{\cdot}6 \times 10^{-7}$	240
m-Tyramine	$5{\cdot}1 \times 10^{-7}$	215
(+)-Methamphetamine	$6{\cdot}7 \times 10^{-7}$	165
(±)-Noradrenaline	$7{\cdot}3 \times 10^{-7}$	150
N-Methyldopamine (Epinine)	$7{\cdot}6 \times 10^{-7}$	145
(±)-Buphenine	$8{\cdot}5 \times 10^{-7}$	130
(±)-Propylhexedrine	$8{\cdot}5 \times 10^{-7}$	130
(−)-Adrenaline	$1{\cdot}0 \times 10^{-6}$	110
Mephentermine	$1{\cdot}0 \times 10^{-6}$	110
β-Phenylethylamine	$1{\cdot}1 \times 10^{-6}$	100
(+)-Noradrenaline	$1{\cdot}3 \times 10^{-6}$	85
(±)-Octopamine	$1{\cdot}3 \times 10^{-6}$	85
(±)-Cyclopentamine	$1{\cdot}4 \times 10^{-6}$	78
Noradrenalone	$1{\cdot}5 \times 10^{-6}$	75
(±)-Adrenaline	$1{\cdot}6 \times 10^{-6}$	70
(±)-Phenylpropanolamine	$2{\cdot}0 \times 10^{-6}$	55
(−)-Ephedrine	$2{\cdot}2 \times 10^{-6}$	50
Hordenine	$2{\cdot}5 \times 10^{-6}$	45
(−)-Amphetamine	$3{\cdot}7 \times 10^{-6}$	30
(±)-Phenylethanolamine	$4{\cdot}8 \times 10^{-6}$	23
(−)-Phenylephrine	$5{\cdot}6 \times 10^{-6}$	20
1-Methylhexylamine (Tuamine)	$5{\cdot}6 \times 10^{-6}$	20
(±)-*N*-Ethylnoradrenaline	$9{\cdot}2 \times 10^{-6}$	12
p-Methoxyphenylethylamine	$1{\cdot}0 \times 10^{-5}$	11
(±)-Methoxyphenamine	$1{\cdot}1 \times 10^{-5}$	10
(±)-Oxedrine	$1{\cdot}2 \times 10^{-5}$	9
(±)-Isoprenaline	$2{\cdot}5 \times 10^{-5}$	4·5
(±)-*N*-n-Butylnoradrenaline	$3{\cdot}5 \times 10^{-5}$	3·2
(±)-*N*-Isobutylnoradrenaline	$4{\cdot}0 \times 10^{-5}$	2·6
(−)-DOPA	$6{\cdot}0 \times 10^{-5}$	1·2
(±)-3-Methoxynoradrenaline	$2{\cdot}0 \times 10^{-4}$	0·55
3,4-Dimethoxyphenylethylamine	$2{\cdot}0 \times 10^{-4}$	0·55
(±)-Methoxamine	$1{\cdot}0 \times 10^{-3}$	0·11
Mescaline	$1{\cdot}5 \times 10^{-2}$	0·007

to the perfusing medium at various concentrations to determine the drug concentration which produced a 50% inhibition of the control noradrenaline uptake. This drug concentration (ID50) was taken as a reciprocal measure of the affinity of the drug molecule for the noradrenaline uptake site, and relative affinities were calculated and expressed on an arbitrary scale on which the wholly unsubstituted parent molecule β-phenylethylamine was assigned a value of 100. Two to four drug concentrations were used, each concentration was tested on a group of four hearts and the mean inhibition for the group was used in the calculation of the ID50. It should be emphasised that this test procedure does not distinguish between substances that inhibit noradrenaline uptake because they are themselves transported, and non-transported inhibitors, it should therefore be regarded as a screening test.

The results obtained with 43 drugs are presented in Table 1. All the substances tested showed some activity as inhibitors of noradrenaline uptake. From these results the following preliminary conclusions have been drawn concerning the structural specificity of the noradrenaline uptake site in the rat heart with β-phenylethylamine as the reference molecule.

1. *N*-Substitution decreases affinity; the optimal chemical structure seems to be a primary amine group.

2. β-Hydroxylation decreases affinity; in compounds with a β-hydroxyl group the (−)-isomer has a higher affinity than the (+)-isomer.

3. α-Methylation increases affinity; in compounds with an α-methyl group the (+)-isomer has a much higher affinity than the (−)-isomer.

4. Phenolic hydroxyl groups increase affinity.

5. *O*-Methylation of phenolic hydroxyl groups leads to a very striking decrease in affinity.

6. The aromatic ring is not an essential structural feature and can be replaced by saturated ring structures or even by a branched aliphatic chain without great change in activity.

The finding that many sympathomimetic amines are extremely potent inhibitors of noradrenaline uptake suggests that at least part of their pharmacological activity may be related to this property. For example, the ability of tyramine to prevent the normal inactivation of noradrenaline by tissue uptake could explain the reported sensitisation to noradrenaline produced by this compound (Lindmar & Muscholl, 1961; Furchgott, Kirpekar, Rieker & Schwab, 1963).

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