J. Pharm. Pharmac., 1966, **18**, 481–484 Received April 12, 1966

Accumulation of α-methyltyramine by the noradrenaline uptake process in the isolated rat heart

LESLIE L. IVERSEN*

In the perfused isolated rat heart ^{3}H-α-methyltyramine is accumulated as rapidly as noradrenaline. The accumulation of ^{3}H-α-methyltyramine is reduced by the presence of noradrenaline or other drugs known to inhibit the noradrenaline uptake mechanism. ^{3}H-α-Methyltyramine and noradrenaline probably compete for a common uptake mechanism. In these experiments less than 5% of the ^{3}H-α-methyltyramine accumulated by the heart was metabolised to α-methyloctopamine.

CATECHOLAMINE uptake in the isolated rat heart has been shown previously to be mediated by a saturable, stereochemically specific mechanism which has the kinetic properties of an active transport membrane carrier system (Iversen, 1963; 1965). This uptake process is thought to be almost entirely localised in the postganglionic sympathetic innervation of the heart, since the uptake of noradrenaline is severely reduced in hearts which lack a normal sympathetic innervation (Hertting & Schiefthaler, 1964; Potter, Cooper, Willman & Wolfe, 1965; Iversen, Glowinski & Axelrod, 1966). A wide range of sympathomimetic amines structurally related to noradrenaline can act as potent inhibitors of noradrenaline uptake in the rat heart (Burgen & Iversen, 1965). However, it is not clear whether these amines inhibit noradrenaline uptake by acting as competitive substrates for the uptake system, or whether they inhibit uptake without themselves being transported into the tissue. Recent findings suggest that at least some of the structural analogues of noradrenaline act as alternative substrates for the catecholamine uptake process. For example, α-methylnoradrenaline and adrenaline are taken up into the isolated rat heart by a process which has very similar properties to that responsible for noradrenaline uptake (Iversen, 1965; Lindmar & Muscholl, 1965; Muscholl & Weber, 1965). The histochemical studies of Hamberger, Malmfors, Norberg & Sachs (1964) and Malmfors (1965) have also demonstrated an efficient uptake of α-methylnoradrenaline into the sympathetic ground plexus of the rat iris. Tyramine uptake into rat salivary glands is mediated in part by a process which is abolished by the chronic sympathetic denervation of the glands (Carlsson & Waldeck, 1963; Fischer, Musacchio, Kopin & Axelrod, 1964). Metaraminol is also avidly accumulated in rat tissues after the administration of small doses of this compound *in vivo*; this uptake is almost completely lacking in the tissues of immunosympathectomised animals (Shore, Busfield & Alpers, 1964). The uptake of metaraminol is furthermore inhibited by drugs known to reduce the uptake of noradrenaline (Carlsson & Waldeck, 1965).

From the Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland, U.S.A.

*Harkness Fellow. Present address: Department of Pharmacology, University of Cambridge, England.

In a previous study it was found that the accumulation of ^{3}H-α-methyltyramine in the rat heart *in vivo* was severely reduced in immunosympathectomised animals (Iversen & others, 1966). This finding, together with the results presented in the present study, suggests that α-methyltyramine is also taken up by the catecholamine transport process in the sympathetic innervation of the rat heart.

Experimental and results

^{3}H-α-Methyltyramine (generally labelled; specific activity = 3 c/mM; chromatographically pure) was generously supplied by Dr. I. J. Kopin. The uptake of α-methyltyramine in the rat heart was studied after perfusing the organ with a low concentration of the labelled amine for 5 min. This short exposure of the tissue to the labelled amine was sufficient to allow a large accumulation of the substance, but did not allow time for any appreciable conversion of the labelled amine into its β-hydroxylated derivative within the tissue.

The uptake of α-methyltyramine was measured by two methods. In the first, uptake was estimated by measuring the amount of radioactive amine disappearing from the perfusion medium during passage through the heart; this approach has previously been employed for measurements of noradrenaline uptake in this preparation (Lindmar & Muscholl, 1964). Uptake was measured in the same experiments by the alternative method of assaying the accumulated radioactive amine in the heart at the end of the perfusion.

TABLE 1. INHIBITION OF α-METHYLTYRAMINE UPTAKE BY DRUGS

Drug	Uptake of ^{3}H-α-methyltyramine-ng/g heart	Inhibition of α-methyltyramine uptake (%)
Control	9·95 ± 0·50	—
10^{-5}M Cocaine	6·85 ± 0·65	31
10^{-5}M Chlorpromazine	6·20 ± 0·25	37
10^{-5}M Phenoxybenzamine	1·55 ± 1·25	84
10^{-6}M Desipramine	5·25 ± 0·60	47
10^{-6}M (−)-Metaraminol	5·35 ± 0·10	46
2×10^{-7}M (−)-Noradrenaline	5·65 ± 0·15	43

Uptake of ^{3}H-α-methyltyramine measured as accumulation of labelled amine in the rat heart after perfusion for 5 min with a medium containing ^{3}H-α-methyltyramine (1 ng/ml). Drugs were added to this medium to yield the concentrations indicated. Results are mean values ± s.e. mean for 6 control hearts and for 4 hearts in each drug treated group.

In control hearts perfused for 5 min with a medium containing 1 ng ^{3}H-α-methyltyramine/ml, the mean uptake of labelled amine from the perfusing medium was 10·2 ± 0·95 ng/g heart (wet weight), and the amount of labelled amine accumulated in the heart during these experiments was 9·9 ± 0·50 ng/g mean ± s.e. (mean for 6 experiments). Ion-exchange chromatography on columns of Dowex-50 (Iversen & others, 1966) indicated that at least 95% of the radioactivity in the heart extracts could be accounted for as unchanged ^{3}H-α-methyltyramine; the remaining radioactivity was recovered as the β-hydroxylated derivative, ^{3}H-α-methyloctopamine. The effects of various drugs on the uptake of ^{3}H-α-methyltyramine are summarised in Table 1. The drugs were added to the

perfusion medium, together with the labelled amine. In all experiments α-methyltyramine uptake was measured by the two methods described above. The two methods always gave closely similar results, and the results quoted in Table 1 are those obtained by measuring the accumulation of labelled amine in the heart at the end of the perfusion. At concentrations of 10^{-5}M or less, chlorpromazine, cocaine, desipramine, phenoxybenzamine and metaraminol all significantly inhibited the uptake of α-methyltyramine. The presence of (—)-noradrenaline at a concentration of 2×10^{-7}M inhibited the uptake of α-methyltyramine by almost 50%. This concentration of noradrenaline was chosen as it is close to the previously determined "Km" value for the uptake of L-noradrenaline in the rat heart (Iversen, 1963). If noradrenaline and α-methyltyramine compete for uptake by a common mechanism, the presence of noradrenaline at a concentration sufficient to saturate approximately 50% of the available uptake sites should produce an inhibition of approximately 50% of the uptake of α-methyltyramine, as was indeed the case.

Discussio

The sensitivity of α-methyltyramine uptake to inhibition by a low concentration of noradrenaline and by several other drugs which are known to be inhibitors of the noradrenaline uptake process, suggests that α-methyltyramine is taken up in the rat heart by the mechanism responsible for catecholamine uptake. It seems likely that several amines which are structurally related to noradrenaline, including tyramine, α-methyltyramine, adrenaline, α-methylnoradrenaline and metaraminol can act as alternative substrates for the catecholamine uptake process. The use of amines such as α-methyltyramine may prove valuable for future studies of the detailed properties of this uptake process. The uptake of noradrenaline is a complex process, probably involving at least two phases. An initial entry of the catecholamine into the nerve terminals of sympathetic fibres is mediated by a carrier system in the axonal membrane. After this initial uptake, however, the accumulated amine undergoes a further redistribution within the nerve terminal involving an uptake or binding in intraneuronal storage particles (Potter & Axelrod, 1963). The uptake of amines such as α-methyltyramine, however, is relatively simple since this substance and other amines which lack a β-hydroxyl group are not appreciably bound in intraneuronal storage particles (Musacchio, Kopin & Weise, 1965). Furthermore, α-methyltyramine, in common with other α-methylated amines, is not a substrate for intraneuronal monoamine oxidase. The influx of this compound can therefore be estimated reliably simply by measuring the accumulation of the labelled amine in the tissue. In accordance with this prediction, the results of the present experiments showed that more than 95% of the amine removed by uptake from the perfusion medium could be accounted for as unchanged α-methyltyramine which accumulated in the tissue. During the short time of perfusion less than 5% of the accumulated amine was converted to α-methyloctopamine. Despite the short duration of the experiments the

concentration of α-methyltyramine accumulated per gram of heart was ten times higher than that present per ml in the perfusion medium, indicating that α-methyltyramine has a high affinity for the catecholamine uptake sites and is accumulated as rapidly as noradrenaline itself under similar conditions.

References

Burgen, A. S. V. & Iversen, L. L. (1965). *Br. J. Pharmac. Chemother.*, **25**, 34–49.
Carlsson, A. & Waldeck, B. (1963). *Acta pharmac. tox.*, **20**, 371–374.
Carlsson, A. & Waldeck, B. (1965). *J. Pharm. Pharmac.*, **17**, 243–244.
Fischer, J. E., Musacchio, J., Kopin, I. J. & Axelrod, J. (1964). *Life Sci.*, **3**, 413–419.
Hamberger, B., Malmfors, T., Norberg, K-A. & Sachs, C. (1964). *Biochem. Pharmac.*, **13**, 841–844.
Hertting, G. & Schiefthaler, T. (1964). *Int. J. Neuropharmac.*, **3**, 65–69.
Iversen, L. L. (1963). *Br. J. Pharmac. Chemother.*, **21**, 523–537.
Iversen, L. L. (1965). *Ibid.*, **24**, 387–394.
Iversen, L. L., Glowinski, J. & Axelrod, J. (1966). *J. Pharmac. exp. Ther.*, **151**, 273–284.
Lindmar, R. & Muscholl, E. (1964). *Arch. exp. Path. Pharmak.*, **247**, 469–492.
Lindmar, R. & Muscholl, E. (1965). *Ibid.*, **249**, 529–548.
Malmfors, T. (1965). *Acta physiol. scand.*, **64**, *Suppl.* 248.
Musacchio, J. M., Kopin, I. J. & Weise, V. K. (1965). *J. Pharmac. exp. Ther.*, **148**, 22–28.
Muscholl, E. & Weber, E. (1965). *Arch. exp. Path. Pharmak.*, **252**, 134–143.
Potter, L. T. & Axelrod, J. (1963). *J. Pharmac. exp. Ther.*, **142**, 291–305.
Potter, L. T., Cooper, T., Willman, V. L. & Wolfe, D. E. (1965). *Circulation Res.*, **16**, 468–481.
Shore, P. A., Busfield, D. & Alpers, H. S. (1964). *J. Pharmac. exp. Ther.*, **146**, 194–199.