it is predominantly inactivated by catechol-*O*-methyltransferase, whilst the other is predominantly degraded by monoamine oxidase (MAO) in the tissue itself (Kopin, 1966). Some of the animal data drawn on to reach this conclusion have, however, been criticized (Smith, 1966) on the grounds that the tyramine dosage used was excessive and might have acted as a competitive MAO inhibitor. The present study was carried out to establish whether data compatible with the hypothesis could be obtained in man, using comparatively low drug dosage.

At intervals, six adult male volunteers were injected intravenously and with suitable precautions, with placebo, tyramine (2 mg), (+)-amphetamine (10 mg), prenylamine (30 mg) and reserpine (1.25 mg). Urine collections made at 1, 3, 6, 9, 12 and 24 hr were analysed for total metadrenalines, 4-hydroxy-3-methoxymandelic acid (VMA) and 4-hydroxy-3-methoxyphenylglycol (HMPG) by methods previously employed (Sandler & Ruthven, 1966).

Compared with placebo, tyramine provoked a significant general increase of urinary catecholamine metabolite output during the first 3 hr of urine collection. There was a relatively greater proportion of *O*-methylated metabolites (total metadrenalines) compared with the more prolonged but also highly significant increase in excretion noted after reserpine, where the oxidatively deaminated *O*-methylated metabolites (VMA and HMPG) predominated. These data mirror the findings in certain human catecholamine-secreting tumours (Sandler & Ruthven, 1966). The increase of HMPG at the expense of VMA observed after reserpine (Sandler & Youdim, 1968) could not be detected after prenylamine although the comparatively low ratio of *O*-methylated to oxidatively deaminated metabolites was similar after each drug. Amphetamine seemed to have little effect on metabolite output.

These observations in man and their implications are compatible with those summarized by Kopin (1966).

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Studies on the uptake and accumulation of ^{3}H-noradrenaline in adrenergic nerves after pretreatment with reserpine, nialamide and a tyrosine-hydroxylase inhibitor

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The uptake and accumulation *in vitro* of ^{3}H-noradrenaline in adrenergic nerves of rat iris during various experimental conditions has been studied. The rats were pretreated with reserpine (10 mg/kg I.P., 16 hours beforehand) and/or nialamide (100 mg/kg I.P., 2 hours beforehand) or with the tyrosine-hydroxylase inhibitor H 44/68 (the methylester of α-methyl-*p*-tyrosine; 500 mg/kg I.P., 16 hours beforehand). The irides were dissected out and incubated *in vitro* in a Krebs-bicarbonate buffer containing different concentrations

of ^{3}H-noradrenaline from 2×10^{-8} M to 10^{-4} M. The incubation was performed at 37° C for 5–180 min in a metabolic shaker. After termination of the incubation, the irides were homogenized in *n*-butanol/0.1% HCl and radioactivity determined in a liquid scintillation spectrometer (Hamberger, Jonsson, Malmfors & Sachs, unpublished). In representative types of experiments, ^{3}H-noradrenaline and its *O*-methylated and deaminated metabolites were determined by specific chemical analytical procedures. Only very small amounts of metabolites could be detected, except when the animals were pretreated with reserpine alone, where a relatively large amount of acid catabolites were identified. The radioactivity values obtained during the various experimental conditions investigated mainly represented ^{3}H-noradrenaline.

In order to determine the extent of ^{3}H-noradrenaline taken up into the adrenergic nerves and that located extraneuronally, the uptake in normal and sympathetically denervated irides were compared. There was only a very small extraneuronal fraction (less than 7%) when the incubation medium contained up to 10^{-4} M ^{3}H-noradrenaline, whereas this fraction increased markedly when the medium concentration was raised above this value.

It is now generally accepted that at least two accumulation mechanisms are operating in adrenergic nerves, one at the level of the axonal membrane and one at the granular level intraneuronally. Four principally different models for the uptake of noradrenaline were investigated. In the untreated animal, both uptake mechanisms are intact. After pretreatment with the monoamine oxidase inhibitor nialamide the intraneuronal catabolism of noradrenaline was eliminated, allowing accumulation of amine extragranularly. Reserpine and nialamide pretreatment gives an experimental model for studies of the uptake at the axonal membrane, since reserpine strongly inhibits the uptake of noradrenaline into the storage granules. Inhibition of tyrosine-hydroxylase by H 44/68 caused a marked depletion of endogenous noradrenaline in iris, while the uptake of exogenous administered noradrenaline into the amine granules was not affected (Malmfors, 1967). In the last case both uptake mechanisms operate, but the storage granules are initially almost empty. It was found that there was an efficient uptake and accumulation of ^{3}H-noradrenaline in all of these experimental models, indicating that the axonal membrane uptake mechanism is most efficient. The granular uptake seemed to be of importance for the retention of noradrenaline intraneuronally. Detailed kinetic data on the uptake of ^{3}H-noradrenaline for the different models investigated will be presented and discussed.

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Possible functional differentiation between the stores from which adrenergic nerve stimulation, tyramine and amphetamine release noradrenaline

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In anaesthetized rats, stimulation of the cervical sympathetic trunk elicits contraction of the lower eyelid. This response has been shown to be essentially adrenergic in nature (Obianwu, 1967). In animals pretreated with the monoamine oxidase inhibitor pargyline (75 mg/kg I.P., 1 hr beforehand), the response of the eyelid to tyramine, α-methyltyramine and (+)-amphetamine were greatly enhanced. In the same animals the response of the eyelid to nerve stimulation was not affected.