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HCl and radioactivity determined in a liquid scintillation spectrometer (Hamberger, Jonsson, Malmfors & Sachs, unpublished).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.

The methylester hydrochloride of (±)-α-methyl tyrosine (H 44/68) is an efficient inhibitor of tyrosine hydroxylase (Corrodi & Hanson, 1966), an enzyme involved in the rate limiting step of biosynthesis of noradrenaline.

In rats pretreated with H 44/68 (250 mg/kg I.P., 6–12 hr, beforehand) and pargyline (75 mg/kg I.P., 1 hr beforehand), amphetamine failed to elicit contraction of the eyelid. In the same animals or similarly treated animals, tyramine still elicited a marked response while the response to nerve stimulation was not affected. The results indicate that an appreciable proportion of noradrenaline released by the indirectly acting sympathomimetic amines are metabolized by intraneuronal mono-amine oxidase and provide evidence which strongly suggests that the stores from which adrenergic nerve stimulation, tyramine or amphetamine releases noradrenaline may be different.

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Histochemical studies on the uptake of noradrenaline in the perfused rat heart

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Iversen has defined two different uptake mechanisms for noradrenaline in rat heart. One ($Uptake_1$) works at low amine concentrations (10–1,000 ng/ml.) and is inhibited by cocaine and desipramine, while the other ($Uptake_2$) works at much higher concentrations (1–40 μg/ml.) and is inhibited by normetanephrine.

We have studied the cellular localization of noradrenaline and also of α-methyl-noradrenaline taken up in the rat heart during perfusion with different concentrations of the amines, using the fluorescence method of Falck and Hillarp. The hearts of adult female albino rats (Sprague-Dawley) were perfused by the method of Langendorff. The amine was added to the perfusion solution.

When hearts from normal or reserpine-pretreated rats (10 mg/kg reserpine I.P. 16 hr beforehand) were perfused with low concentrations of noradrenaline or α-methyl-noradrenaline (0.02–0.2 μg/ml.) no evidence for an extra-neuronal binding of the catecholamines was found. In the hearts from the reserpine-pretreated animals, however, where the endogenous noradrenaline had been depleted, an accumulation of catecholamines into the adrenergic nerves could be found. The accumulation was prevented when desipramine (10^{-6} M) was added to the perfusion medium, but not when normetanephrine (10^{-4} M) was added. These results support the view of Iversen (1965) that the $Uptake_1$ in the rat heart is localized in the adrenergic nerves and are consistent with the results obtained from the adrenergic nerves in the rat iris (Malmfors, 1965).

When the hearts were perfused with high concentrations of noradrenaline or α-methyl-noradrenaline (20 μg/ml.) there was a markedly increased background fluorescence in the muscle cells due to the presence of catecholamines. Furthermore, small non-neuronal cells with a strong specific green to yellow-green fluorescence could be found, mainly in the connective tissue. These cells were only found in large numbers in the atria. The increase of the specific fluorescence in the muscle cells was objectively measured by means of microspectrophotofluorometry. Addition of desipramine (10^{-4} M) to the perfusion medium prevented the neuronal but not the extraneuronal accumulation, while addition