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Chapter 6

Synthesis, Uptake and Storage of Catecholamines in Adrenergic Nerves, The Effect of Drugs

U.S. v. Euler

With 9 Figures

A. Introduction

The finding that the sympathomimetic agents in chromaffin cells and in adrenergic nerves are stored in apparently similar subcellular particles has sometimes led to the belief that the mechanisms for uptake, storage and release of the active compounds are identical or at least very similar. Detailed studies, mainly on isolated particles, have shown, however, that although similarities occur, important differences between the properties of the chromaffin cell particles and those in the adrenergic nerves exist. Thus nerve particles after partial depletion readily take up amines from a suspension medium to the original content or even higher in the presence of ATP-Mg^{++}, whereas chromaffin cell particles containing either adrenaline or noradrenaline lack this property. Some drugs like phenoxybenzamine inhibit the release of noradrenaline from nerve particles but enhance the release from adrenal medullary granules. Striking differences in osmotic behaviour between chromaffin cell particles and nerve particles have also been described.

Moreover, there is increasing evidence that not all adrenergic nerve particles are of the same kind. Those appearing in the short adrenergic neurons in the male accessory reproductive organs show a different behaviour from those present in the ordinary adrenergic neurons, e.g. in the spleen. It is possible that the adrenergic nerve particles in the CNS have special properties, although little is known in this respect.

The important question of the mechanism of amine release from the chromaffin cells and adrenergic nerve terminals is still under debate and will not be discussed in this Chapter; it is only mentioned here since certain data indicate that the release mechanisms may differ, not only as regards the spontaneous release rate *in vitro*, but also more fundamentally, with respect to the nature of the releasing process *in vivo*.

B. Adrenergic Nerves

I. Synthesis

1. Main Synthetic Pathway

Synthesis of adrenaline occurs *in vivo* from phenylalanine and tyrosine (Gurin and Delluva, 1947; Udenfriend et al., 1953) and from dopa and dopamine (Udenfriend and Wyngaarden, 1956). The formation of noradrenaline as a step in the synthetic pathway was originally suggested by Blaschko (1939) and by

HOLTZ (1939) following the discovery of dopa decarboxylase by HOLTZ et al. (1938). This was confirmed *in vitro* by GOODALL and KIRSHNER (1957) who showed that tyrosine is converted in a sequence to dopa, dopamine, noradrenaline and adrenaline by enzymes in the adrenal medulla. This pathway could later be confirmed also for adrenergic nerves (GOODALL and KIRSHNER, 1958) and for organs containing adrenergic nerves (SPECTOR et al., 1963a).

In the first step tyrosine serves as substrate for the enzyme tyrosine hydroxylase which is present in the axoplasm (NAGATSU et al., 1964). The DOPA formed is transformed to dopamine by L-DOPA decarboxylase which also occurs in the axoplasm. Dopamine is subsequently oxidized to noradrenaline with the aid of the enzyme dopamine-β-hydroxylase which is present in the storage particles (Fig. 1).

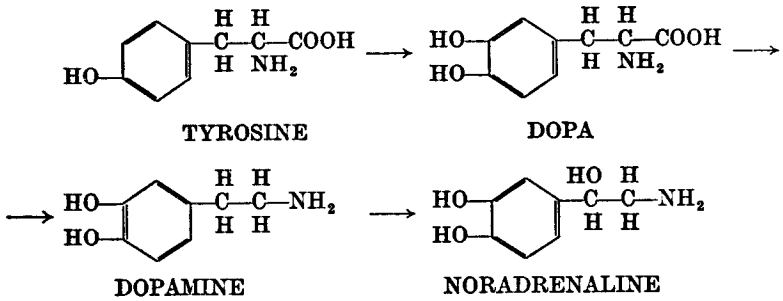

Fig. 1. Biosynthetic pathway for noradrenaline

a) Tyrosine Hydroxylase

Since the formation of tyrosine from phenylalanine occurs in a variety of tissues outside the adrenergic neuron it seems appropriate to regard the formation of DOPA from tyrosine as the first synthetic step in this context. Tyrosine, which is generally available in the body fluids, can enter the adrenergic axon, where tyrosine hydroxylase is present. Whether or not special "pump" or "carrier" mechanisms are required for the entrance of tyrosine into the axon is not known, but it has been assumed that this process is facilitated by a "permease".

While tyrosine generally is a poor substrate for amino acid decarboxylases it is transformed into an efficient substrate by hydroxylation in the 3-position of the ring. The active enzyme was isolated and characterized by NAGATSU et al. (1964) (cf. UDENFRIEND, 1966). It is present in tissues which normally contain noradrenaline or adrenaline. The enzyme requires tetrahydropyridines, e.g. tetrahydrofolic acid, and is activated by divalent iron (IKEDA et al., 1965). Tyrosine hydroxylase catalyzes the oxidation of phenylalanine to tyrosine as well as the following step from tyrosine to DOPA.

The hydroxylation of tyrosine to DOPA is slower than the subsequent decarboxylation and β-hydroxylation and therefore rate limiting (SPECTOR et al., 1963a; LEVITT et al., 1965). The activity of the enzyme is inhibited by phenylalanine as well as by DOPA and noradrenaline, which may well constitute a feedback control mechanism, regulating the synthesis rate (NAGATSU et al., 1964).

b) DOPA-Decarboxylase

The second step in the biosynthesis of the adrenergic neurotransmitter, the formation of dopamine from L-DOPA, is catalyzed by an efficient enzyme, L-DOPA decarboxylase, discovered in the mammalian kidney by HOLTZ et al.

(1938) (cf. Blaschko, 1959; Holtz, 1959; Sourkes, 1966). The enzyme, which requires pyridoxal-5'-phosphate as co-factor (cf. Holtz and Palm, 1964), is cyanide-sensitive; it seems to occur in all tissues containing adrenergic neurons (Holtz et al., 1942) or chromaffin cells (Langemann, 1951).

Incubation of noradrenaline or dopamine (but not adrenaline) with the enzymic co-factor causes inactivation of the enzyme and of the amine through formation of Schiff's bases and transformation to tetrahydro isoquinoline derivatives (Schott and Clark, 1952; Holtz and Westermann, 1957).

Since DOPA but not dopamine passes the blood brain barrier, the amino acid has been used in patients with Parkinsonism in order to supply substrate for dopamine formation which is deficient in these cases (Birkmayer and Hornykiewicz, 1962).

DOPA-decarboxylase is not highly substrate specific, even if L-DOPA appears to be the best substrate. Ortho and metatyrosine also serve as substrates (Blaschko et al., 1949; Blaschko, 1950; Sourkes, 1955; Blaschko and Chrusciel, 1960), whereas tyrosine is not decarboxylated by the enzyme prepared from the kidney or liver of some animals (Holtz et al., 1939; Awapara et al., 1964). The finding that N-methyl DOPA is not attacked by mammalian decarboxylase actually formed the basis for the concept of adrenaline formation via a primary amine like dopamine or noradrenaline (Blaschko, 1939). On the other hand 5-HTP is a good substrate for the enzyme (Holtz and Westermann, 1957; cf. also Hagen and Cohen, 1966), and also 3,4-dihydroxyphenylserine (DOPS) which is directly decarboxylated to noradrenaline (Blaschko et al., 1950; Schmiterlöw, 1951).

Decarboxylation of *erythro*-dihydroxyphenylserine and *erythro-m*-hydroxyphenylserine to noradrenaline has been demonstrated with hog kidney extract, and of *erythro-* or *threo*-dihydroxyphenylserine with rat liver homogenate. After injection of *threo*-dihydroxyphenylserine in rats, the (—) isomer of noradrenaline was recovered in urine (Hartman et al., 1955b).

The decarboxylation product, dopamine, was first detected in the mammalian heart and the suprarenal medulla (Goodall, 1950, 1951) and occurs as a natural product in urine (Euler et al., 1951) and in a variety of organs and tissues. In studies of the dopamine content of organs it must be kept in mind, however, that it is not only a precursor for noradrenaline but also occurs as a specific product in certain cells of the chromaffin type in various organs (Falck et al., 1959), sympathetic nerves (Schümann, 1956), lungs (Euler and Lishajko, 1957), sinus node (Angelakos et al., 1963) and carotid glomus (Chiocchio et al., 1966; Lishajko, 1970). Increased amounts of dopamine are found in the urine of patients with phaeochromocytoma. Dopamine also occurs normally in the brain (Montagu, 1957) and appears to serve as neurotransmitter in certain areas such as basal ganglia, where its presence has been studied both by direct analysis and by the histochemical fluorescence technique (Bertler and Rosengren, 1959; Hornykiewicz, 1962; Fuxe, 1965; Carlsson, 1966).

Of considerable theoretical and practical interest is the observation that α-methyl DOPA and α-MMT are readily decarboxylated in the mammalian organism and stored in adrenergic nerves as α-methyldopamine, α-methylnoradrenaline and metaraminol (after β-oxidation) (Carlsson and Lindqvist, 1962; Maître and Staehelin, 1963; Muscholl and Maître, 1963; Muscholl, 1965).

c) Dopamine ß-hydroxylase

On incubation of homogenates of sympathetic nerves and ganglia with dopamine, the formation of noradrenaline has been established (Goodall and

KIRSHNER, 1958), indicating that a hydroxy group is introduced in β-position in the side chain. The transforming enzyme, dopamine-β-hydroxylase was first prepared from adrenal medullary extracts (LEVIN et al., 1960) and is a copper proteid. The enzyme, which requires ascorbic acid and fumaric acid as co-factors, has been studied in detail by GOLDSTEIN et al. (1965) (cf. KAUFMAN and FRIEDMAN, 1965). Its activity is high and similar to that of DOPA-decarboxylase.

The formation of noradrenaline from dopamine has also been demonstrated in isolated nerve granules (EULER and LISHAJKO, 1968a; STJÄRNE et al., 1967a).

2. Other Synthetic Pathways

Even meta-tyrosine can be hydroxylated to DOPA, as shown by its subsequent conversion to dopamine (SOURKES et al., 1961b). By ring hydroxylation, metaraminol can be transformed *in vivo* to α-methylnoradrenaline in the guinea pig (MAÎTRE and STAEHELIN, 1965). Interestingly, the amount of α-methylnoradrenaline in the heart exceeded that of noradrenaline.

As already mentioned, 3,4-dihydroxyphenylserine (DOPS) may serve as a precursor to noradrenaline, although no evidence for its presence in the organism has been given. Phenylethylamine (ASATOOR and DALGLIESH, 1959) as well as tyramine (SJOERDSMA et al., 1959) occur normally in urine indicating that these amines are formed by decarboxylation of the corresponding amino acids (cf. LOVENBERG et al., 1962). After inhibition of monoamine oxidase their excretion is increased. Most of the tyramine found is present in the central nervous system. Apparently the greater part of the tyramine formed in peripheral nervous tissue is oxidized to octopamine (MUSACCHIO and GOLDSTEIN, 1963) which is also a natural excretion product in urine (PISANO et al., 1960; SPECTOR et al., 1963b).

In the heart and kidney octopamine could only be demonstrated after inhibition of MAO (KAKIMOTO and ARMSTRONG, 1962). Posterior salivary glands from *Octopus vulgaris* contain both octopamine (ERSPAMER and BORETTI, 1951) and noradrenaline (EULER, 1953) but the exact stage at which the ring hydroxylation occurs is not known (cf. BLASCHKO, 1959).

Tyramine and octopamine are apparently to some extent transformed to noradrenaline (CREVELING et al., 1962), since administration of these compounds cause an increased excretion of noradrenaline. Some of this may be due to the noradrenaline-releasing effect of the two amines, however.

Liver microsomes from the rabbit can form catechols from the corresponding phenols (dopamine from tyramine, noradrenaline from octopamine) reactions which require NADH (AXELROD, 1963). Non-enzymatic conversion of tyrosine to DOPA (UDENFRIEND et al., 1954) or of tyramine to dopamine (HOLTZ and CREDNER, 1943) has also been demonstrated.

3. Localization of Synthesis. Effect of Precursors

Noradrenaline synthesis takes place in the adrenergic neurons in immediate relation to the storage granules. Of the different enzymes partaking in the formation of the neurotransmitter, only the dopamine-β-hydroxylase has been found to be directly associated with the granules (POTTER and AXELROD, 1963a, b) and these are a prerequisite for the final synthetic step (STJÄRNE and LISHAJKO, 1967). The newly synthesized noradrenaline is taken up and retained by the granules. The preceding enzymatic transformations from tyrosine are mediated by enzymes present extragranularly in the axoplasm (STJÄRNE, 1966). It is generally assumed that the enzymes engaged in the synthesis of noradrenaline are transported to the periphery by the axoplasmic flow (cf. WEISS, 1963) like much other material.

After inhibition of synthesis with the decarboxylase inhibitor decaborane-14 in the rabbit, dopamine but not DOPA, restored the deficient response to adrenergic nerve stimulation (BYGDEMAN and EULER, 1966). Although transmitter synthesis normally is adequate to maintain the stores at a level not far from the storage capacity, a certain deficit appears to exist, as concluded from the finding of increased amounts of transmitter in organs after inhibition of nerve activity by decentralization (REHN, 1958) or a neuronal blocker like bretylium (RYD, 1962). An increase of the order of 25—50% over the "normal" content is frequently found on injection of noradrenaline, its precursors, or by exclusion of nerve stimuli.

Access of precursors may also play a role in the rate of synthesis. Thus BALZER and PALM (1962) observed that pretreatment of mice with the MAO inhibitor iproniazid causes an increased influx of ^{14}C-α-aminobutyric acid in the brain. This may also hold true for amino acids serving as noradrenaline precursors (cf. HESS et al., 1959). Alterations in the permeability of the cell membrane may therefore play a part in the action of some MAO inhibitors (EHRINGER et al., 1961).

4. Synthesis Inhibitors

By inhibition of one or more of the enzymatic synthetic steps it should be theoretically possible to diminish the production of the adrenergic neurotransmitter. Such inhibition has been extensively studied partly with the aim to achieve therapeutic results (cf. MUSCHOLL, 1966a).

a) Tyrosine Hydroxylase Inhibitors

Of the three enzymatic steps, the formation of DOPA by tyrosine hydroxylase is the slowest and therefore rate-limiting. Consequently it would seem that an inhibition of this step should be most useful for the purpose of reducing noradrenaline formation. Several kinds of inhibitors have been described.

A certain degree of tyrosine hydroxylase inhibition has been observed with dopacetamide and some congeners in doses of 300 mg/kg (CARLSSON et al., 1963a).

α-Methyltyrosine, which like tyrosine is not a substrate for DOPA decarboxylase (SPECTOR et al., 1965), inhibits tyrosine hydroxylase in contrast to α-methyl DOPA and α-MMT. When α-methyltyrosine is given in amounts that lead to its accumulation in tissue in concentrations higher than those of the natural analogue (5×10^{-5}M), biosynthesis of DOPA is inhibited and the noradrenaline content of the organs lowered. (Fig. 2). On the other hand α-methyltyrosine does not inhibit the formation of dopamine from DOPA or 5-hydroxytryptamine from 5-HTP (Fig. 2).

The inhibition of tyrosine hydroxylase by α-methyltyrosine may cause severe signs of noradrenaline deficiency (SPECTOR et al., 1965). Notwithstanding its inhibitory effect, α-methyltyrosine is to some extent itself hydroxylated in the ring to α-methyl-DOPA and subsequently decarboxylated and β-oxidized to α-methylnoradrenaline (MAÎTRE, 1965).

Effects similar to those of α-methyltyrosine have been demonstrated for its dimethyl ester (H 44/68) (CORRODI and MALMFORS, 1966) which in a dose of 250 mg/kg lowers the noradrenaline stores in rat tissues. Strong inhibitory action has also been reported for 3-iodo-L-tyrosine (GOLDSTEIN and WEISS, 1965).

The noradrenaline depleting effect of H 44/68 has also been shown for adrenergic nerve fibres in the periphery, using the histochemical fluorescence technique of FALCK and HILLARP (CORRODI and MALMFORS, 1966). Of great functional interest is the observation that noradrenaline as well as dopamine strongly inhibit the

tyrosine hydroxylase which may form the basis for a feedback control mechanism (NAGATSU et al., 1964; STJÄRNE, 1966).

Inhibition of tyrosine hydroxylase (and of dopamine-β-hydroxylase) has been observed with the pigment antibiotic chrothiomycin (AYUKAWA et al., 1969).

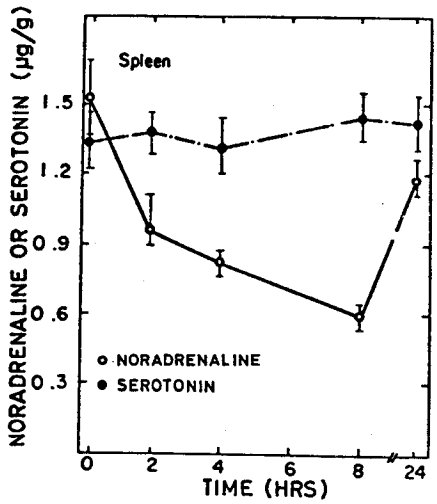

Fig. 2. Levels of noradrenaline and 5-hydroxytryptamine in the spleen of guinea pigs after administration of a single dose of α-methyltyrosine (80 mg/kg i.p.), mean and range (SPECTOR et al., 1965)

b) DOPA Decarboxylase Inhibitors

A number of DOPA decarboxylase inhibitors have been investigated by CLARK and his group (HARTMAN et al., 1955a; CLARK, 1959; CLARK and POGRUND, 1961). Active *in vitro* was e.g. 3-hydroxycinnamic acid although the inhibition *in vivo* was only moderate.

α-Methyl-DOPA and α-MMT exerted a marked inhibitory effect on DOPA decarboxylase as discovered by SOURKES (1954), while α-methylphenylalanine and α-methyltyrosine had only a weak effect. The inhibitory effect could also be shown *in vivo*; thus α-methyl-DOPA diminished the effect of DOPS on the blood pressure which depends on decarboxylation to noradrenaline (SCHMITERLÖW, 1951; DENGLER and REICHEL, 1957). After administration of DOPA to rats *in vivo*, α-methyl-DOPA decreased the excretion of dopamine in urine (MURPHY and SOURKES, 1961).

The fall in noradrenaline-content in various organs observed after α-methyl-DOPA and α-MMT (SOURKES et al., 1961a) does not seem to depend on decarboxylase inhibition since more active hydrazine derivatives lack the effect on the noradrenaline content of the heart and brain. It is therefore assumed that the noradrenaline depleting effect of α-methyl-DOPA and α-MMT is caused by partial substitution of the normal noradrenaline content through the decarboxylated products α-methyldopamine and α-methyl-meta-tyramine which after β-hydroxylation act as false transmitters (WEISSBACH et al., 1960; CARLSSON and LINDQVIST, 1962). This may contribute to the therapeutic effect of α-methyl-DOPA in the treatment of hypertension (OATES et al., 1960), although the β-hydroxylated products are quite efficient as sympathomimetic amines. α-methylnoradrenaline is readily taken up by the normal storage sites in the nerve granules and given off

at a similar rate as noradrenaline. This explains the nearly quantitative exchange of noradrenaline for α-methylnoradrenaline (Carlsson and Lindqvist, 1962; Schümann and Grobecker, 1964). It has been assumed that α-methylnoradrenaline is lost from organs at a slower rate than noradrenaline and also that it causes, by substituting noradrenaline in the CNS, some alteration in the function of the adrenergic system (Holtz, 1965; Henning, 1969).

α) Derivatives of Hydrazine and Hydroxylamine

Strong inhibitory action *in vitro* on the DOPA decarboxylase is exerted by e.g. N-methyl-N (3-hydroxybenzyl)-hydrazine (NSD 1034) (Brodie et al., 1962; Levine and Sjoerdsma, 1964), but this and similar compounds (NSD 1039, 1045) have only little effect on the endogenous amines. These compounds also inhibit the decarboxylation of α-methyl-DOPA and α-MMT (Udenfriend and Zaltzman-Nirenberg, 1962), and counteract their blood pressure lowering effect in the hypertensive rat (Davis et al., 1963).

β) Decaborane

Merritt et al. (1965) have shown that the boron hydride decaborane-14 ($B_{10}H_{14}$) lowers the noradrenaline content in the rat brain in doses of 10—20 mg/kg. Decaborane also causes noradrenaline depletion in a variety of tissues, notably the heart, brain and kidney (Euler and Lishajko, 1965a). After decaborane treatment the dopamine content in the brain after administration of DOPA is greatly diminished, and in other experiments Merritt and Schultz (1966) have shown that the decarboxylase activity is greatly lowered. The noradrenaline-depleting effect on the rabbit heart reaches a maximum about 24 hrs after a dose of 4—8 mg/kg intraperitoneally and remains for another 24 hrs whereafter the noradrenaline content gradually returns to normal. In the rabbit decaborane causes a rapid diminution of the response to sympathetic stimulation (Bygdeman and Euler, 1966). The effect of decaborane appears to depend on the inactivation of the co-factor pyridoxal-5′-phosphate and can be counteracted by injection of pyridoxine (Wykes and Landez, 1967).

c) Dopamine ß-hydroxylase Inhibitors

Arylalkylamines such as phenylethylamine inhibit the transformation of dopamine to noradrenaline (Goldstein and Contrera, 1961) by competing for the hydroxylating sites of the storage granules.

α) Hydroxybenzyloxyamine

m-Hydroxybenzyloxyamine (NSD 1024) inhibits *in vivo* the formation of noradrenaline from dopamine in the heart (Nikodijevic et al., 1963) and exerts similar inhibitor action on the oxidation of α-methyl-dopamine to α-methylnoradrenaline.

Although N-methyl-N-(3-hydroxybenzyl)-hydrazine (NSD 1034) in low doses is able to prevent the usual rise in noradrenaline in rat brain following a MAO-inhibitor (pargyline) even 200 mg/kg only lowers the normal noradrenaline content by 60% for a few hours (Kuntzman et al., 1962).

β) Disulfiram, Tropolone

Disulfiram (antabuse) inhibits aldehyde dehydrogenase and the hydroxylation of dopamine to noradrenaline by dopamine-β-hydroxylase. The active compound is diethyl-dithio-carbamate which is readily formed from disulfiram in the presence

of ascorbic acid (ALBERT, 1961; GOLDSTEIN et al., 1964a). It serves as chelator like 8-hydroxy-quinoline, 2,2'-dipyridyl, and EDTA, and inhibits the dopamine-β-hydroxylase which probably is a metal proteid. Also active as inhibitors in this group are 4-methyl- and 4-isopropyltropolone (GOLDSTEIN et al., 1964b)

Disulfiram in a dose of 400 mg/kg lowers the noradrenaline content of the rat heart by about 50% and also prevents the formation and storage of octopamine after administration of tyramine (MUSACCHIO et al., 1964).

The enzyme was found to be relatively insensitive to hormones and reserpine, but was markedly inhibited by thyroxine or benzyloxyamines (NAGATSU et al., 1968).

d) Reserpine

The rapid depletion of noradrenaline stores in organs and adrenergic nerves by reserpine has raised the question as to whether this drug affects synthesis as well as binding to the granules. In experiments with homogenized splenic nerves and with splenic nerve trunk particles the conversion of dopamine to noradrenaline was found to be inhibited by reserpine (STJÄRNE and LISHAJKO, 1966; ROTH and STONE, 1968), suggesting that an altered ability of the granules to take up catecholamines after reserpine treatment prevents the final synthetic step. On the other hand, some observations seem to indicate that reserpine does not seriously impair the noradrenaline synthesis in tissues. Thus GLOWINSKI et al. (1966a) found a considerable degree of noradrenaline synthesis from various precursors in the brain of the reserpinized rat and concluded that synthesis proceeds essentially in a normal fashion, although it may be somewhat hampered by diminished availability of dopamine. Observations on the excretion of free noradrenaline and of 3-methoxy-4-hydroxy mandelic acid in the urine of rats treated with reserpine (WENNMALM, 1968) also support the view that reserpine interferes only to a moderate extent with noradrenaline synthesis (cf. RUTLEDGE and WEINER, 1967).

The fact that noradrenaline synthesis continues even after reserpine depletion of the noradrenaline stores suggests that the ability of the storage particles to take up and bind the product, noradrenaline, is not a prerequisite for its formation. If noradrenaline is not taken up this may be assumed to hold true also for dopamine. Oxidation of dopamine may therefore occur without the amine entering the storage particle.

In the experiments of ROTH and STONE (1968) it was observed that the inhibitory effect of reserpine on noradrenaline synthesis was partly reversed by the MAO inhibitor pargyline in intact nerves, in contrast to isolated nerve particles (STJÄRNE and LISHAJKO, 1966), possibly by increasing the intraaxonal dopamine levels.

The membrane-active drugs prenylamine and phenoxybenzamine also inhibit noradrenaline synthesis at the β-hydroxylation step (STJÄRNE and LISHAJKO, 1966), presumably by preventing access of dopamine to the active sites in the storage particles.

5. Induction and Regulation of Synthesis

Increased adrenergic nerve activity enhances the synthesis of noradrenaline in the nerve terminals as evidenced by the rise in the excretion of noradrenaline in urine (EULER, 1954) and of its metabolites (ARMSTRONG and MCMILLAN, 1959), without lowering of the amounts in the stores.

The effect of nerve stimulation on the synthesis of noradrenaline was first demonstrated for the cat spleen *in vivo*; after 10 min stimulation of its nerves there was no significant reduction of its noradrenaline content in spite of a con-

siderable release of transmitter (EULER and HELLNER-BJÖRKMAN, 1955). Similar results have been obtained on the isolated perfused spleen by DEARNALEY and GEFFEN (1966), who observed a fall of only 7.7% of the original noradrenaline content after nerve stimulation. The outflow of transmitter caused by nerve stimulation (cf. BROWN, 1965) directly indicates that synthesis is increased under these conditions since the transmitter content is practically unchanged. The relative constancy of the noradrenaline content in organs during varying conditions suggests that the regulatory mechanism possesses a high degree of precision. As a likely system it has been proposed that, after noradrenaline release, the lowered concentration at some strategic point in the storage system elicits resynthesis, presumably by removal of an inhibitory action (negative feedback control). This concept has received support by the experimental results of NAGATSU et al. (1964) and UDENFRIEND et al. (1965). Thus the hydroxylation of tyrosine, the rate-limiting step, is significantly inhibited by noradrenaline 2×10^{-4}M in the medium. STJÄRNE (1966) and STJÄRNE et al. (1967b) found that noradrenaline inhibits synthesis to more than 90% even at 2.4×10^{-5}M concentration, and since dopamine formation was less affected, the results suggest inhibition also at the final step (Fig. 3). Binding of noradrenaline to the tyrosine hydroxylase has been reported by IKEDA et al. (1966).

If the concentration of transmitter is increased by a MAO inhibitor, the conversion of labelled tyrosine to noradrenaline is decreased (SPECTOR et al., 1967), which is in harmony with the above concept.

The remarkable and continuous increase in adrenergic transmitter release observed after administration of reserpine in conjunction with a MAO inhibitor (CHESSIN et al., 1957), suggesting increased synthesis, may partly be due to derailment of the regulating system caused by the two drugs in combination. Increased tyrosine hydroxylase activity has been observed in the rat superior cervical ganglia and the rabbit brain stem after reserpine with increased V_{max} for both tyrosine and the pteridine co-factor (MUELLER et al., 1969 a).

The effect of direct and reflex nerve stimulation in increasing noradrenaline synthesis has been observed on various organs (GORDON et al., 1966a, b; ALOUSI and WEINER, 1966; OLIVERIO and STJÄRNE, 1965; AUSTIN et al., 1967; SEDVALL and KOPIN, 1967; WEINER and RABADJIJA, 1968). The effect is not accompanied by an increase in tyrosine hydroxylase activity *in vitro*, which supports the concept of a functional interaction of the product with the synthesizing system.

Noradrenaline synthesis from tyrosine in the isolated guinea pig vas deferens was found to increase 3 times by nerve stimulation (ROTH et al., 1967). The enhancement of synthesis was not dependent on the response of the organ since the same effect was seen when the motor effects of stimulation were inhibited by Hydergine. The effect is not due to increased conversion of dopamine to noradrenaline but is presumably a result of increased tyrosine hydroxylase activity. On the other hand, no increase in synthesis rate occurred on stimulation of the isolated bovine splenic nerve. It is doubtful, however, whether stimulation of the nerve trunk causes release of transmitter as in the isolated organ.

A compensatory increase in adrenal tyrosine hydroxylase activity has been found after chemical sympathectomy produced by 6-hydroxydopamine which destroys the adrenergic nerve endings and depletes the noradrenaline stores (MUELLER et al., 1969b). As shown by DEQUATTRO et al. (1968) sinoaortic denervation of the rabbit caused an increased enzyme activity.

Since the tyrosine hydroxylase activity may also be altered by preganglionic nerve stimulation (THOENEN et al., 1969) it has been suggested that the neural control can be accomplished by two mechanisms 1. rapid changes in end-product

inhibition or availability of substrate at the enzyme site, and, 2. gradual alterations in the amount of tyrosine hydroxylase in response to prolonged nerve stimulation (MUELLER et al., 1969a).

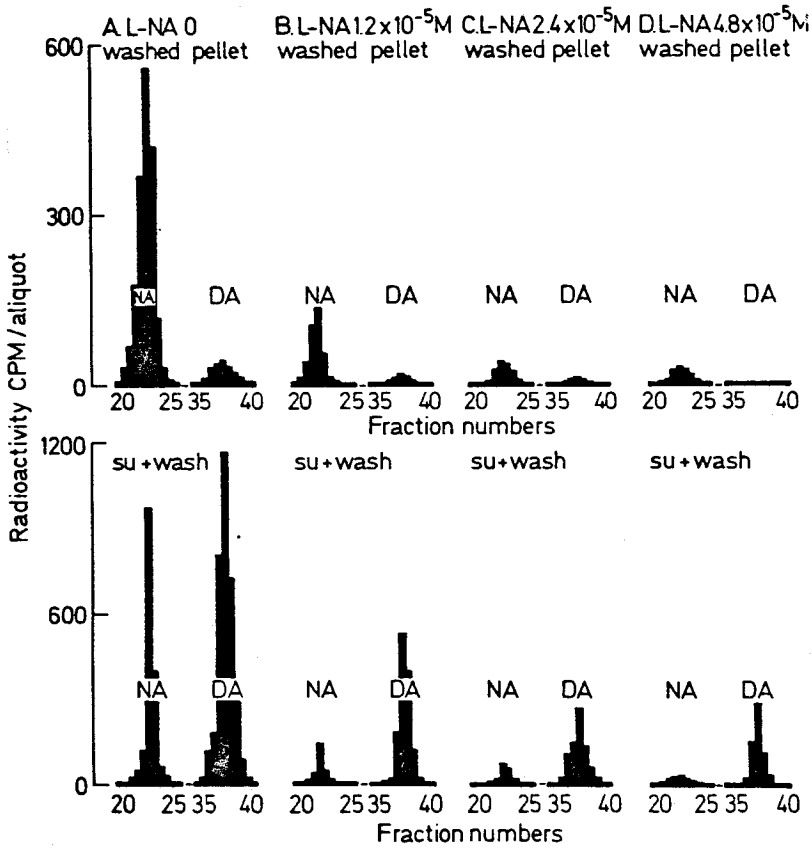

Fig. 3. Inhibition of the noradrenaline and dopamine formation from tyrosine in microsomal fractions of splenic nerve homogenate by addition of noradrenaline to the incubation medium in different concentrations (*A—D*). Ion exchange chromatogram fractions. *L-NA*, *NA*, (—)-noradrenaline; *DA*, dopamine; *SU*, supernatant (STJÄRNE et al., 1967b)

Muscular exercise, which strongly increases the noradrenaline output (KÄRKI, 1956), also enhances the activity of tyrosine hydroxylase (AXELROD et al., 1970).

Of drugs which have been reported to stimulate tyrosine hydroxylase, there may be mentioned thyroxine (BAGCHI and MCGEER, 1964) and α-adrenergic blocking agents (DAIRMAN et al., 1968). With the blocking agents no increase in enzyme activity *in vitro* was observed. Since α-adrenergic blockers inhibit noradrenaline uptake in isolated nerve particles as well as release (EULER and LISHAJKO, 1968b) the effect may be the result of either reflexly induced nerve activity or diminished end-product inhibition.

In isolated rat and guinea pig atria and in rat vas deferens angiotensin increases the formation of noradrenaline from tyrosine (BOADLE et al., 1969).

Increased *de novo* synthesis has been seen in the rat heart following hypophysectomy (LANDSBERG et al., 1969). This occurred concomitantly with increased

turnover, reflecting thyroid and adrenal deficiency (LANDSBERG and AXELROD, 1968a) and was accompanied by reduced accumulation of ^{3}H-noradrenaline in the rat heart (LANDSBERG and AXELROD, 1968b).

6. Synthesis Rate and Kinetics

The rate of noradrenaline synthesis has been estimated *in vivo* and *in vitro* during different experimental conditions. The rate (measured as μg/g.hr) can be expected to vary widely in different organs as a result of variations in both the rate of release and the size of the stores; the latter may vary from near zero to values of about 10 μg/g depending on the organ.

In order to make results more comparable, data for synthesis rate may be expressed in % of the normal noradrenaline content per g tissue per hr.

PAULSEN and HESS (1963) have reported synthesis rates for noradrenaline of about 0.03 μg/g.hr in the guinea pig heart, depleted after administration of the benzoquinolizine compound, RO 4—1284, or of the methyl ester of methyl reserpate, Su 9064. A similar value for resynthesis was found in the guinea pig brain in which the noradrenaline content was reduced by electroshock.

The amounts of noradrenaline synthesized from tyrosine in the isolated guinea pig heart have been calculated to 0.03—0.05 μg/g.hr (SPECTOR et al., 1963a) but values may be considerably higher at high release rates. Synthesis rate has been calculated on the basis of the disappearance of radioactive noradrenaline incorporated when the organ content was constant (UDENFRIEND and ZALTZMAN-NIRENBERG, 1963; MONTANARI et al., 1963) or by means of the rate of loss of transmitter after synthesis.

Values between 0.03 and 0.10 μg/g.hr have been reported for various organs (CROUT, 1962; POTTER and AXELROD, 1963b; MONTANARI et al., 1963). In the isolated bovine splenic nerve the rate of noradrenaline synthesis from tyrosine was 0.15—0.20 μg/g.hr (ROTH et al., 1967).

At a half-life of 7 hrs for incorporated ^{3}H-noradrenaline, the rate of loss is about 10% per hr and, given a constant total noradrenaline value of 1 μg/g and a regular distribution and release of the label, synthesis proceeds at the rate of 0.1 μg/g.hr. There is some evidence, however, that the release does not affect the stores in a proportionate way but that there is a preference for the release of newly synthesized material. In such case the loss of label is delayed and the rate of synthesis higher than indicated by the half-life (KOPIN et al., 1968).

In perfusion experiments on the isolated guinea pig heart a K_m value for the overall conversion of tyrosine to noradrenaline has been found which is the same as that for tyrosine conversion to DOPA, or 10^{-5}M (SPECTOR et al., 1963a). A more detailed study of the kinetics of bovine adrenal tyrosine hydroxylase has been reported by IKEDA et al. (1966).

The kinetics of the dopamine β-oxidation reaction has been examined in detail by GOLDSTEIN et al. (1968).

The approximate K_m values for the enzymes taking part in catecholamine synthesis are, according to UDENFRIEND (1968)

Tyrosine hydroxylase 1×10^{-5}M
DOPA decarboxylase 4×10^{-4}M
Dopamine-β-hydroxylase 5×10^{-3}M

From these constants UDENFRIEND has concluded that the enzymes must occur in close connection since the intermediates do not accumulate.

II. Uptake

1. Introduction

Normally the adrenergic nerves, like the organs which receive a supply of these nerves, contain a characteristic amount of neurotransmitter, subject to only moderate changes both in loss and gain even under greatly varying activity conditions (EULER, 1951; EULER and HELLNER-BJÖRKMAN, 1955; DEARNALEY and GEFFEN, 1966). This suggests a limited storing capacity, presumably structurally and chemically determined, and normally utilized to an extent allowing only a small or moderate complementary uptake.

Exogenous noradrenaline can be taken up to various extents (RAAB and GIGEE, 1953) but is only temporarily stored. Thus after a limited time the organ content returns to its normal value. The granular stores are capable of taking up and storing also adrenaline; this explains why the uptake in tissues after administration of this amine is more readily detectable: it partly substitutes for noradrenaline in the specific stores (STRÖMBLAD and NICKERSON, 1961; EULER and LISHAJKO, 1963a; ANDÉN, 1964; WESTFALL, 1965).

The availability of radioactively highly labelled noradrenaline has afforded a method of high precision and usefulness, introduced and successfully used in its present form by AXELROD and his co-workers since 1959.

A problem which has not been fully elucidated is whether tracer amounts of highly labelled amines are distributed in a way truly representative of the endogenous distribution in the organism under all conditions.

While uptake studies with the use of semi-tracer amounts of noradrenaline appear at present to give consistent and accurate results, it may under some circumstances be advantageous to study bulk uptake after previous depletion of the stores (cf. p. 202).

For the interpretation and evaluation of uptake experiments distinction has to be made between uptake through the axonal membrane and uptake into the specific stores, particularly since some drugs have different actions on the uptake at these two sites. So far there seems to be no direct method which allows studies to be made of the effect of various drugs and other factors on the uptake through the axonal membrane. However, by comparing the effect of drugs on the total uptake in nerves, for instance by the fluorescence histochemical technique of FALCK and HILLARP, with that in the storage particles, it is sometimes possible to decide whether an uptake inhibition is located at one or the other site. Thus reserpine inhibits noradrenaline uptake in storage particles but not in the axon (MALMFORS, 1965). On the other hand isopropylnoradrenaline is hardly taken up by tissues (ANDÉN et al., 1964; HERTTING, 1964), while it is readily incorporated in isolated particles (EULER and LISHAJKO, 1967b).

Catecholamine uptake in the tissues has become the subject of numerous studies of which only a limited number can be discussed in the present survey. It has been attempted to include those studies in the first place which deal with specific uptake in the normal physiologically relevant stores. An extensive bibliography on uptake is found in the monograph of IVERSEN (1967).

2. Uptake of Catecholamines in Organs and Tissues

Functional uptake of catecholamines was demonstrated as early as 1933 by BURN, who observed a modification of the effect of nerve stimulation after adding adrenaline to the perfusion fluid used for the hindleg of the dog. In most of the studies of amine uptake (e.g. BURN and RAND, 1958) various proportions of the

injected amines were in all probability unspecifically located and disappeared relatively rapidly from their deposits. Using highly radioactive catecholamines Axelrod et al. (1959) were able, however, to distinguish for the first time between a specific and a non-specific uptake after injection of 0.1 mg/kg ^{3}H-adrenaline intravenously to mice. They observed that a large proportion of the dose, about 70%, disappeared from the body in some 5 min, whereafter the remaining 30% disappeared more slowly. The experiment thus showed that a certain proportion became bound more firmly and was released at a slow rate. In experiments with highly labelled noradrenaline the results were similar (Whitby et al., 1961), but an interesting difference could be noted, in that a higher proportion of noradrenaline than of adrenaline was specifically bound. As already mentioned, isoprenaline is taken up even less efficiently (Andén et al., 1964). Since all three amines are equally well taken up, and stored by the specific storage particles the differentiation must occur at the axon membrane which therefore appears to constitute a neuronal barrier, allowing preferential uptake of the natural neurotransmitter.

After injection of a large dose of noradrenaline into puppies most of the amine recovered from the heart was located in the soluble fraction of a homogenate after sedimentation, indicating that it was not specifically bound (Wegmann and Kako, 1961). Binding of noradrenaline, administered in large doses, to certain tissues such as smooth muscle cells has been studied by Gillespie (1968) with the aid of the fluorescence technique. For further references see Trendelenburg (1971).

Uptake of catecholamines in brain has recently become the subject of numerous studies after introduction of a technique allowing administration of amines directly into the brain ventricles (Glowinski et al., 1965; Glowinski and Axelrod, 1965). From these and other studies it has become evident that the brain contains specific stores capable of binding catecholamines.

3. Uptake in Adrenergic Nerves

The main specific storage sites for noradrenaline are found in the adrenergic nerves, and it is in agreement with this fact that the distribution of labelled noradrenaline after injection is roughly proportionate to the amount of the transmitter normally present in the different organs (Whitby et al., 1961); this in turn is closely related to the supply of adrenergic nerves. In experiments on the developing rat the accumulation of ^{3}H-noradrenaline occurred parallel to the endogenous noradrenaline content (Iversen et al., 1967). For the distribution of injected label both turnover rate and blood flow in relation to the size of the store are important. If these parameters have low values the conditions for labelling are correspondingly less favourable, as for instance in the adrenal medulla (cf. Wurtman et al., 1964).

Uptake in nerves has been well illustrated by autoradiographic technique. Samorajski et al. (1964) found a deposition of silver over adrenergic nerves in the heart after injection of ^{3}H-noradrenaline in the cat (Fig. 4).

The uptake of radioactive noradrenaline in isolated splenic nerve bundles has been studied by Stjärne et al. (1970a). The concentration of the amine was relatively small, which may be associated with the limited stores.

After degeneration of the adrenergic nerves the transmitter stores are greatly diminished or disappear (Cannon and Lissák, 1939; Goodall, 1951; Euler and Purkhold, 1951). Under these conditions there is a greatly reduced specific uptake of noradrenaline in the organs (Hertting et al., 1961a; Strömblad and Nickerson, 1961). Similarly, after immuno-sympathectomy in rats and mice, the uptake of ^{3}H-noradrenaline is greatly reduced (Zaimis et al., 1965).

A certain uptake nevertheless occurs in the absence of adrenergic nerves but this is presumably of non-specific character (ANDÉN et al., 1963). Nerve free tissue like the placenta lacks endogenous noradrenaline and consequently presents no specific storage sites for uptake of circulating amines under normal conditions (EULER, 1951). It is not known to what extent the placenta takes up catecholamines unspecifically after an injection.

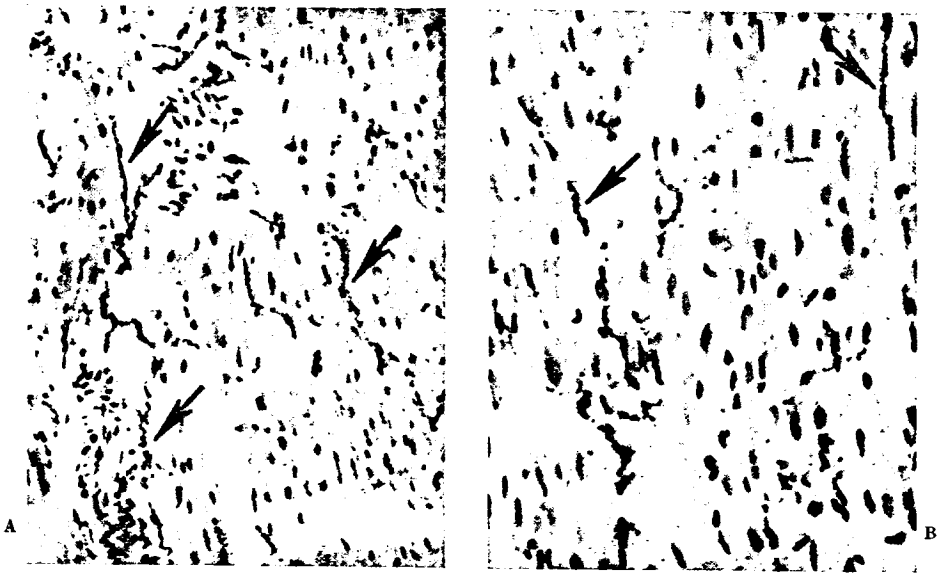

Fig. 4. A Autoradiography of heart muscle 2 min after intravenous injection of ^{3}H-noradrenaline; loci of uptake indicated by arrows. 162×. B Localization of radioactivity in heart muscle 2 hrs after intraperitoneal injection of ^{3}H-noradrenaline. 162× (SAMORAJSKI et al., 1964)

With the histochemical fluorescence technique of FALCK and HILLARP it has been shown that in the rat iris, previously depleted of noradrenaline by reserpine, noradrenaline after injection accumulates in the adrenergic nerve fibres, including the cell bodies and the terminals (HAMBERGER et al., 1964). Combined radioautography and fluorescence technique have also been used, with similar results (GILLESPIE and KIRPEKAR, 1965).

The ease with which noradrenaline is taken up and incorporated in the specific neuronal stores, as evidenced by the rapid uptake in depleted stores (EULER and LISHAJKO, 1965a; BHATTACHARYA, 1968), suggests that some of the released transmitter may be recaptured in the axon, and, depending on the available sites, either bound to specific stores or metabolized.

Precise information as to the proportion of released transmitter recaptured by the axon terminal is for obvious reasons difficult to obtain. Some authors claim that more than 90% of the released transmitter is recaptured (HAEFELY et al., 1964) which would mean that less than 10% of the released transmitter is lost and also that release and uptake are simultaneous processes during continuous adrenergic nerve activity.

As a method of estimating the recapture, use has often been made of drugs which inhibit the passage of the amines through the axonal membrane. Such drugs are cocaine, desipramine, protriptyline and its congeners, and phenoxybenzamine.

It has been reasoned that if re-uptake occurs, a blocking of the membrane should increase the outflow from a perfused organ as a result of nerve stimulation.

While the results obtained with uptake blockers like cocaine (BROWN, 1965) or desipramine (GEFFEN, 1965) at times indicate a weak action on the outflow in the perfused, stimulated cat spleen they generally cause an increase of 2—3 times (cf. STJÄRNE and WENNMALM, 1971). Phenoxybenzamine and other α-blockers, on the other hand, regularly cause a marked increase in the outflow under these conditions.

Although it may be safely assumed that under favourable conditions a certain re-uptake at the axonal level takes place, the importance of this mechanism under normal conditions is difficult to assess. Noradrenaline and its metabolites are continuously present in blood plasma and excreted in urine in amounts which are roughly parallel to the activity of adrenergic nerves. An increased outflow after certain drugs might also depend on releasing action of these drugs per se.

For exogenous circulating amines the temporary uptake in adrenergic axons as well as in extraneuronal tissue probably represents the most important mechanism for the inactivation of the amines (AXELROD, 1965) although the uptake in the specific binding sites may be small.

4. Specific Uptake in Storage Particles

After uptake in adrenergic nerves a certain proportion of released or injected catecholamines is deposited in the storage particles and specifically bound (EULER and LISHAJKO, 1965a; MACKENNA, 1965), as readily observed after previous depletion. This part is not freely diffusible as evidenced by the stability of the particles at low temperature (EULER and LISHAJKO, 1963a), their insensitivity to oxidizing agents (EULER and LISHAJKO, 1967a) and inability to become adsorbed on alumina.

Evidence for the uptake of catecholamines into the specific storage sites has been provided by electron microscope autoradiography, using radioactive noradrenaline which accumulated in sympathetic axons in the rat pineal body (WOLFE et al., 1962). Accumulation of silver grains in the region of the particles in bovine splenic nerves is shown in Fig. 5. The results obtained with the histochemical fluorescence technique are also compatible with this conclusion (CORRODI et al., 1966).

More detailed information on the specific uptake has been obtained by studies on isolated nerve particles. On incubation of a suspension of splenic nerve particles in isotonic sucrose or potassium phosphate there is a continuous release of noradrenaline, increasing in rate with temperature (EULER and LISHAJKO, 1963a, 1967a). The release is partly compensated by re-uptake, depending on the concentration of noradrenaline in the medium as evidenced by the incorporation of labelled noradrenaline in the particles. At a noradrenaline concentration of about 10^{-4}M the reuptake almost wholly compensates for the loss by release at 20°C.

After partial depletion of the noradrenaline content of the particles a net uptake occurs, whereby the uptake of noradrenaline from the incubation medium is greatly facilitated by ATP and other nucleotides in the presence of Mg (EULER and LISHAJKO, 1963b, 1969) (Fig. 6). After re-uptake as well as net uptake the noradrenaline incorporated in particles is released in the same way as the endogenous noradrenaline.

Uptake in particles as well as in organs shows a certain degree of stereoselectivity. Thus the uptake of the (+)-isomers of noradrenaline and adrenaline is considerably smaller than that of the (—)-isomers (ANDÉN, 1964; WESTFALL, 1965;

EULER and LISHAJKO, 1965a) whereas the two isomers are given off at the same rate *in vivo* and from isolated particles.

The amine uptake in nerve particles is not strictly specific since in addition to noradrenaline also adrenaline, isoprenaline, octopamine, *α*-methylnoradrenaline

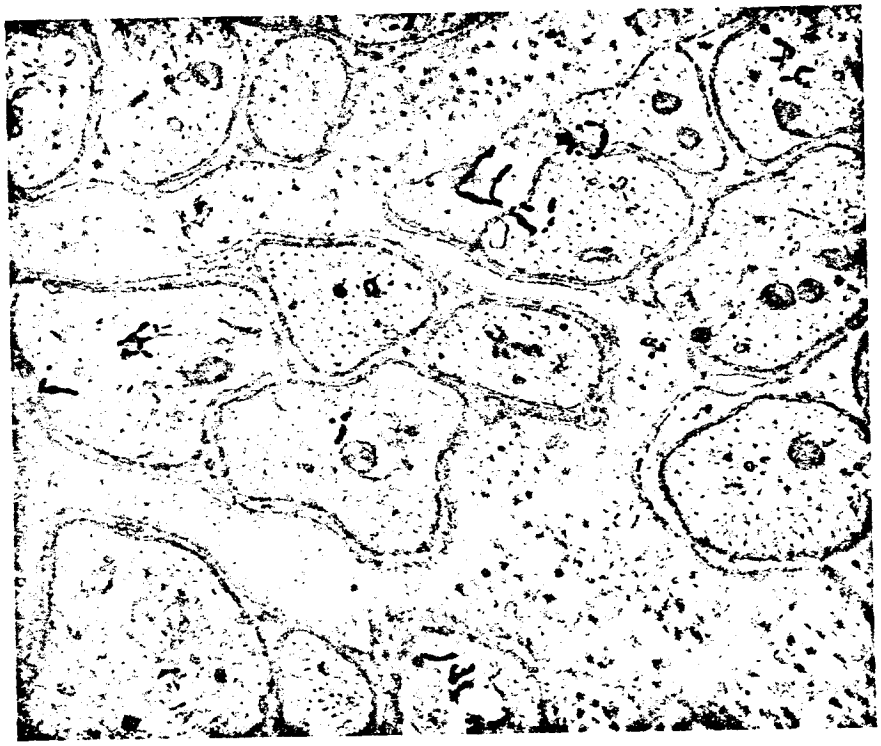

Fig. 5. Autoradiographic electron micrograph of bovine splenic nerve after incubation with ^{3}H-noradrenaline. ×25000 (STJÄRNE et al., 1970a)

and metaraminol can be taken up and stored (EULER and LISHAJKO, 1968a; 1970). A small uptake of dopamine has also been observed after treatment with disulfiram and a MAO-inhibitor (GOLDSTEIN et al., 1964a; MUSACCHIO et al., 1964, 1965a, b). However, even after previous depletion of the noradrenaline stores with decaborane, inhibition of dopamine *β*-oxidase with disulfiram, and a MAO inhibitor, the uptake of dopamine in the stores is small while noradrenaline is readily taken up (SHAHAB et al., 1971). Infusion of dopamine 1 μg/ml in the isolated rat heart did not cause any substitution of noradrenaline (PESKAR et al., 1968). Some of the dopamine taken up may be stored in dopamine particles present in the heart (ANGELAKOS et al., 1963).

The proportion of uptake of adrenaline in the nerve particles depends on the relative concentrations of adrenaline and noradrenaline (cf. STRÖMBLAD and NICKERSON, 1961; WESTFALL 1965). Whether the specific stores in nerves contain adrenaline in cases of phaeochromocytoma where the plasma concentration may reach values of 3×10^{-7}M adrenaline, is not known, but the proportion of adrenaline would in any event be low.

If uptake of administered catecholamines has taken place at the specific sites it should be possible to demonstrate release of the amine upon nerve stimulation.

This has been shown in the early experiments of Burn (1933) who found that the vasoconstrictor effect of nerve stimulation reappeared in the perfused leg after adding adrenaline to the blood reservoir. Release of labelled amine upon nerve stimulation was shown by Hertting and Axelrod (1961). After decentralization of the rat salivary gland and the iris the disappearance of labelled noradrenaline was slower than in the normally innervated organs, indicating that the release is enhanced by the normally occurring nerve impulses. Similarly, ganglionic blocking agents slowed the release of ^{3}H-noradrenaline from the heart (Hertting et al., 1962).

5. Bulk Uptake of Catecholamines after Depletion

While the physiological uptake process is obviously less open to study when the stores are filled to capacity or nearly so, it is possible to study specific uptake after depletion, particularly after giving inhibitors of synthesis which do not seem to interfere with uptake. Thus after decarboxylase inhibition by decaborane-14, which causes a severe depletion of the noradrenaline content of rabbit heart and kidney 24—48 hrs after administration, the injection of noradrenaline in doses of 0.05 mg/kg i.v. or 0.3—0.4 mg/kg intramuscularly rapidly refills the stores to nearly normal values (Euler and Lishajko, 1965a). Analysis of subcellular fractions showed that distribution of noradrenaline between particles and supernatant was normal.

The effect of various drugs on the bulk uptake of noradrenaline in the isolated perfused rat heart previously depleted by decaborane has been studied by Bhattacharya (1968) (cf. Section B. II. 6).

The histochemical fluorescence technique has also been used for the demonstration of bulk uptake after previous depletion of the endogenous noradrenaline by synthesis inhibitors (Corrodi et al., 1966; Malmfors and Euler, 1971).

Bulk uptake in the rabbit heart has also been demonstrated after noradrenaline-depletion with prenylamine (Mackenna, 1965), while the subcellular distribution in different fractions after homogenization was essentially normal.

6. Uptake Estimated by Perfusate Deficit Studies

By measuring the loss of amine in the perfusion fluid after passage through an organ it has been possible to arrive at an estimation of the uptake through the organ. As observed by Pak (1926) and Lund (1951), perfusion of a solution of adrenaline or noradrenaline through the liver leads to an almost complete disappearance of the biological activity of the outflowing solution. Bacq (1937) has found that the kidney also possesses strong inactivating power, and subsequently several organs have been found to remove catecholamines from the perfusion fluid to different extents. An inactivation of this kind is presumably operating continuously under physiological conditions. If no net uptake occurs, removal equals inactivation with the exception of the small percentage excreted unchanged. In man only about 1.5—2% of the infused catecholamines is excreted as such (Euler and Luft, 1951), indicating that the major part is rapidly metabolized and inactivated.

Of the amount of catecholamines eliminated by the isolated rat heart 90% was retained (Lindmar and Muscholl, 1964). In the cat spleen 40—70%, depending on the concentration, is lost during the passage, partly by retention, partly by inactivation (Gillespie and Kirpekar, 1965). In bulk uptake studies after depletion, as much as 40% of the noradrenaline perfused may be taken up and recovered as noradrenaline in the organ when noradrenaline was perfused in a

concentration of 10^{-7}M (BHATTACHARYA, 1968). At increasing concentrations of noradrenaline the total amount taken up by the organ as a rule increases (cf. IVERSEN, 1963) and a part of this is temporarily distributed in various kinds of cells (cf. GILLESPIE, 1968). (For discussion of extraneuronal uptake, see TRENDELENBURG, 1971). The ability to remove catecholamines from the perfusing fluid varies considerably in different organs, depending on cell types, vascularization, adrenergic nerve supply and other factors (cf. LUND, 1951).

7. The Uptake Process

It has frequently been stated that the uptake of catecholamines in adrenergic nerves occurs against a concentration gradient. This is true insofar as the concentration of noradrenaline in the plasma after injection of the amine may be considerably lower than the amount of noradrenaline found per unit weight in the tissue, and presumably very much lower than that present at the specific uptake sites. Yet an uptake, e.g. of labelled amines, can be readily demonstrated. Part of this uptake is of exchange character since a steady release and re-uptake appears to take place both at the axonal membrane and at the level of the storage particle. The gradient on the other hand may be largely apparent, since the transmitter amine bound in the storage particles is osmotically inert. Uptake of the transmitter in the stores will then proceed as long as storage sites are available.

As observed by DENGLER et al. (1961) the uptake of ^{3}H-noradrenaline in slices of heart and brain from the cat becomes saturated at a concentration in the medium of 25 ng/ml (1.5×10^{-7}M), when uptake seems to assume the characteristics of diffusion.

The uptake of ^{3}H-noradrenaline in the isolated rat heart has been extensively studied by IVERSEN (1963) using concentrations of 10—1000 ng/ml. The net uptake in the heart increased with time (over a period of 30 min) and with the concentration of ^{3}H-noradrenaline in the medium and reached values of 1.5 μg/g, about three times the normal noradrenaline content. A large part of this noradrenaline is presumably located outside the specific stores, since these amounts cannot be maintained without continuous supply.

From the initial uptake rates the Michaelis-Menten constants have been computed (IVERSEN, 1963). As seen in Table I the K_m for (—)-noradrenaline was estimated at 0.27×10^{-6}M. In later experiments on the iris and ciliary body IVERSEN (1968) found an "affinity constant" of 1.4×10^{-6}M.

Table I. *Kinetic constants for catecholamine uptake in rat heart at perfusion concentrations 10—500 ng/ml* (IVERSEN, 1963)

Compound	K_m M$\times10^{-6}$	V_{max} μg/min/g heart
(±)-noradrenaline	0.67	0.23
(—)-noradrenaline	0.27	0.20
(+)-noradrenaline	1.39	0.29
(±)-adrenaline	1.40	0.19

In isolated storage particles (EULER and LISHAJKO, 1967a; cf. EULER, 1970) the K_m values for the reuptake of noradrenaline as well as for the ATP-stimulated net uptake were about 1.5×10^{-6}M, which is close to the value reported by IVERSEN for the iris/ciliary body. Similar values were obtained for the uptake of noradrenaline in the perfused lung (HUGHES et al., 1969) and rat iris (JONSSON et al., 1969).

Kinetics of the dopamine-uptake in the isolated rat heart have been studied by PESKAR et al. (1968) who found a value of 0.68×10^{-6}M.

A second uptake process, operating when high concentrations of catecholamines are used in the perfusion medium, has been described by IVERSEN (1965). Binding occurred to some as yet unidentified structures, and at a perfusion concentration of adrenaline of 5 μg/ml the uptake of adrenaline in the rat heart was about 10 μg/g in 5 min or about 15 times the normal content. A large uptake of catecholamines in the spleen has been described by AVAKIAN and GILLESPIE (1968), using analytical and histochemical techniques. Except in adrenergic nerves, uptake was observed in arterial smooth muscle and collagen. For a histochemically visible uptake of this kind, concentrations above 10 μg/ml in the perfusion fluid were required. (For detailed accounts of the uptake studied with high concentrations of catecholamines see IVERSEN [1967] and GILLESPIE [1968]).

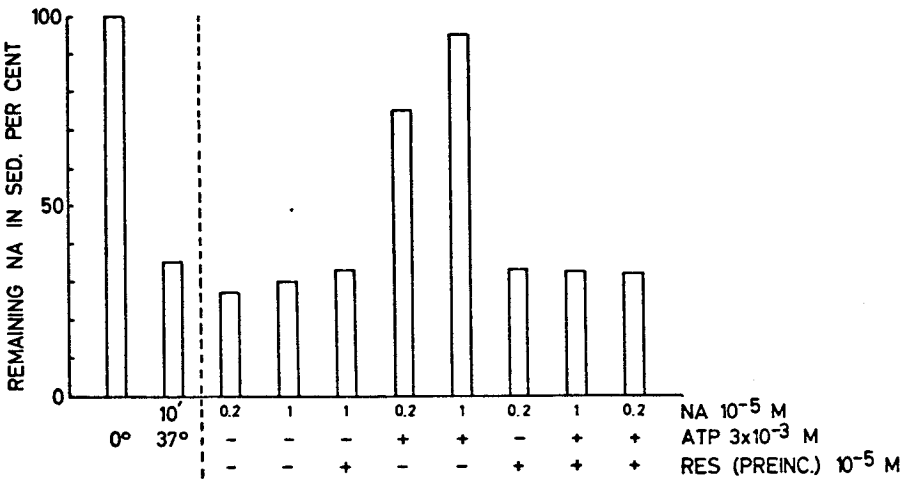

Fig. 6. Isolated splenic nerve particles incubated in phosphate buffer. Per cent noradrenaline of original. After partial depletion (10 min 37°) continued incubation (30 min 20°) with net uptake after addition of ATP. Note no net uptake after reserpine (EULER and LISHAJKO, unpubl.)

The uptake of ^{3}H-noradrenaline in heart slices is also dependent on the presence of Na^+ in the medium. Substitution of Na^+ for Li^+ or K^+ almost completely blocked the ability of the tissue to concentrate the amine. In isotonic sucrose, however, some uptake occurred (BOGDANSKI and BRODIE, 1966). (For reference see also IVERSEN and KRAVITZ, 1966).

The importance of ATP for catecholamine uptake has been shown for adrenal medullary particles by CARLSSON et al. (1963b) and by KIRSHNER (1962). In isolated adrenergic nerve granules ATP strongly facilitates reuptake as well as net uptake of noradrenaline (EULER and LISHAJKO, 1963b, 1969) (Fig. 6).

Stimulation of noradrenaline uptake in isolated storage particles is also observed with ADP, ITP, CTP and UTP, but not with AMP or cyclic AMP. An interesting difference is noted between nerve particles and adrenal medullary particles, in that the latter show no net uptake of catecholamines after partial depletion (LISHAJKO, 1969). Moreover, uptake in medullary particles is not enhanced by ADP.

It has been assumed that the ATPase present in nerve particles as well as in adrenal medullary particles is in some way involved in the amine uptake process (Taugner and Hasselbach, 1966; Philippu et al., 1967; Burger et al., 1968). Some observations on the action of metabolic inhibitors are compatible with this concept (Euler and Lishajko, 1969).

From the data accumulated it appears that the amine "uptake" is of two principally different kinds, specific uptake in special stores and non-specific accumulation in neurons as well as in other tissue cells. The specific stores seem to be of different kinds and it is generally assumed that the main granular store is supplemented by a more "peripheral" store, perhaps in the axon membrane, from which the immediate release occurs (cf. Stjärne, 1964; Euler, 1970).

The final uptake in storage particles is subject to a large number of influences of physical and chemical nature. In addition to temperature, pH, and metabolic factors a variety of drugs act on this process. Reserpine is a potent inhibitor of the ATP-stimulated uptake, but several other drugs have similar though less persistant actions. Most of these cause transmitter depletion *in vivo*.

There are some indications that the uptake process in particles is an active, energy-requiring process. Thus the uptake is greatly facilitated by ATP and apparently dependent on a functioning respiratory chain and oxidative phosphorylation (Euler and Lishajko, 1969). The effect of uncouplers suggest that phosphorylation processes are of importance for uptake as well as for retention of the transmitter. Interference with one or several of the mechanisms involved, for instance by drugs, may therefore cause disturbances in uptake and release, and consequently influence the transmitter content of an organ.

The finding that the noradrenaline uptake — both *in vivo* and in isolated particles — is inhibited by α-blockers may be of significance for elucidating the binding mechanism of the transmitter to the receptors in particles as well as in effector cells.

It is worth noticing that many of the findings reported as regards uptake of noradrenaline and other amines in adrenergic neurons and their specific transmitter stores seem to have a certain parallel in the 5-hydroxytryptamine uptake in platelets and their intracellular storage particles (cf. Da Prada and Pletscher, 1968). The platelets in these respects thus appear to behave like "synaptosomes" or free-moving nerve terminals as it were. Blood platelets may therefore in certain ways serve as a model for the more inaccessible adrenergic nerve endings. For amine uptake in "synaptosomes" see the review of Whittaker (1966).

8. Uptake of False Transmitters. Multi-Amine Storage

Specific uptake of amines, different from the normal neurotransmitter but capable of binding to the same sites, has been studied both *in vivo*, in perfused organs, and in isolated storage particles. Since these amines may be released in a way similar to that of specifically bound noradrenaline they are often referred to as false transmitters (Day and Rand, 1963).

Evidence for a stoichiometric exchange of endogenous noradrenaline for metaraminol formed *in vivo* from α-methyl-metatyrosine by decarboxylation and β-oxidation has been provided by Carlsson and Lindqvist (1962). Muscholl and Maître (1963) showed release of α-methylnoradrenaline by nerve stimulation after administration of α-methyl DOPA. Similarly tyramine may be taken up in the nerves and stored and released as octopamine after β-oxidation (Kopin et al., 1965; cf. Muscholl, 1966a).

False transmitters may be said to have occurred in experiments where adrenaline was injected into an organism in amounts large enough to cause an uptake in adrenergic nerves (Raab and Gigee, 1953). Uptake experiments with different catecholamines were made by Strömblad and Nickerson (1961), Andén (1964) and Andén et al. (1964), who observed that both (—)- and (+)-adrenaline were taken up by the heart, although in different proportions. When used in sufficiently high doses, (+)-adrenaline or metaraminol could even competitively replace the

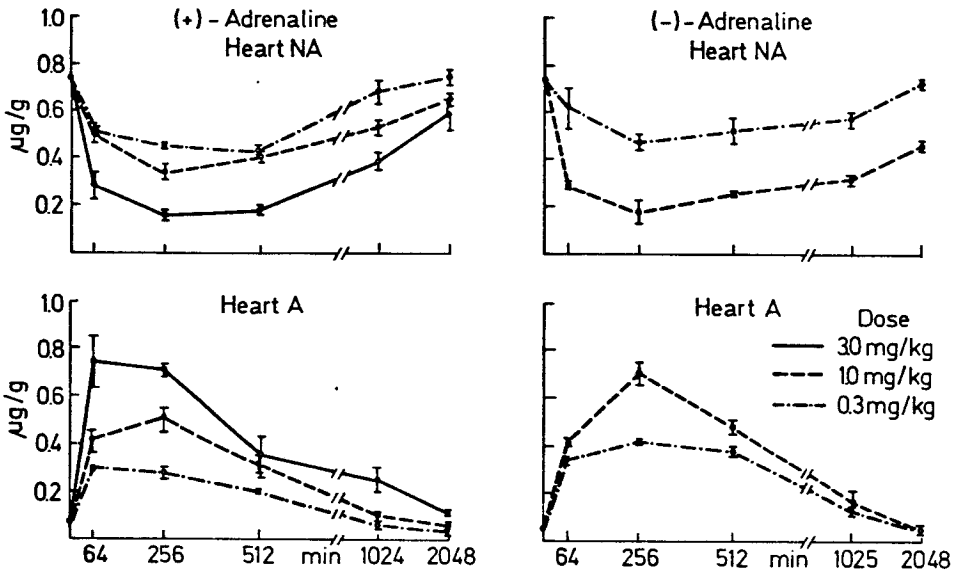

Fig. 7. Noradrenaline and adrenaline in the rat heart after i.m. injection of (—) and (+) adrenaline. Mean and S.E.M. (Westfall, 1965)

endogenous noradrenaline to 50—100% in various organs of the rat (Andén and Magnusson, 1963). The substitution process between endogenous noradrenaline and the potential false transmitters (—)- and (+)-adrenaline was studied by Westfall (1965) who found an approximately stoichiometric exchange for both isomers in the rat heart. As a sign of the different uptake rates for (—)- and (+)-adrenaline, the adrenaline values after injection of the (—)-isomer were approximately twice as high as for the (+)-isomer (Fig. 7).

The sympathetic nerves of the rat pineal gland contain both noradrenaline and 5-hydroxytryptamine (Owman, 1964). As shown by Zweig and Axelrod (1969) treatment with the tyrosine hydroxylase inhibitor α-methyltyrosine causes an increase in the pineal 5-hydroxytryptamine, which is synthesized by the parenchymal cells and taken up by the adrenergic nerve terminals. This rise is prevented by dopamine or noradrenaline, suggesting competition for storage sites. On the other hand, tryptophan increases the pineal 5-hydroxytryptamine. These results indicate that adrenergic nerve fibres may also take up and store 5-hydroxytryptamine if this — as in the pineal gland — is normally available in the adjacent cells.

Similar multi-amine storage ability has been described for nerves in a variety of tissue and organs (for references see Zweig and Axelrod, 1969) — but in these cases the nerves have been shown to take up foreign amines only after exogenous administration and apparently lack the biosynthetic machinery. An uptake may

take place, however, during conditions of increased plasma levels such as may occur in cases of amine-producing tumours.

However, a marked degree of specificity in the ability to take up amines or their precursors has been noted for central neurons (HILLARP et al., 1966). The same is true for organs innervated by peripheral adrenergic nerves (SHAHAD et al., 1971).

9. Effect of Drugs on Catecholamine Uptake in Organs, Nerves and Isolated Particles

Extensive studies on the action of drugs on the uptake of noradrenaline in organs and in isolated nerve particles have been made. Some of these drugs are listed below:

a) Sympathomimetic amines
b) Adrenergic blocking agents
c) Neuronal and ganglionic blockers
d) Psychotropic drugs
e) Enzyme and metabolic inhibitors
f) Various drugs

a) Sympathomimetic Amines

BURGEN and IVERSEN (1965) studied systematically the effect of a large number of sympathomimetic amines on the uptake of ^{14}C-noradrenaline in the isolated perfused rat heart from a solution containing 10 ng/ml (0.6×10^{-7} M). The effects were expressed as ID 50 (drug concentration producing 50% inhibition of noradrenaline uptake). The strongest inhibitory actions on the noradrenaline uptake were exerted by (—)-metaraminol, dopamine, (+)-amphetamine, α-methylnoradrenaline, noradrenaline and tyramine, while isoprenaline had a much weaker effect. This is of interest since isoprenaline is hardly taken up by the axonal membranes. Normetanephrine has also only a moderate inhibitory effect on the uptake.

Amphetamine and tyramine are both noradrenaline releasers and their strong inhibitory effect on noradrenaline uptake may contribute to their relatively strong biological action. The most active inhibitory amines are also readily taken up into tissues.

The following conclusions from the results with the sympathomimetic amines are quoted from BURGEN and IVERSEN (1965) (L stands for (—)):

"(a) β-Hydroxylation produced a decreased affinity for the uptake site. In such compounds the L-enantiomer had a higher affinity than the D-enantiomer.

(b) α-Methylation resulted in a considerable increase in affinity for the uptake site. This effect was also stereochemically specific, in this case the D-enantiomer had considerably more activity than the L-enantiomer.

(c) Phenolic hydroxyl groups enhanced the affinity for the uptake site, para- and meta-substitutions having approximately equal effects. The optimal structure was the 3,4-dihydroxyphenyl group.

(d) N-Substitution decreased the affinity of the drug for the uptake site. This effect was dependent on the size of the N-substituent, bulky substituents having correspondingly greater effects in depressing affinity.

(e) O-Methylation of phenolic hydroxyl groups produced a striking decrease in affinity for the uptake site. Meta-methoxy-compounds had considerably lower affinities than the corresponding para-methoxy compounds.

(f) The phenylethylamine structure could be replaced with saturated five- or six-membered ring structures as in propylhexedrine and cyclopentamine without

producing a marked decrease in the affinity for uptake. Even the long chain aliphatic amine tuamine and the indoleamine serotonin had appreciable affinities for the uptake site".

Using the rabbit heart Muscholl and Weber (1965) tested the effect of various amines on the uptake of α-methyl-noradrenaline with similar results. Amphetamine also inhibits the uptake of ^{3}H-noradrenaline from a depot injected into the lateral ventricles of the brain (Glowinski and Axelrod, 1965).

In a study of the uptake of ^{3}H-noradrenaline in cat tissue slices Dengler et al. (1961) found that tyramine, ephedrine and (+)-amphetamine in concentrations of 5×10^{-6}M inhibited the uptake 32—57%.

The inhibition of uptake of (—)-metaraminol in rat heart slices by noradrenaline (Giachetti and Shore, 1966) probably signifies that noradrenaline in the concentration used (1.7×10^{-5}M) effectively competes with (—)-metaraminol (5×10^{-7} M) for uptake sites.

On isolated nerve particles Euler and Lishajko (1968a) found that indirectly acting amines like tyramine, phenethylamine and amphetamine increase the rate of noradrenaline loss on incubation in potassium phosphate. Most of this effect was due to inhibition of reuptake. Tyramine also inhibits the ATP-facilitated reuptake and net uptake of noradrenaline, partly by competition through the octopamine formed (cf. Kopin et al., 1965). Uptake of tyramine itself was small. Of other arylamines prenylamine was strongly active as uptake inhibitor.

Incubation of nerve particles with ^{3}H-dopamine caused a considerable uptake of radioactivity which, however, quantitatively corresponded to the extra uptake of noradrenaline. Even when present in a concentration 10 times higher than noradrenaline in the medium, dopamine did not appear bound as such in nerve particles. The inhibitory action of dopamine on the noradrenaline reuptake is presumably a result of a competitive action. When dopamine and noradrenaline both are present in the incubation medium in a concentration of 3×10^{-6}M the reuptake of noradrenaline is inhibited by about 25% (Euler and Lishajko, 1968a).

α-Methyl-metatyramine 10^{-4}M inhibits noradrenaline uptake in nerve particles by 65%, while α-methyl-*m*-tyrosine has no effect. The effect of metaraminol, α-methylnoradrenaline and other amines, which are stored in the nerve particles, on the uptake of noradrenaline is dependent on the relative concentrations of noradrenaline and the amine (Euler and Lishajko, 1970).

b) Adrenergic Blocking Agents

An inhibitory action of the ^{3}H-noradrenaline uptake in various organs by phenoxybenzamine was observed by Hertting et al. (1961b). Phentolamine and dichloroisoproterenol in the doses used had no significant action. On testing a number of phenothiazine derivatives Rosell and Axelrod (1963) found that the inhibitory effect on the noradrenaline uptake in the rat heart was related to their antiadrenergic effect. Chlorpromazine had only a negligible effect on the ^{3}H-noradrenaline uptake in brain (Iversen et al., 1966) although it was quite active on the noradrenaline uptake in the heart. Dibenamine had no action on the uptake of noradrenaline in the heart (Muscholl, 1961) or of ^{3}H-noradrenaline in slices of spleen (Dengler et al., 1961).

Brown and his co-workers (cf. Brown, 1965) found that in the perfused cat spleen the α-blockers dibenamine, phenoxybenzamine, phentolamine and Hydergine increased the transmitter outflow at low stimulation frequencies by a factor of 5. This effect was at first interpreted as the result of blockade of the α-receptors

in the effector organ but was later attributed to inhibited reuptake (BROWN, 1965). Phenoxybenzamine, as well as Hydergine and phentolamine increased the recovery of infused noradrenaline in the outflow from the perfused spleen from about 30% to about 60—80% (GILLESPIE and KIRPEKAR, 1965).

EISENFELD et al. (1967) found that adrenergic blocking agents, particularly the α-blockers, inhibit also the extraneuronal uptake of noradrenaline in tissues. These agents, in addition, decrease the concentration of the metabolites, which is interpreted as the result of an inhibited extraneuronal transport mechanism.

A number of adrenergic blocking agents have also been tested on the noradrenaline-uptake in isolated nerve particles. All α-blockers tested, including dibenamine, and the majority of β-blockers inhibited reuptake as well as the ATP-stimulated net uptake of noradrenaline to a varying degree (EULER and LISHAJKO, 1968b) suggesting that nerve granules possess an uptake mechanism with certain properties in common with the adrenergic receptors. On the other hand a lack of correlation between receptor and uptake blockade has been reported both with α-adrenergic blockers (THOENEN et al., 1964a) and with β-adrenergic blockers (MUSCHOLL, 1966b). Adrenergic blockers also inhibit the uptake of (—) adrenaline (BORN, 1968) and of ^{3}H-noradrenaline in human blood platelets (BYGDEMAN and JOHNSEN, 1969).

c) Neuronal and Ganglionic Blockers

Of the neuronal blockers, bretylium and TM 10 have little effect on the uptake of ^{3}H-noradrenaline in cat organs, while guanethidine markedly inhibited uptake (HERTTING et al., 1961b; HERTTING, 1965). Bretylium and guanethidine also inhibit noradrenaline uptake in spleen slices (DENGLER et al., 1961).

According to SHORE and GIACHETTI (1966) guanethidine at 5×10^{-6}M blocks an intracellular uptake mechanism and at 10^{-4}M uptake through the axonal membrane. On the other hand guanethidine at 10^{-5}M has no action on release or uptake of noradrenaline in isolated nerve particles and only a weak inhibitory action at 10^{-4}M. This is also seen with β-TM 10 and TM 6 whereas bretylium has no action even at 3×10^{-4}M (EULER and LISHAJKO, 1970).

Hexamethonium seems to have little action on noradrenaline uptake in the heart (LINDMAR and MUSCHOLL 1964), brain (WEIL-MALHERBE et al., 1961) or on isolated particles.

d) Psychotropic Drugs

α) Reserpine

Reserpine strongly inhibits uptake of ^{3}H-noradrenaline in various organs *in vivo* (HERTTING et al., 1961b) and the bulk uptake of noradrenaline after depletion in the perfused rat heart (BHATTACHARYA, 1968). The effect of reserpine is less dramatic in the brain; it blocks the uptake in the pituitary but not in the hypothalamus, cortex or medulla (WEIL-MALHERBE et al., 1961).

An inhibitory action of reserpine on the noradrenaline uptake is also noted on tissue slices (DENGLER et al., 1961).

The initial uptake of catecholamines through axonal or other cell membranes does not seem to be inhibited by reserpine, however (IVERSEN et al., 1965). This is in agreement with histochemical evidence (HAMBERGER et al., 1964) and earlier observations (KOPIN and GORDON, 1962; WEINER and TRENDELENBURG, 1962; LINDMAR and MUSCHOLL, 1964). These results are also in harmony with the findings of ANDÉN et al. (1963) that ^{3}H-noradrenaline can be taken up in tissues of a reserpine-treated animal even after denervation. It is then rapidly metabolized.

Glowinski and Axelrod (1965) observed that whereas amphetamine raises the normetanephrine levels in brain, reserpine augments the O-methylated deaminated metabolites.

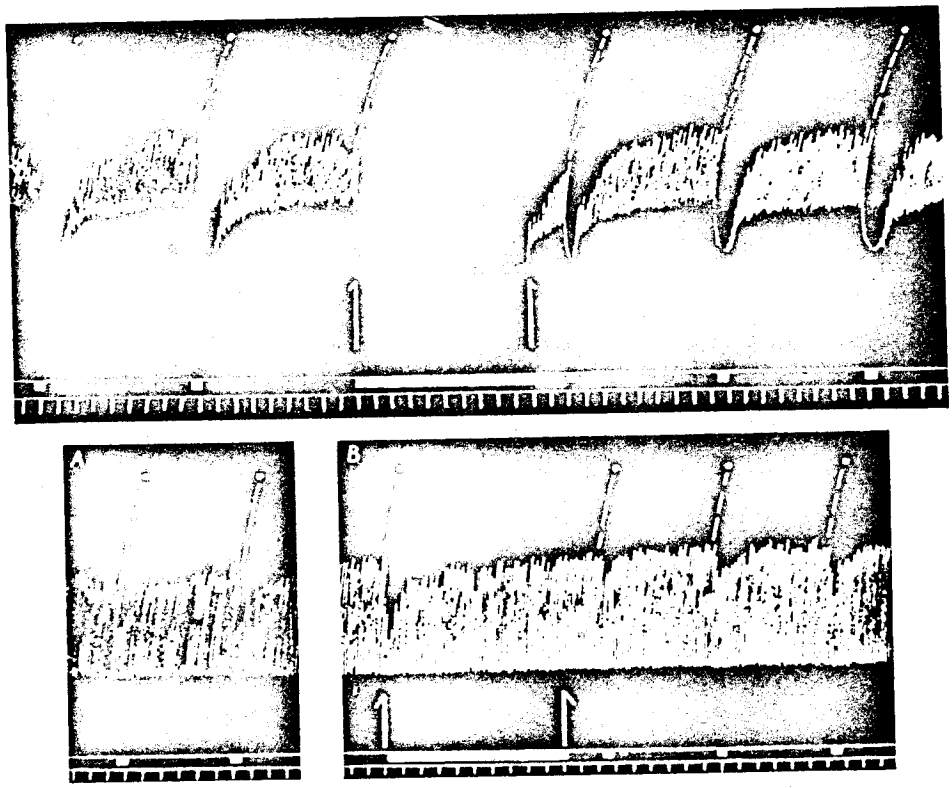

Fig. 8. Upper trace: response of isolated rabbit ileum to pre-arterial nerve stimulation 50/sec for short periods and 5 min. Lower traces: ileum from rabbit pretreated with reserpine. Response to stimulation partially restored by soaking in noradrenaline but readily fatigued (Gillespie and Mackenna, 1961)

Unchanged (Lindmar and Muscholl, 1964) or even increased removal of noradrenaline from the perfusion fluid passing through organs from reserpine-treated animals (Gillespie and Kirpekar, 1965) indicates that specific uptake is not essential. Newly synthesized noradrenaline is then partly deaminated by intraneuronal MAO (Kopin and Gordon, 1962). However, even in reserpine-treated animals a small uptake of noradrenaline can occur at sites which allow temporary restoration of the nerve stimulation effect in an isolated intestinal preparation (Gillespie and Mackenna, 1961) (Fig. 8) or on the circulation of the cat (Rosell and Sedvall, 1961; Burn and Rand, 1958). The restored effect is easily fatigued by continuous stimulation of the nerves, suggesting that the uptake in specific stores is very limited, or occurs only in a small pool. The recovery observed after „neuronal rest" may be explained by refilling of these stores by resynthesis. Gillespie and Mackenna (1961) also observed an acute blocking effect of reserpine added to the organ bath on the effect of adrenergic nerve stimulation, presumably due to delayed refilling of some specific pool even in the presence of

nearly normal stores. Effects of a similar kind have been observed by DAY and OWEN (1968) on the rabbit ear and EULER (1969) on guinea pig vas deferens.

Reserpine inhibits the uptake of ^{3}H-noradrenaline in 10 and 18 days old rats but considerably less than in adult animals which may indicate a difference in the specific stores (IVERSEN et al., 1967).

LINDMAR and MUSCHOLL (1965) made the interesting observation that after pretreatment with small doses of reserpine the α-methylnoradrenaline taken up in the rabbit heart after administration of α-methyl-DOPA is not depleted whereas the noradrenaline stores decreased. Whether this difference is due to a less affected uptake or reuptake in particles of α-methylnoradrenaline or depends on firmer binding of this amine has not been established. Similar findings have been made by CARLSSON et al. (1965) on stores in the brain.

The effect of reserpine on isolated nerve particles is to block the net noradrenaline uptake in the presence of ATP (cf. Fig. 6). Whether reuptake is blocked is difficult to ascertain since the normal release is strongly retarded (EULER and LISHAJKO, 1965b).

β) Cocaine

Inhibited tissue uptake as a cause of increased catecholamine action was first suggested by BLASCHKO (1954) who presented this possibility as an explanation of the potentiation of the response to adrenaline by cocaine.

In 1959 MACMILLAN suggested that cocaine, by preventing the uptake of noradrenaline into the tissue stores, increased the amount available for the receptors and thus potentiated its effect. This concept was supported by the finding of TRENDELENBURG (1959) that cocaine delayed the disappearance of noradrenaline from the blood after injection. Direct evidence for the correctness of MACMILLAN's hypothesis was given by MUSCHOLL (1960) and by WHITBY et al. (1960) who observed that cocaine reduced the uptake of circulating noradrenaline in organs and confirmed the increase of its concentration in plasma after injection. This finding has since been amply confirmed in a variety of ways. HUKOVIĆ and MUSCHOLL (1962) showed that cocaine increased the noradrenaline-content of the perfusate from the rabbit heart after sympathetic nerve stimulation by 122%.

Cocaine has been used repeatedly in experiments designed to study recapture of noradrenaline released from organs by nerve stimulation. The results have been variable. Thus TRENDELENBURG (1959), NASMYTH and ANDREWS (1959) and KIRPEKAR and CERVONI (1963) observed only a very modest increase in the outflow of noradrenaline from the stimulated cat spleen after cocaine, certainly not comparable with that seen after phenoxybenzamine. Similar results were obtained by BLAKELEY et al. (1963) who could not detect any action of cocaine on the outflow of noradrenaline after nerve stimulation, while the α-blocker Hydergine caused a large increase like dibenamine and phenoxybenzamine (BROWN and GILLESPIE, 1957). THOENEN et al. (1964b) found an increase of noradrenaline in the outflow of the stimulated spleen after cocaine but still far lower than that observed after phenoxybenzamine. It may be recalled that cocaine increases the effect of adrenaline on the vascular resistance of the perfused human placenta (EULER, 1938). If the effect of cocaine is due to inhibition of reuptake this must occur in non-neuronal tissue in this case.

While cocaine clearly inhibits the uptake of noradrenaline in the tissues, including the adrenergic neurons, this is of a moderate degree, since even large doses of cocaine did not prevent the deposition of radioactive noradrenaline in adrenergic fibres of the heart and vas deferens in mice although the uptake was delayed (SAMORAJSKI et al., 1964) (Fig. 4).

As shown by Hertting and Schiefthaler (1963) the noradrenaline output of the perfused cat spleen during sympathetic nerve stimulation greatly depends on the flow rate. At low flow rates during vasoconstriction a larger proportion of the released transmitter is re-bound. By blockade of the vasoconstrictor effects or at elevated perfusion pressure the noradrenaline output of the spleen is increased. An enhancement by cocaine of the vasoconstrictor effect of nerve stimulation tends to mask the elevation of overflow of noradrenaline brought about by inhibition of re-uptake at the same time. If the cat spleen is perfused with a constant volume cocaine causes an increase in transmitter output (Thoenen et al., 1964b). The results obtained on the spleen are therefore in accord with those obtained on the heart (Huković and Muscholl, 1962). In this preparation sympathetic stimulation with or without cocaine did not appreciably alter the coronary flow.

Several years ago Blaschko (1954) suggested the possibility that potentiation of adrenaline-like action might be brought about by a competitive interference with entry across the cell membrane, causing an amine to be present in an effective concentration for a longer time.

The inhibitory action of cocaine on noradrenaline uptake depends on the concentration ratio of the two compounds (Muscholl, 1961).

Cocaine only moderately inhibited the bulk uptake of noradrenaline in the depleted rat heart (Bhattacharya, 1968) particularly when noradrenaline was used in higher concentrations (10^{-6}M). This is in agreement with the conclusion of Furchgott et al. (1963) that cocaine may compete with noradrenaline for common uptake sites.

Cocaine also inhibits the uptake of α-methylnoradrenaline by the isolated rabbit heart (Weber and Muscholl, 1965). Tetracaine had no effect on the uptake in a concentration with twice the local anesthetic effect. In brain slices no inhibitory action of cocaine is seen on the uptake of ^{3}H-phenylethanolamine, which is an active noradrenaline releaser. The antagonistic action of cocaine on this indirectly acting amine could therefore not be explained by inhibition of amine uptake (Ross et al., 1968). As observed by Barnett et al. (1968), cocaine does not inhibit the effect of tyramine on the nictitating membrane in concentrations which potentiate the response to noradrenaline. Similar observations were made by Lagercrantz (1968) on the isolated iris.

γ) Imipramine, Desipramine, Amitriptyline

Imipramine inhibits the uptake of ^{3}H-noradrenaline in heart and spleen (Hertting et al., 1961b) and after administration in the lateral ventricle the uptake in the brain (Glowinski and Axelrod, 1965). Desipramine (DMI) and amitriptyline acted similarly.

DMI, which is normally a potent inhibitor of noradrenaline uptake (Titus and Spiegel, 1962) and more efficient than cocaine (Barnett et al., 1968), is ineffective in preventing uptake in the reserpine-pretreated heart (Iversen et al., 1965). It is conceivable that reserpine has altered the properties of the cell membrane.

The finding of Geffen (1965), previously referred to, that DMI does not increase the outflow of noradrenaline from the isolated perfused spleen on nerve stimulation suggests either that the postulated recapture of transmitter is negligible under the prevailing experimental situation or that desipramine does not reach the release sites.

As reported by Iversen et al. (1966) the uptake of noradrenaline in the brain is inhibited by DMI and by amphetamine, but not the uptake of dopamine.

On isolated particles DMI (STJÄRNE et al., 1968), amitriptyline and nortriptyline strongly inhibit release as well as uptake of noradrenaline. DMI also inhibits the noradrenaline releasing effect of tyramine on isolated nerve particles (EULER and LISHAJKO, 1968a).

δ) d-Lysergic Acid Diethylamide

Lysergic acid diethylamide does not affect noradrenaline uptake in organ slices (DENGLER et al., 1961), or in the brain (GLOWINSKI and AXELROD, 1965) but has an increasingly larger inhibitory effect on the noradrenaline uptake in isolated nerve particles when added to the incubation medium to 10^{-6}M or higher.

e) Enzyme and Metabolic Inhibitors

Of the MAO inhibitors, iproniazid inhibits noradrenaline uptake in the hypothalamus but not in the pituitary, whereas pheniprazine was without effect in both cases (WEIL-MALHERBE et al., 1961; HERTTING et al., 1961b). Nialamide and pargyline had no effect on uptake in isolated nerve particles in concentrations up to 10^{-4}M, whereas pheniprazine had a slight releasing effect.

Noradrenaline uptake *in vivo* or in isolated nerve particles was not found to be inhibited by ouabain (WEIL-MALHERBE et al., 1961; HERTTING et al., 1961b; EULER and LISHAJKO, 1965b) whereas inhibition is observed in tissue slices (DENGLER et al., 1961). Recent observations indicate, however, that uptake may be inhibited after a time lag of 10—20 min *in vivo*. (TISSARI et al., 1969; LEITZ and STEFANO, 1970).

Eserine and prostigmine have no action of the outflow of noradrenaline from the perfused spleen on stimulation of its nerves (BLAKELEY et al., 1963) and are also inactive when tested on the release and noradrenaline uptake in isolated nerve particles.

Arsenate and fluoride in high concentrations (10^{-2}—10^{-3}M) do not seem to affect noradrenaline uptake in particles (EULER and LISHAJKO, 1969).

In their study of various metabolic inhibitors on the incorporation of radioactive catecholamines in isolated adrenal medullary granules CARLSSON et al., (1963b) observed that the facilitating effect of ATP plus Mg was inhibited by various compounds. Most active as inhibitors were SH-reagents.

KIRSHNER and SMITH (1966) found that cyanide and iodoacetic acid given together strongly inhibited the catecholamine secretion by acetylcholine from the adrenal gland whereas each substance alone had only little effect. Antimycin or oligomycin also acted as inhibitors only when given in combination.

KIRPEKAR and WAKADE (1968) observed that while iodoacetic acid or dinitrophenol (DNP) 5×10^{-4}M used singly, had a moderate inhibitory effect only, their combined action was marked.

In a study of the action of various metabolic inhibitors on the release and uptake of noradrenaline in nerve particles, EULER and LISHAJKO (1969) found that while cyanide or azide had only little effect on these functions, a number of other agents were active.

Sulphydryl reagents like N-ethylmaleimide (NEM), iodoacetate, iodobenzoate and mercuribenzoate were slightly to moderately active and required high concentrations in order to exert an action. NEM at 3×10^{-4}M inhibited noradrenaline uptake by 40%.

Inhibitors at various sites of the respiratory chain and of oxidative phosphorylation were all active inhibitors of the uptake of noradrenaline in isolated particles, both on reuptake and on the ATP-stimulated net uptake. Thus rotenone, anti-

mycin A, chlorpromazine and oligomycin strongly inhibited uptake. In a concentration of 3×10^{-4}M antimycin completely prevented the ATP-stimulated uptake. Oligomycin was also strongly active.

Uncouplers of oxidative phosphorylation, DNP, carbonyl cyanide *m*-chlorophenylhydrazone (CCP), desaspidin and pentachlorophenol all strongly enhanced spontaneous release and inhibited noradrenaline uptake (Euler and Lishajko, 1969).

f) Various Drugs

Nicotine and atropine seem to have no effect on the noradrenaline uptake in tissues or in particles (Muscholl, 1961; Euler and Lishajko, 1965b).

A large number of other substances have been studied with regard to their action on the uptake of ^{3}H-noradrenaline in organs *in vivo* or on isolated nerve particles *in vitro*. Of special interest are perhaps the actions of drugs, known to exert actions on the functions of the autonomic nerve system. To this group belong various amines and naturally occurring substances. 5-Hydroxytryptamine, histamine, prostaglandin E_2 (PGE_2) (Hedqvist, 1970), GABA, angiotensin, vasopressin and oxytocin, insulin and various other peptides were without action on amine uptake.

III. Storage

1. Storage in Subcellular Particles

From analytical data, based on the relative amounts of noradrenaline in splenic nerves and splenic tissue and the probable amount of nerve tissue in the spleen, it was inferred that the amine is accumulated in the nerve terminals (Euler, 1954). Later experiments showed (Euler and Hillarp, 1956) that noradrenaline was bound to a small subcellular particle fraction obtained in the sediment from homogenates of organs and adrenergic nerves by high speed centrifugation. The noradrenaline present in the particles[1] occurred in a bound form as shown by the facts that it was not susceptible to oxidants, nor was it adsorbed on passage through alumina. Moreover, it was not biologically active until released from the granules (Euler, 1958).

Incubation of a suspension of particles in phosphate buffer at 37°C causes a rapid release of the transmitter with a halftime of about 5 min, whereas it is firmly bound at 0°, no loss being observed in several hours (Euler and Lishajko, 1963a).

The cytology of these particles has been studied by electron microscopy. After squeezing of bovine splenic nerves and removal of larger particles a suspension is obtained which contains osmiophilic granules with a diameter of 0.03—0.15 μ (Fig. 9a). (cf. Euler and Swanbeck, 1964). Tissue sections have shown granules of this kind in sympathetic nerve terminals in various organs (De Robertis and De Iraldi, 1961; Lever and Esterhuizen, 1961; Richardson, 1962; Hökfelt, 1969). The bovine splenic nerve particles have been studied with regard to their physical and chemical properties by Laduron et al. (1966), Hörtnagl et al. (1969) and De Potter et al. (1969).

While the association of the transmitter with the osmiophilic granules rests beyond doubt, detailed knowledge is still lacking as regards the relative noradrenaline content in granules from the nerve trunk and terminal granules or in granules of different size. After administration of radioactive noradrenaline to animals it has been possible to show that silver grains are located over nerve

Fig. 9.\
genizat\
splenic

1 The subcellular particles storing noradrenaline in adrenergic nerves are often referred to as nerve granules.

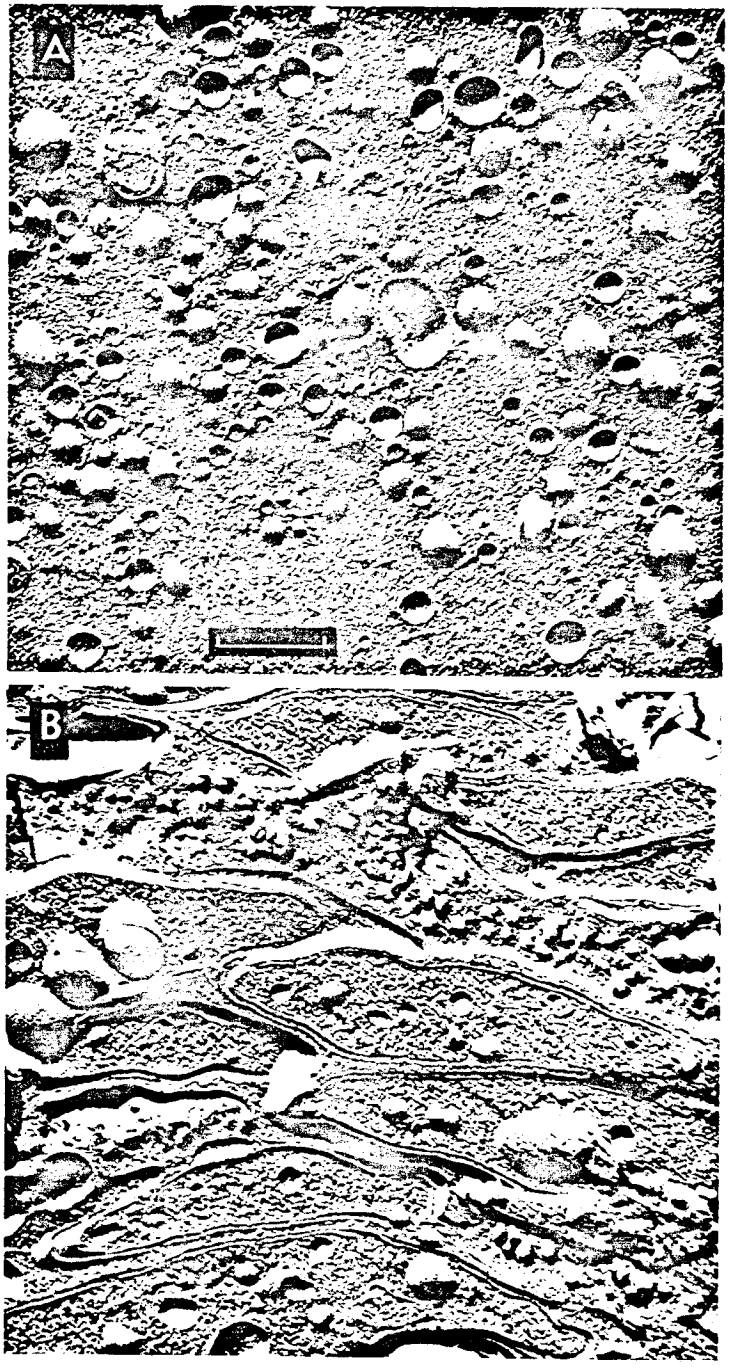

Fig. 9A and B. A: Bovine splenic nerve particles from high speed sediment after homogenization and removal of coarse particles by centrifugation 10 min at 10000× g. B: Bovine splenic nerve axons. Glutaraldehyde fixation. Freezeetching. Marker: 0.2 μ (GEMNE and EULER, unpubl.)

particles in preparations studied by combination of autoradiography and electron microscopy (WOLFE et al., 1962).

Transmitter storage particles appear in the highest number in the terminal swellings of the adrenergic nerves, sometimes tightly packed. In the axon they occur much more sparsely (Fig. 9b); this explains the fact that they have eluded detection for considerable time although their presence in the axoplasm was known. From their relative dispersion and assuming the same relative noradrenaline content in the particles the concentration would be about 100 times higher in the terminal swellings than in the axon. Since the splenic nerve trunk contains about 10 μg/g the concentration in the varicosities would be of the order of 1 mg/g which is similar to that in chromaffin tissue (cf. NORBERG and HAMBERGER, 1964).

Although several possibilities have been suggested for the storage mechanism this is still unknown. Of the various possible modes of storage, binding to nucleotides, proteins, lipoproteins and phospholipids have been suggested (cf. BELLEAU, 1960). For an extensive discussion of this problem the reader is referred to the review by GREEN (1962).

From the results of experiments on uptake *in vivo* and *in vitro* it appears that the catechol group and the β-hydroxyl group in the side chain are essential for the binding in adrenergic storage particles (cf. MUSACCHIO et al., 1965b). As pointed out in section II B 4 the binding characteristics are different in the dopamine particles.

2. Storage Capacity, Noradrenaline Content in Particles

Precise data relating to the noradrenaline storage capacity of the granules at different locations (terminal and extraterminal) and their size in the adrenergic axons cannot be given at present. It has been computed from morphological studies, however, that the average number of granules in a varicosity is of the order of 1000, and from estimations of the number of varicosities and the noradrenaline content of the tissue it is calculated that the average noradrenaline content per granules is 5×10^{-6} pg. or 1.5×10^{4} molecules (DAHLSTRÖM et al., 1966; FOLKOW et al., 1968).

The storage capacity is usually not wholly utilized as judged from the moderate increase of 25—50% observed in the total noradrenaline store in an organ after decentralization, ganglionic blockers or neuronal blockade (cf. Uptake p. 209). Apparently a moderate deficit is upheld during normal impulse traffic.

Whether the lower noradrenaline content in the axon in comparison to that in the terminal swelling simply depends on the greater dispersion of granules in the axon or upon a difference in storage capacity is not known.

3. Stability of Storage Particles

The very high degree of stability of the transmitter storage particles at low temperature is illustrated by the observation that storage of an organ at refrigerator temperature (2—5° C) for 24 hrs does not diminish the yield of noradrenaline extractable from an organ. On storing isolated nerve particles in phosphate buffer for 24 hrs the loss in noradrenaline content is less than 50% of the original content.

At higher temperatures the noradrenaline content of an organ as well as in isolated particles diminishes. Release increases rapidly with temperature. Thus the release constant at 20° C in isotonic phosphate, ph 7.0, is approximately 0.01 or 1% per min, but increases to about 0.15 at 37° (EULER and LISHAJKO, 1967a). The high temperature coefficient (Q_{10} 3—4), has so far not been adequately explained.

4. Free and Particle-bound Noradrenaline in Homogenates of Adrenergic Nerves and in Organs

In the press juice from bovine splenic nerves about 30—40% of the total noradrenaline was recovered in the high speed sediment whereas the remaining part was found in the soluble fraction and in the residue (EULER and LISHAJKO, 1963a). Several authors, using different homogenization procedures have found noradrenaline from various organs, usually the heart, about equally distributed in the coarse particles, the high speed sediment and the soluble fraction (cf. CAMPOS and SHIDEMAN, 1962; BHAGAT, 1963; STITZEL and LUNDBORG, 1967; WESTFALL, 1965; MACKENNA, 1965) and others. These fairly consistent results at first seem to indicate that a certain proportion of the noradrenaline in the adrenergic neurons is either free or loosely bound. In a study of the influence of homogenization on the distribution of noradrenaline between the subcellular fraction, EULER (1966) found, however, that continued treatment of the particle suspension with the homogenizer rapidly lowered the proportion of particle-bound noradrenaline and increased the amount in the soluble portion. Extrapolating back from the rate of continued release from particles, it appeared that the original proportion of soluble noradrenaline only amounted to around 10% or less. The "soluble" fraction may therefore partly be an artifact depending on the mechanical treatment during homogenization. The concept that only a small fraction of the total noradrenaline in the terminal is present in free form is supported by the observation that newly synthetized noradrenaline which cannot be stored, e.g. after reserpine, is either rapidly metabolized or leaves the axon (KOPIN and GORDON, 1962). However, using a standardized technique it seems possible to obtain comparable values which might serve as guidelines for changes caused by various physico-chemical factors or by drugs.

Although most or practically all of the noradrenaline in the terminals may normally occur in a bound form, several observations suggest that there is more than one pool containing the transmitter. TRENDELENBURG (1961) used the term "available noradrenaline" for a certain small fraction which seemed to be released in response to nerve stimulation and various agents, e.g. tyramine. Observations by CROUT et al. (1962) and TRENDELENBURG (1963) are in harmony with the concept that the small available pool can be replenished from a larger pool. It has also been suggested that storage particles near the axonal membrane form a tyramine-releasable pool and take up ^{3}H-noradrenaline preferentially (POTTER and AXELROD, 1963a; CROUT, 1964). Experiments on the isolated guinea pig vas deferens have shown that reserpine in concentrations of 0.25—1 μg/ml rapidly causes neuromuscular inhibition or block (EULER, 1969) which may be due to deficient refilling of a peripheral "available" pool since it is known that reserpine retards the release from the storage granules (EULER and LISHAJKO, 1965b). It has been suggested that the peripheral pool may be associated with or even form a part of the axon membrane (cf. EULER, 1970).

Studies on the specific protein chromogranin A, first demonstrated in adrenal medullary granules, have shown its presence also in the noradrenaline-containing granules of bovine splenic nerve (BANKS et al., 1969). On stimulation of the nerve to the isolated perfused spleen of the calf it was observed (DE POTTER et al., 1969; GEFFEN et al., 1969) that the perfusates contained far more noradrenaline in proportion to chromogranin A and dopamine-β-hydroxylase than the vesicles. These results, like those of STJÄRNE et al. (1970b), which demonstrated that release of transmitter from the stimulated perfused spleen was not accompanied

by a release of previously labelled nucleotides, do not speak in favour of exocytosis as a principal mechanism for transmitter release from adrenergic nerves.

5. Effect of Drugs

While the amount of noradrenaline stored in reserpinized tissue is diminished, due to deficient uptake in the particles, there is a striking increase in deaminated metabolites (KOPIN and GORDON, 1962; GLOWINSKI and AXELROD, 1965; GLOWINSKI et al., 1966b). After administration of a MAO inhibitor to the reserpinized animal before giving ^{3}H-noradrenaline, it accumulates to almost normal levels. How this noradrenaline is "stored" is not known; it is not even certain that it occurs mainly intraneuronally but it may be present also extraneuronally. In the acutely reserpinized spinal cat preparation the transmitter, protected by a MAO-inhibitor, is constantly leaking out from the terminals and may increase the tissue content of noradrenaline.

The small reserpine-resistent noradrenaline "store", described by SEDVALL (1964), HÄGGENDAL and LINDQVIST (1964), GLOWINSKI et al. (1966b) may be due to retention in particles still able to take up a certain amount of newly synthesized noradrenaline, but releasing it slowly. In this case an additional dose of reserpine might or might not affect this amount, which may represent the balance between a small uptake and slow release. In the reserpine-treated rat heart the small store can be partially released by DMPP (IVERSEN et al., 1965).

In the reserpine-treated brain DMI or amphetamine hardly affect the accumulation of ^{3}H-noradrenaline, but if the rapid metabolism of the noradrenaline is prevented by a MAO inhibitor like pheniprazine the effects of these drugs again become apparent and the uptake is smaller (GLOWINSKI et al., 1965). Amphetamine in these experiments seems to have a MAO inhibiting effect.

The results of injections of noradrenaline in reserpine pretreated rats have revealed the important fact that the heart of such rats is able to take up and store in a normal manner tracer doses of noradrenaline, but cannot store larger amounts, which are recovered in the supernatant fraction after homogenization (IVERSEN et al., 1965). Uptake experiments with tracer doses of noradrenaline must therefore be judged with caution.

After administration of amphetamine the normetanephrine level increases in the rat brain whereas after reserpine the O-methylated deaminated metabolites increase. The differences in the metabolic pattern of noradrenaline observed after amphetamine and reserpine moreover suggest the presence of more than one storage form for catecholamines in the brain (GLOWINSKI and AXELROD, 1965).

From studies on the rat brain it has been suggested that reserpine releases noradrenaline mainly from the "supernatant fraction" i.e. from the whole neuron while amphetamine releases from the varicosities (GLOWINSKI et al., 1966b).

MAÎTRE and STAEHELIN (1968) have observed that while cocaine, imipramine and desipramine strongly inhibit the uptake of ^{3}H-noradrenaline in the rat heart, the amount found in the vas deferens was considerably increased, possibly indicating a special storage form in this organ.

The storage in adrenergic nerve particles is influenced by a large number of drugs, as judged by their action on the "spontaneous" release rate (EULER and LISHAJKO, 1965b). Many of these cause a retardation of the release as observed with reserpine, prenylamine, adrenergic α-blockers, desipramine, nortriptyline, LSD, metabolic inhibitors at the first and second stage of the respiratory chain, oligomycin. Several of these compounds are releasers at higher concentrations.

A releasing action is seen with indirectly acting amines, and uncouplers of oxidative phosphorylation (EULER and LISHAJKO, 1968a, 1969). The releasing effect (also seen with detergents, increased temperature, lysis, and sonication) is apparently in some way associated with the condition of the particle membrane or physico-chemical state of the particle, but may also be induced by metabolic changes.

Several biologically highly active compounds like acetylcholine, histamine, 5-hydroxytryptamine, nicotine, atropine have no effect on the storage and release from particles, whereas cocaine, bretylium and guanethidine have only a weak effect.

Interestingly, there is an exchange between endogenous noradrenaline in the nerve storage particles and exogenous adrenaline in the incubation medium, the rate of which is dependent on the release and the relative concentrations of the amines. This is true also for dopamine (stored as noradrenaline) and tyramine (stored as octopamine).

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