ADRENERGIC MECHANISMS[1,2]

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Several important aspects of the vast and rapidly expanding field of "adrenergic mechanisms" were adequately covered in three excellent articles in a previous volume of the *Annual Review of Pharmacology* (1–3). We have therefore chosen to elaborate further on certain aspects in which we have been particularly interested: the basic mechanisms underlying transmitter storage and release by the nerve impulse, and the localization and function of the monoamines of the central nervous system. Our reference list is deliberately far from complete. For further references the reader should consult the reviews mentioned above.

Some Basic Aspects of the Monoamine-Storing Neurons

The present discussion on some fundamental properties of the monoamine-storing neurons will be based mainly on data derived from the peripheral adrenergic neuron, which for obvious reasons can be more easily studied than the corresponding central neurons.

The intraneuronal distribution of monoamines.—As shown by the histochemical fluorescence technique, the adrenergic transmitter noradrenaline (NA) occurs in nearly all parts of the adrenergic neuron, although the concentration varies considerably. The highest concentration occurs in characteristic enlargements, so-called varicosities, within the terminal fibre network. In other parts of the neuron the amine level is considerably lower. The uneven amine distribution appears to be due to the accumulation of amine granules, i.e., the intraneuronal amine-storing organelles within the varicosities. In other parts of the neuron these granules are scarce. The amine granules isolated by differential centrifugation of tissue homogenates are probably identical with small "dense cored" vesicles (diameter 40 to 60 mμ) seen in the electron microscope, and with the less abundant large "dense cored" vesicles (diameter 80 to 120 mμ).

In the peripheral adrenergic neuron the total NA content in the cell body is about 4×10^{-13} g, while in the terminal net of the neuron it is at least 300 times higher, about 1.3 to 3×10^{-10} g (4). The total length of the terminal net of one neuron is of the order of 10 to 30 cm. The NA content in one varicosity of the adrenergic nerve terminal is about 5×10^{-15} g, correspond-

[1] The survey of literature pertaining to this review was concluded in June 1968.

[2] Abbreviations used in this review are: CA [catecholamine(s)], COMT (catechol-O-methyl transferase), DA (dopamine), DOPA (L-3,4-dihydroxyphenylalanine), MAO (monoamine oxidase), NA (noradrenaline), 5-HT (5-hydroxytryptamine), 5-HTP (DL-5-hydroxytryptophan).

ing to a wet weight concentration of about 0.1 to 0.3 per cent (5). Based on figures for the catecholamine (CA) concentration in the amine granules of the adrenals, the approximate number of amine granules within one varicosity can be calculated to be about 1000 and the number of NA molecules to be about 15,000 per granule.

In the central nervous system the monamine-containing neurons have a similar characteristic appearance, with the largest amounts of amine present in numerous varicosities of the nerve terminals. The distribution of dopamine (DA) in the nigro-neostriatal neurons has been examined in particular detail (6) and was found to be similar to that of NA in the peripheral adrenergic neurons. Thus, about 99 per cent of the DA in the nigro-neostriatal neurons occurs in a concentration of 0.1 to 1.0 per cent in the varicosities of the nerve terminals, which form a very large plexus. In electron-microscope studies (7) it was found that DA, like NA and 5-HT, is mainly stored in small granular vesicles (diameter about 50 mμ) which occur in large numbers in the varicosities or boutons (diameter 0.5 to 0.8 μ).

Synthesis, transport and life-span of the amine granules.—The amine granules appear to be synthesized in the cell bodies and transported at a rate of several millimeters per hour down the axon to the varicosities of the nerve terminals (8–11). If a ligation is made on a nerve carrying adrenergic fibres, e.g., the sciatic nerve, a rapid and pronounced accumulation of NA occurs above the ligation. Pharmacological and electron-microscope evidence supports the view that this accumulation is due to piling up of amine granules steadily transported down the axon (11–13). The mechanism of this proximo-distal transport is still not clarified, but seems to be independent of both perikaryon and nerve terminals, since it still occurs within an isolated part of the nerve (14). The life-span of the amine granules has been calculated to be 5 to 10 weeks in different mammals (10, 15).

Function of the amine granules.—As is well-known, reserpine is capable of blocking adrenergic neurotransmission by inhibiting the amine uptake by the storage granules, which leads to depletion of the store. During recovery from a single large dose or repeated small doses of reserpine, however, normal function reappears long before the tissue levels of monoamines have increased markedly. Thus, almost normal behaviour is observed in rabbits having very low amine levels in brain and peripheral tissues after repeated small doses of reserpine (16, 17). A major fraction of the monoamines appears to be present in reserve pools without immediate functional importance. Functional changes appear to be better correlated with changes of a small monoamine fraction (functional pool) disclosed by chronic reserpine treatment. After administration of a single dose of reserpine the adrenergic transmission in peripheral tissues of rat and cat has been shown to recover within 48 to 72 hr. This coincides with an increase in the ability of the tissue to take up and retain labelled NA (18–20). The monoamine levels in the tissues remain very low when the function recovers but the urinary NA exception in rats is markedly increased at this time (21). Subcellular distribution data give direct support for the view that recovery of adrenergic

neurotransmission coincides with recovery of the uptake mechanism of the granules (22, 23).

The data quoted above provide strong evidence that the reserpine-sensitive uptake mechanism of the amine granules is essential for maintaining neurotransmission. In further support of this conclusion it has been found that exogenous NA taken up and accumulated in adrenergic nerve fibres of reserpine-treated animals is not available for release by the nerve impulse (24, 25).

The time required for total recovery of the NA levels in different tissues of rat and rabbit after a large dose of reserpine is about the same as the calculated life-span of amine storage granules for both species (26). Furthermore, the recovery of the NA levels is about linear. The data thus suggest that the action of reserpine on the storage mechanism of the granules is largely irreversible and that the recovery is mainly due to a steady down-transport of newly formed amine granules. Some evidence suggests that newly formed down-transported granules may be of importance also for the early functional recovery after reserpine (26), but at present it is not possible to exclude a reversible effect on a small functional pool of the granules.

In addition to the well-known ATP-Mg^{++}-dependent reserpine-sensitive uptake mechanism, reserpine-resistant uptake by the amine granules has been demonstrated (24, 27, 28). Metaraminol seems to use the reserpine-resistant mechanism to a greater extent than NA (29), while DA seems to use both mechanisms (30). Functional response to sympathetic stimulation can be obtained after reserpine when a large dose of DA is given after monoamine oxidase (MAO) inhibition (25). Under these conditions DA appears to utilize a reserpine-resistant mechanism, and to be transformed to NA by β-hydroxylation in the granules. This NA can then be released on nerve stimulation, presumably from a small functional pool.

Recent studies on subcellular distribution have shown that ^{3}H-metaraminol is first taken up into a reserpine-resistant pool of the granules and thereafter transferred to a reserpine-sensitive storage pool (31), thus demonstrating different pools in the granules.

Amine granules and "quantal release".—On stimulation of the adrenergic nerves to cat skeletal muscle, the average amount of transmitter released per nerve impulse and individual varicosity has been calculated to be approximately 400 molecules of NA (32, 33). This amount of NA corresponds to only about 3 per cent of the calculated NA content in a single amine granule.

Katz (34, see also 35) has presented evidence that acetylcholine is released from motor nerve endings in "qunatal packets". Similar evidence for quantal release of NA from adrenergic nerve endings has been presented (36). Katz suggested that one "quantal packet" would correspond to the total transmitter content of one acetylcholine vesicle. However, the direct measurements quoted above do not support this hypothesis, insofar as the adrenergic nerves are concerned.

According to recent experimental data on adrenal medullary granules, the nerve impulse causes release of proportional amounts of other granular components, i.e., adenine nucleotides and soluble protein, along with the catecholamines (37–39, see also 40). Although these data underline the importance of the amine granules, it should be emphasized that they do not tell whether the whole or a fraction of the content of one granule is released by the nerve impulse. In this connection it is of interest to recall that the life-span of the amine storage granules is of the order of 5 to 10 weeks, whereas the turnover time of the adrenergic transmitter is at most a few days. Thus it is improbable that the whole granules are released along with the transmitter.

There are two main possibilities to explain the important role of the amine granules in the release mechanism. The nerve impulse may directly or indirectly influence the granule to give off part of its content into the synaptic cleft. In this case the granule in question must be assumed to be in very close contact or even fused together with the cell membrane. Alternatively, the nerve impulse may cause release of a hypothetical transmitter pool attached to specific sites of the cell membrane. Reloading of these sites could then be achieved specifically by the amine granules. Both alternatives appear to be compatible with the concept of "quantal release."

Inactivation of the adrenergic transmitter.—The released transmitter may be inactivated by different mechanisms including the following:

(*a*) Local enzymatic destruction.—This inactivation mechanism presumably plays only a minor quantitative role. There now seems to be general agreement that MAO exerts its function mainly intraneuronally, whereas COMT is important for inactivation of extracellular NA. However, no net change in the outflow of NA metabolites has been observed following sympathetic stimulation of skeletal muscles in dogs (41). Other observations also speak in favour of only a minor role for COMT in the inactivation of released NA (cf. 42, 33).

Extraneuronal binding may also occur to some extent but is probably only of small importance under normal conditions.

(*b*) Membrane pump.—Recapture of the released transmitter by the membrane pump appears to be an important mechanism under normal conditions (43–46, 24, 33). When the membrane pump is blocked, the normally swift relaxation of the effector cells after cessation of a stimulation period fails; at higher impulse frequencies the nerve fibres are fairly rapidly exhausted, and, furthermore, the released amount of NA per impulse is decreased. Under normal conditions the active NA reuptake, combined with NA synthesis, generally appears to keep the readily available transmitter pool constant and sufficient.

(*c*) Diffusion to the blood.—The released transmitter may diffuse from the synaptic gap into the blood stream. A marked "overflow" may occur, particularly when the blood flow is high and when the inactivating mechanisms are blocked. Under certain physiological conditions, e.g., during hard muscular exercise, rather high NA levels are found in the blood (e.g. 46,

47), and it seems likely that in these cases the "overflow" is the dominating local inactivating mechanism. It is not unlikely that this circulating NA then exerts a sort of "hormonal" function, e.g., plays a role in fat metabolism. Circulating NA seems to be metabolized mainly by liver COMT.

Central Monoamine Neurotransmission

Demonstration and mapping out of central monoamine neurons.—By means of the histochemical fluorescence method of Hillarp, Falck, and coworkers, in combination with biochemical determinations, it has been possible to demonstrate that the NA, DA and 5-HT in the central nervous system occur in neurons and that only one of the amines is stored in each monoamine neuron (48–50). Practically all the cell bodies of these neurons are present in the lower brain stem, i.e., the medulla oblongata, pons, and mesencephalon. The monoamine nerve terminals are found in almost all parts of the brain and the spinal cord although with large variations in density. The entire monoamine neurons can be observed under the fluorescence microscope when their intraneuronal amine content is increased as after MAO inhibition or after intracerebral monamine injections (49, 51). After lesions of the monoamine neurons, the nerve terminals with their amine content disappear due to anterograde degeneration, whereas the amine content increases in the cell bodies and in the axons proximal to the damage, probably due to accumulation of amine granules formed in the cell bodies (*vide supra*). By these techniques the following three large categories of central monamine neurons have been detected: (*a*) bulbospinal NA and 5-HT neurons with the cell bodies in the medulla oblongata and the nerve terminals in the ventral, dorsal and lateral horns of the gray matter in the spinal cord (52, 53); (*b*) short NA and 5-HT neurons with both the cell bodies and the nerve terminals in the lower brain stem; (*c*) the ascending monoamine neurons of the forebrain (diencephalon + telecephalon) which can be divided in two subgroups (54): nigro-neostriatal DA neurons originating mainly in the pars compacta of the substantia nigra, ascending in the mesencephalic tegmentum, crus cerebri, and internal capsule and terminating in the neostriatum, i.e., the caudate nucleus and putamen; and in the neurons of the medial forebrain bundle (ascending DA, NA and 5-HT neurons) of which the DA terminals are found mainly in the tuberculum olfactorium, the nucleus accumbens, and the dorsolateral part of nucleus interstitialis striae terminalis, whereas the NA and 5-HT terminals are more widely distributed, e.g., in the hypothalamus, most parts of the limbic system, and the neocortex.

Criteria for central monoamine neurotransmission.—These criteria have been extensively described by Bloom & Giarman (1) and therefore only the five most important points will be briefly discussed.

(*a*) Storage of monamines in neuronal synaptic structures.—As discussed above, the central monoamine neurons have in all essential respects the same morphological characteristics as the peripheral NA neurons. The monoamine neurons in the brain and the spinal cord thus have a highly charac-

teristic appearance with the largest amounts of amine present in numerous enlargements or varicosities of the nerve terminals. The monoamine varicosities contain large amounts of granules and they lie in synaptic contact with cell bodies or dendrites. The morphology is therefore very suggestive for a transmitter function of the monoamines in the central nervous system. Certainly there are other central neurotransmitters, which is evident from the fact that in the neostriatum where the monoamine nerve terminals form the densest network in the central nervous system the DA varicosities probably account for only 16 per cent of all the varicosities (*7*).

(*b*) Synthesis of monoamines in the monoamine neurons.—After transection of the nigro-neostriatal DA neurons or the bulbo-spinal monoamine neurons, the DOPA/5-HTP decarboxylase activity disappears almost completely in the neostriatum and in the caudal spinal cord, respectively, simultaneously with the DA and the NA, indicating that this decarboxylase is present in monoamine neurons (54, 55). Similarly, the neostriatum is depleted of its tyrosine hydroxylase activity after lesions of the nigro-neostriatal DA neurons (56). In all probability also, the other synthesizing enzymes are localized in the monoamine neurons. The synthesis of monoamines is much faster in the central than in the peripheral neurons as judged from the more rapid amine loss in the brain and the spinal cord than in the sympathetically innervated organs after inhibition of one of the synthesizing enzymes (57, 58).

(*c*) Release of monoamines from the monoamine neurons.—Naturally it is much harder to demonstrate release of monoamines from central than from peripheral neurons for at least two reasons. First, the former neurons do not release amines to the blood, because of the blood-brain barrier. Diffusion of free amines to the cerebrospinal fluid is also difficult because of the relatively long distances and inactivation mechanisms. Second, selective electrical stimulation of the central monoamine neurons is technically very difficult because they are mingled with many other neurons. Electrical stimulation of the descending pathways in the mouse or frog spinal cord can, however, produce *in vitro* release of NA and 5-HT to a solution surrounding the isolated spinal cords provided that the MAO of the animals is inhibited (59, 60). Tritiated NA or 5-HT accumulated in brain slices is released on electrical stimulation or on depolarization induced by K^+ ions in the presence of Ca^{++} ions (61, 62).

(*d*) Functional effects of monoamines in the central nervous system.—The most convenient way to reveal the effects of the monoamines in the brain and in the spinal cord appears to be through studies of the functional changes induced by injection of the NA and DA precursor, DOPA, or the 5-HT precursor, 5-HTP. The functional effects are the result of formation of the corresponding amines, since they are potentiated by MAO inhibitors and inhibited by decarboxylase inhibitors. Another way to cause stimulation of the monoamine receptors is to give reserpine after MAO inhibition. In this case the amines coming from the granules will not be degraded by the MAO but can reach the receptors of the effector cells in free and active

form. The functional significance of the monoamines in different parts of the central nervous system will be discussed below.

(*e*) Efficient inactivation of monoamines in the central nervous system.—In order to serve as neurotransmitter, the monoamines in free form must be inactivated efficiently. Such mechanisms exist in the central nervous system and they seem to be similar to those in the periphery, i.e., uptake by the neurons and degradation by means of the MAO and the COMT (*vide infra*). In the periphery the released amines can also be eliminated by diffusion to the blood, but such an inactivation mechanism presumably does not operate in the central nervous system because of the blood-brain barrier.

Inactivation of Monoamines in the Central Nervous System

(*a*) Membrane pump.—As discussed above the membrane pump of the peripheral NA neurons is of great importance for the inactivation of the transmitter released from the postganglionic sympathetic nerves. Therefore the possible existence of membrane pumps of the central monoamine neurons has been studied. The main problem in such studies is that the blood-brain barrier prevents the passage of the three monoamines from the blood to the central nervous system. The following methods have been worked out to overcome this difficulty and have been found useful in investigations of this kind: (i) *in vitro* uptake of amines from an incubation medium by monoamine neurons of brain slices from animals pretreated with reserpine (and sometimes also a MAO inhibitor) (63, 64); (ii) displacement of endogenous monoamines from the brain neurons *in vivo* by α-methyl-metatyramine and related compounds passing the blood-brain barrier (65, 66); (iii) *in vivo* accumulation of monoamines in brain neurons after intraventricular injection of the amines (65, 67); (iv) *in vivo* accumulation of NA after DOPA in brain monoamine neurons of reserpine-treated animals (63). By these techniques it has been observed that all three types of central monoamine neurons have a membrane pump. There are, however, differences between the neuronal membrane pumps since they are blocked by different drugs. The membrane pump of the central, like the peripheral, NA neurons is inhibited by compounds of the imipramine group, of which protriptyline and desipramine appear to be the most potent (63). The membrane pump of the DA neurons seems to be unaffected by all members of the imipramine group (63). The membrane pump of the 5-HT neurons is blocked by imipramine and amitriptyline but apparently not by desipramine (65). The effects of the imipramine derivatives on the membrane pump of the 5-HT neurons are possibly correlated with their mood-elevating or antidepressive properties. On the other hand, the motor hyperactivity and exophthalmus, seen when a membrane-pump blocking agent is given together with reserpine, seem to be more pronounced after compounds which act mainly on the membrane pump of the NA neurons. In this connection it is of interest that the head movements, tremor, and limb hyperextensions seen in mice after treatment with MAO inhibitors, the other large group of anti-

depressive drugs, are essentially linked to accumulation of 5-HT, to judge from data obtained after selective amine-synthesis inhibition (68).

(*b*) MAO and COMT.—Both the MAO and the COMT are of importance for the inactivation of monoamines in the central nervous system, which can be seen, *inter alia,* from the fact that the 5-HT metabolite 5-hydroxyindoleacetic acid and the DA metabolites 3,4-dihydroxyphenylacetic acid, homovanillic acid, and methoxytyramine can be found at the same locations as the corresponding amines (69-72). Of the possible NA metabolites, normetanephrine is the only one detected in normal brain tissue (73, 74) although there is evidence that NA is metabolized almost as fast as the other amines. This paradox has been explained in recent studies on the metabolic fate of DA and NA during incubation with brain slices (75–77). In slices of rabbit cerebral cortex, the oxidatively deaminated DA is primarily transformed to the two phenolic acids, 3,4-dihydroxyphenylacetic acid and homovanillic acid, whereas the oxidatively deaminated NA is primarily transformed to the two phenolic alcohols, 3,4-dihydroxyphenylglycol and 3-methoxy-4-hydroxyphenylglycol. Since the slices from the cerebral cortex contain NA but not DA nerve terminals, the same type of experiment has also been carried out on slices from the rabbit caudate nucleus which contains a very rich network of DA but not NA nerve terminals. The DA and NA seem to be metabolized in the caudate nucleus essentially as in the cerebral cortex except that DA is not β-hydroxylated to NA and that 3-O-methylation seems to play a smaller role in the caudate nucleus. Therefore, the differences in the metabolism between DA and NA are in all probability due to the amines and not to the neuron type. The glycols formed from NA are lipid-soluble and should therefore easily pass from the central nervous system to the blood. On the other hand, the acids formed from DA are only slightly lipid-soluble at physiological pH and thus accumulate in the brain.

After pretreatment of the animals with protriptyline, a blocking agent of the membrane pump in the NA neurons, there is a pronounced reduction of the deamination of DA and NA in the slices from the cerebral cortex but not from the caudate nucleus. This difference in protriptyline effect is probably due to differences in the membrane pump of the DA and NA neurons and not to the amines themselves. The data also indicate that the oxidative deamination to a large extent takes place in the CA neurons since it is markedly reduced by a membrane pump blocking agent in the cerebral cortex. The 3-O-methylation seems to occur mainly extraneuronally since a large amount of normetanephrine is formed during incubation with NA but only a small amount when the NA is formed from DA in the NA neurons. The same conclusions regarding the locations of MAO and COMT have previously been drawn from the observations that after MAO inhibition or DOPA administration the 3-O-methylated products accumulate later than the CA and dihydroxyphenylacetic acid, respectively (78, 79, 71).

Functions of the monoamines in the spinal cord and the corpus striatum.

—The spinal cord and the corpus striatum have proved particularly suitable for analyzing central monoaminergic mechanisms.

(*a*) Spinal cord.—The functions of the monamines in the spinal cord can conveniently be studied in a spinal animal. After transection of the spinal cord, the connections between the brain and the caudal part of the spinal cord are disrupted and therefore drug-induced changes in the hind-limb reflexes cannot be due to actions in the brain. In addition, it is easier to detect changes in the hind-limb reflexes in a spinal animal since there are no disturbing influences from other descending pathways and since without drug treatment there is probably little or no release of monoamines from the monoamine pathways which all originate in the brain stem. Injection of DOPA or 5-HTP causes formation of CA (DA plus NA) and 5-HT, respectively, also in the caudal part of the spinal cord following transection. The amines are formed in large amounts in the monamine nerve terminals and presumably diffuse to the receptors of the effector cells.

Injection of DOPA stimulates flexor reflex activity in spinal animals (80). This effect has been analyzed electrophysiologically in the spinal cat (81–86). In the spinal cord the released catecholamines cause inhibition of the transmission of impulses from pain afferents to motoneurons, dorsal roots, certain ascending spinal pathways. At the same time a pronounced flexor reflex is evoked which is concealed in the spinal animal. Presumably it is present in the intact animal when the descending NA pathway is activated. The DOPA injection can thus be said to replace the activity of the descending NA neurons from the brain.

In the cat, 5-HTP also stimulates flexor reflex activity and acts like DOPA (80, 87). Furthermore, 5-HTP increases the excitability of the motoneurons and causes an increased monosynaptic reflex. Thereby the injection of 5-HTP presumably replaces the effect of stimulation of a descending 5-HT pathway directly innervating the motoneurons. In the rat, 5-HTP evokes a somewhat different action on the hindlimbs, characterized by athetoid movements and hyperextensions. Similar effects are produced by lysergic acid diethylamide (LSD) and some other hallucinogenic indole compounds (88). This effect of LSD is in all probability because of 5-HT receptor stimulation and not owing to release of 5-HT from the nerve terminals, since it is observed also when all known 5-HT stores have been depleted by treatment with reserpine and a tryptophan hydroxylase inhibitor.

The effects of DOPA on the spinal reflexes are inhibited by the neuroleptics, haloperidol and chlorpromazine, and the adrenergic α-receptor blocking agent, phenoxybenzamine, but not by β-receptor blocking agents (82, 89). On the other hand, the effects of 5-HTP and LSD on the spinal reflexes are not significantly altered. These findings and the dissimilar actions of DOPA and 5-HTP indicate that NA and 5-HT stimulate different receptors.

The insulin-induced depletion of the adrenaline in the adrenals can be inhibited by treatment with 5-HTP but not by systemically given 5-HT.

This effect seems to be best explained by an inhibitory effect of 5-HT released from the nerve terminals to the preganglionic sympatho-adrenal neurons in the lateral horns (90).

(*b*) Corpus striatum.—The nigro-neostriatal DA neurons are completely uncrossed. Therefore the functional role of these neurons should be revealed by observing asymmetries following unilateral lesions. In monkeys, electrolytic lesions in the ventromedial tegmentum produce ipsilateral loss of neostriatal DA and contralateral hypokinesia and tremor (91). The observations are very similar to those seen in human hemiparkinsonism. In the rat, a lesion in the internal capsule or removal of the corpus striatum unilaterally by itself does not produce any clearcut asymmetry, perhaps because of involvement of other pathways to or from the corpus striatum (92). However, after treatment of unilaterally operated rats with DOPA or nialamide plus reserpine (after allowing time for the DA nerve terminals to degenerate) the animals rotate to the side of the lesion. This effect is presumably evoked from the neostriatum on the intact side because, on the operated side, there are no DA terminals from which DA can be released. Similar asymmetries are produced by apomorphine and amphetamine. Apomorphine seems to act directly on the DA receptors since its action is not reduced by depletion of the DA stores by reserpine plus a tyrosine hydroxylase inhibitor (93, 94). Apomorphine has no action on spinal reflexes and, thus, in all probability lacks effect on NA receptors. Amphetamine seems, however, to produce its striatal effects by release of DA since its activity is lost after the same pretreatment (*vide infra*).

If reserpine, haloperidol, or chlorpromazine is given to rats with a unilateral lesion of the nigro-neostriatal DA pathway an asymmetry opposite to that seen after DOPA is observed, i.e., the animals turn the head and the tail to the unoperated side (92). The effects are probably due to increased excitability in α- and decreased excitability in γ-motoneurons on the unoperated side (95). In intact rats these signs are seen on both sides after the drugs mentioned (96, 97). All the reserpine effects described are counteracted by treatment with DOPA. These actions of DOPA are inhibited by haloperidol and chlorpromazine without changes in the formation of DA. These findings indicate that haloperidol and chlorpromazine also block DA receptors. Phenoxybenzamine, promethazine, and barbiturates do not evoke any asymmetries or reduce the DOPA-induced ones and, thus, probably do not block the DA receptors. The inability of phenoxybenzamine to block the spinal but not the striatal actions of DOPA indicates that central NA and DA receptors are different.

Impulse flow in central monoamine neurons.—Since the axons of the central monoamine neurons are fine (about 1 μ) and mingled with many other nerve fibres it is probably very hard to record their action potentials electrophysiologically. A new possibility was presented when it was found in the spinal cord that the inhibitors of NA synthesis (e.g., the tyrosine hydroxylase inhibitor α-methyltyrosine methylester) or the 5-HT synthesis (e.g., the tryptophan hydroxylase inhibitor α-propyldopacetamide) depend

on nerve impulses for their monoamine-depleting action (57, 98, 99). These drugs cause a rather uniform monoamine loss throughout the intact spinal cord, as well as in the brain, indicating that the impulse flow is normally roughly the same in the different regions of the central nervous system (57, 100). In a transected spinal cord there is about the same monoamine reduction cranial to the lesion as normally, but caudal to it the monoamine concentrations are almost unchanged. Furthermore, electrical stimulation accelerates the monoamine disappearance induced by synthesis inhibitors both in peripheral and central tissues (101, 102). Therefore it appears that the synthesis inhibitors can be used to reveal changes in nerve impulse activity.

Another possibility for investigating the activity in the monoamine nerves is to determine the concentrations of some monoamine metabolites which cannot diffuse from the central nervous system to the blood easily. Such compounds are the 5-HT metabolite 5-hydroxyindoleacetic acid, the DA metabolites methoxytyramine, dihydroxyphenylacetic acid, and homovanillic acid, and the NA metabolite normetanephrine. It has been observed that caudal to a transection of the spinal cord there is a significant reduction of the 5-hydroxyindoleacetic acid (103) and of the normetanephrine accumulated after MAO inhibition (104).

Haloperidol and chlorpromazine enhance the accumulation of methoxytyramine and normetanephrine after MAO inhibition (105) and produce increases in the levels of the acid DA metabolites (106). These drugs also accelerate the loss of DA and especially NA in the brain induced by a tyrosine hydroxylase inhibitor (107). In the spinal cord this acceleration is observed only cranial to a transection, indicating that these drugs act by increasing the impulse flow in the monoamine neurons (89). Phenoxybenzamine has the same action as haloperidol and chlorpromazine on the NA but not on the DA turnover. Haloperidol and sometimes also chlorpromazine seem to increase the amine levels in some NA and DA cell bodies (108).

Apomorphine causes retardation of the DA, but not the NA, loss induced by a tyrosine hydroxylase inhibitor (94). Similarly, the 5-HT turnover studied by tryptophan hydroxylase inhibition is slowed down by LSD and some other hallucinogenic indole compounds (88). The chemical effects of apomorphine and LSD are dose- and time-related to the functional effects reported above.

Feedback control of synaptic activity.—There is an interesting correlation between these biochemical actions and the above mentioned effects of the various drugs on central monamine receptors. Thus, haloperidol and chlorpromazine appear to block central DA and NA receptors and to accelerate the turnover of these CA. For phenoxybenzamine a similar relationship holds true, though limited to NA. Apomorphine appears to stimulate DA receptors while inhibiting DA turnover selectively, and LSD to stimulate 5-HT receptors while inhibiting 5-HT turnover, again selectively. The conclusion appears inescapable that a causal relationship exists between the activity of the postsynaptic receptor and the turnover of the presynaptic transmitter. Since the latter seems to be mediated via the impulse flow in

the presynaptic fiber, it is tempting to suggest that this impulse flow is under the influence of a feedback mechanism, brought into play by changes in receptor activity.

Central monoamines and behaviour.—Many of the drugs interfering with the brain monoamine metabolism strikingly influence human mental functions and the behaviour of man and animals. These drugs can be used as tools for elucidating the role of monoamines for higher integrative brain functions. Although observations of gross behaviour have yielded valuable information, the fact that the neuroleptic drugs efficiently disrupt various conditioned responses (e.g., avoidance) without necessarily blocking the unconditioned responses (e.g., escape) in animals has proved particularly useful for obtaining accurate quantitative data on these relationships.

(a) Conditioned avoidance response.—Reserpine causes an almost complete disruption of conditioned avoidance responses in mice, rats, and cats, and the behaviour is restored after treatment with DOPA (109–111). The 5-HT precursor 5-HTP appears inefficient in this respect (112, 113). Further evidence for the view that catecholamines are of importance for conditioned avoidance responses is that the tyrosine hydroxylase inhibitor α-methyltyrosine also disrupts this behaviour (114–116). This agent influences the biosynthesis of DA and NA but not that of 5-HT. Treatment with DOPA restores the conditioned avoidance behaviour after α-methyltyrosine, as after reserpine.

If dexamphetamine is given after reserpine, the conditioned avoidance response recovers. However, if α-methyltyrosine is also administered beforehand, dexamphetamine fails to influence this behaviour and other central effects as well (*vide supra*) (117, 118). The restorative action of dexamphetamine reappears if DOPA is given in a dose which by itself is ineffective. These functional results indicate that dexamphetamine acts centrally as well as peripherally by releasing CA from nerve terminals. Presumably dexamphetamine can release CA from a small extragranular store which is immediately dependent on continuous CA biosynthesis in the central nervous system. This concept is strengthened by the chemical findings that dexamphetamine is a potent releaser of CA accumulated extragranularly in the CA nerve terminals (63).

(*b*) Gross behaviour.—The well-known sedation seen after reserpine treatment can be eliminated by the CA precursor DOPA but not by the 5-HT precursor 5-HTP (119, 120). Since DOPA induces formation of both DA and NA and since DA may stimulate both DA and NA receptors, it is not possible to determine by DOPA injection only whether the reserpine effects are caused by lack of DA or NA or both. Recently, however, two ways have been described to study selectively the importance of brain DA loss for the reserpine syndrome.

If the granules in the monoamine nerve terminals are surrounded with amines in high concentrations when reserpine is injected, reserpine will not be bound to the ATP-Mg^{++}-dependent uptake mechanism because of competition with the amines. The functional significance of such protection can be

revealed 24 hr after the reserpine injection when the immediate effects of the protective agent have worn off. In this way it is possible to protect the central DA and NA stores but not the 5-HT stores against reserpine by metatyramines formed after repeated injections of metatyrosine (121). The DA stores can be selectively protected if protriptyline is given before metatyrosine (121). Since protriptyline blocks the membrane pump of the NA but not of the DA neurons, the metatyramines formed in the NA neurons are rapidly lost when they leave the NA neurons and a sufficiently high concentration in the NA neurons is not achieved. In the DA neurons the protective action of metatyramine is naturally uninfluenced. Also the 5-HT stores can be selectively protected against reserpine by the 5-HT formed from 5-HTP (122). Experiments of this kind have shown that the gross reserpine syndrome is weak or absent in mice whose CA stores have been selectively protected against reserpine. DA appears to have a dominating influence in this respect (121).

As described above, apomorphine directly stimulates the central DA but not the NA receptors. Another way to stimulate the DA receptors selectively is to give DOPA or dexamphetamine after phenoxybenzamine, since phenoxybenzamine in all likelihood blocks the NA but not the DA receptors (*vide supra*). Such selective DA receptor stimulation after reserpine treatment produces a certain increase in spontaneous motility and stereotyped movements (123). The animals do not move in a normal way, e.g., they often fall from the edge of a table. After phenoxybenzamine pretreatment, DOPA and dexamphetamine do not produce hyperkinesia and aggressiveness. These signs may therefore be due to stimulation of central NA receptors. Injection of apomorphine, DOPA or dexamphetamine to reserpine-treated animals does not give the same almost normal behaviour as seen after selective protection of the DA stores against reserpine. This is not unexpected since in the latter case the amines are released in a physiological manner by means of the nerve impulses and, thus, feedback mechanisms can control the magnitude of the receptor stimulation. Naturally such a regulation does not occur after administration of apomorphine, DOPA or dexamphetamine and, therefore, the receptors are easily "overstimulated."

It can be concluded, both from the experiments with selective protection of the DA stores against reserpine and from the experiments with selective DA-receptor stimulation, that the DA loss is of importance for the gross syndrome after reserpine. Needless to say, it is likely that some less conspicuous effects of reserpine are caused by depletion of the NA and 5-HT stores.

General characteristics of the functional role of the central monoamine neurons.—As described above, monoamine neurons innervate practically all parts of the central nervous system. It has even been observed that an individual NA neuron may send axons to the cortex cerebri, the cerebellum, and the spinal cord as well (124). All the central monoamine neurons appear to be tonically active and to have a rather high turnover of the transmitters, to judge from the rather uniform and fast disappearance of the

monoamines after inhibition of their synthesis. These facts are probably the reason why the drugs influencing the monoamine neurotransmission have such widespread effects as on the central regulation of the autonomic nervous system (spinal cord, lower brain stem), on the motor function (spinal cord, neostriatum), on the endocrine system (hypothalamus), as well as on integrative mechanisms like conditioned avoidance response and mood (probably limbic system and cortex cerebri). The monoamine neuron systems may also normally react more or less as a unity, e.g., to increase the excitability of large parts of the central nervous system. On the other hand, the different monoamine neuron systems can also react differently to special stimuli, which is evident from the changes after the drugs which act selectively on one monoamine receptor type. No doubt further investigations of the monoamine mechanisms in the different central regions will give more informative answers to these important questions.

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