TRANSPORT AND STORAGE OF BIOGENIC AMINES 6538

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The remarkable evolution of knowledge of the metabolism, transport, and storage of biogenic amines has come about to a large extent because of studies with drugs that interact with these processes. There are few better examples of the contribution of pharmacologic research to basic biology.

Various aspects of biogenic amines and adrenergic mechanisms have been reviewed from time to time, and several excellent reviews may be found in previous issues of *Annual Review of Pharmacology* as well as other sources (1–8). The interested reader is referred to them.

The present review will restrict itself generally to aspects of biogenic amine transport and storage in nervous tissue and with the interactions of selected drugs with these mechanisms. The discussion will address itself only to the biogenic monoamines, that is, the catecholamines and 5-hydroxytryptamine (5HT). It will focus mostly on transport and storage of norepinephrine at the adrenergic neurone, as it appears that this system serves generally as a model of the mechanisms involved in transport and storage of the other monoamines, although, to be sure, differences arise. Amine biosynthesis and metabolism will not be touched upon except as pertinent to questions of transport and storage. As will be apparent to the reader, no attempt has been made to present a complete review of the vast literature on this subject.

Two distinctly separate but linked systems exist at the level of monoamine neurones: an intraneuronal amine concentrating-storage mechanism associated with amine granules, and a neurone membrane amine pump.

UPTAKE AND STORAGE BY AMINE GRANULES

Although norepinephrine (NE) is found throughout the adrenergic neurone, studies, mainly of peripheral adrenergic neurones, have revealed that this amine is present in highest concentration in the nerve terminal, with lesser amounts in the more proximal portion of the axon and in the cell body. The histochemical fluorescence technique, which allows the visualization of NE stores as well as other monoamines, reveals the presence of a filamentous network studded with enlargements or varicosities where NE is especially concentrated (see, for example, references 9, 10). These varicosities seem to be groupings of the amine storage granules or vesicles as seen

under the electron microscope or isolated by appropriate centrifugation techniques. The storage vesicles appear as small dense-cored granules of about 500Å, with less abundant larger granules of about twice that size (11–14).

Essentially the same picture is true for monoamine-containing neurones in the brain where the monoamines are located in granulated vesicles in highest concentration in the varicosities of nerve terminals (15–17).

The storage of catecholamines within adrenergic neuronal or adrenal medullary granules appears to be in association with ATP (18–20) and Ca^{+2} (21) to form a complex which, by nuclear magnetic resonance studies, appears to be a tetracatecholamine-ATP complex (20). This, in turn, may be bound to a granular soluble protein, chromogranin (22–26). In blood platelets, 5-hydroxytryptamine (5HT) is also found localized in granules in association with ATP (27–29). A most interesting biophysical model of ATP-amine complexes has been developed recently whereby it has been demonstrated that NE or 5HT in solution with ATP form spontaneous aggregates in the presence of small amounts of bivalent cations such as Ca^{+2} or Mg^{+2}. Formation of the NE-ATP aggregate is inhibited by tyramine or amphetamine and is also altered by small quantities of reserpine which appears to combine with and precipitate some of the system constituents (30).

Although generally a stable ratio of amine to ATP content is found in the storage granules, suggesting that ATP serves as a complexing agent, isolated adrenal medullary or splenic nerve granules also can take up a variety of amines such as NE, epinephrine (E), dopamine (DA) or 5HT, provided that ATP and either Mg^{+2} or Mn^{+2} are present in the incubation medium (31–34). Recent detailed investigation of the action of metabolic inhibitors on NE uptake by adrenergic nerve granules has led to the suggestion that an electron transport-coupled phosphorylation is involved in granular amine uptake (35).

Perhaps the most convincing evidence that the Mg^{+2}-ATP-dependent granular amine uptake has a role in vivo is the finding that low concentrations of reserpine, but not isoreserpine, inhibit amine uptake in this system (33). It remains somewhat surprising that the granular uptake system is so nonspecific in its selectivity, as the granular mechanisms are generally conceded to be the more structurally specific of the determinants of amine uptake and storage. It would seem, however, that granular amine uptake may be distinguishable from granular amine storage, the latter being more specific even though both functions of the granule are sensitive to reserpine.

There are a number of indications that amine transport and storage at the level of the granules may be separate, though related functions. For example, it has long been noted that the pharmacological effects of reserpine are not related temporally to tissue monoamine concentrations, but are far more closely related to the relatively short-lived reserpine-induced inhibition of granule amine uptake. Thus the Mg^{+2}-ATP-dependent amine accu-

mulation by rabbit adrenal medullary granules is inhibited at 24 hours after a large dose of reserpine, but recovers after 48 hours (33). Similarly, the ability of brain to retain injected NE or 5HT recovers within about this time, even though levels of endogenous amines remain severely depressed much longer (36). Recovery of animals from the gross behavioral and pharmacological effects of reserpine generally occurs within 48 hours.

Recent studies with labeled reserpine also support the concept that reserpine may affect two distinct but linked granular functions, granular amine uptake, and granular amine storage. For example, after administration of small quantities of ^{3}H-reserpine, two distinct phases of reserpine disappearance can be detected in several adrenergically innervated organs (37). The first is a relatively slow disappearance (half life about 12–18 hours) followed by a second phase of semipermanent reserpine binding lasting for many days. The gross pharmacological effects of reserpine correlate well temporally with the first phase, while the slow recovery of amine stores correlates well with the semi-permanent phase of reserpine binding. Still another indication that the more important of reserpine's pharmacological actions is relatively short-lived and is correlated with amine transport rather than with storage is recent work indicating that reserpine, even after large doses, inhibits, for less than two days, a Na^{+}-dependent amine uptake which may be operant intraneuronally since only very low Na^{+} concentrations are required for its action (38, 39).

Reserpine action on the granular uptake mechanism seems to be competitive with amines as the presence of high amine concentrations in the vicinity of the reserpine receptor site can compete with the drug, whereas once reserpine has interacted with the storage system, the latter remains inhibited even in the presence of amine. Thus, in vitro it has been noted that initial reserpine action on the Mg^{+2}-ATP granular uptake mechanism of adrenal medullary granules is competitive with catecholamines (33), and in vivo the flooding of a tissue with NE or the false adrenergic transmitter, metaraminol, inhibits, in part, the long-lasting effects of reserpine on amine storage (40, 41). After reserpine has acted, however, amine granules cannot be refilled. Again, studies with labeled reserpine support the likelihood that reserpine initially acts reversibly and later, irreversibly. Thus the injection of a large dose of unlabeled reserpine 18 hours after labeled reserpine administration dislodges a portion of the labeled drug, whereas when the injection of unlabeled drug occurs 36 hours after labeled reserpine, no labeled drug is dislodged (37).

The functional recovery of adrenergic neurones after reserpine, a recovery related to restoration of granular amine uptake function, is one of the arguments for the existence of a small functional amine pool important in neurotransmission. However, a portion of the recovery of uptake function may be attributed to the arrival in the nerve terminals of new, undamaged amine granules. In fact, it has been suggested that the arrival of new

granules is the major reason for restored uptake (42), but it is not clear as to how the arrival of a relatively few new granules could, by themselves, allow the almost complete restoration of uptake function.

Origin and life span of amine storage granules.—Studies combining histochemical fluorescence with nerve ligation experiments have been invaluable in the better understanding of the origin and life span of amine storage granules. Ligation of cat or rat sciatic nerve leads to a rapid accumulation of NE above the ligation, and, indeed, proximal to both ligations in a double ligation experiment (43, 44). These findings have been interpreted as indicating a proximal-distal flow of preformed amine storage granules which move from the site of granule synthesis in the neurone cell body to concentrate, normally, in nerve terminals. These conclusions are supported by observations that the specific proteins also known to occur in amine granules, namely chromogranin and dopamine-β-oxidase, also accumulate proximal to a nerve ligation (26). Calculation of the speed of proximal-distal axoplasmic granule flow has been estimated in the rat to be about 5 mm per hour with a granule life span of about 3 weeks.

It should be noted that the concept of axoplasmic flow of preformed amine granules is not universally accepted. It has been suggested, for example, that amine granules might be formed distally in the neurones from microtubules and that ligation might lead to the local disintegration of microtubules (45).

Based on these measurements of granule life span and the duration of reserpine effect on stored amine concentration, it has been suggested that after reserpine the affected granules do not recover their storage function, but full recovery of amine stores must await the downward transport of an entirely new complement of fresh granules, a process requiring many days (46, 47). This concept was questioned on the basis of the half-life of bound reserpine in adrenergically innervated tissues (48). Initial experiments with labeled reserpine showed a half-life of about 18 hours, and since this rate of disappearance of reserpine was more rapid than the rate of recovery of norepinephrine stores, it was suggested that after recovery from reserpine, amine storage granules may be reused. Reinvestigation with labeled reserpine of higher specific activity, however, has revealed the second semipermanent sojourn of reserpine as described above (37), a finding that supports the concept that reserpine permanently damages amine granule storage function by virtue of its presence in the affected granules, thus maintaining neuronal amine depletion until the organelles are finally replaced by new ones.

The questions of exocytosis and the functional pool.—The evidence for the quantal release of acetylcholine, and the indication that each quantal packet represents the transmitter content of a single storage vesicle, has naturally led to the suggestion that the same might hold true in the case of

adrenergic transmission. The possibility that the entire content of an amine granule might be released following nerve stimulation is supported by the observation that stimulation of the adrenal medulla leads to the release of catecholamines and AMP in the same ratio as catecholamine to ATP in the gland, and the distinctive soluble proteins of adrenal medullary amine granules, chromogranin and dopamine-β-oxidase, are similarly released (49–52). In addition, all of the enzyme seems to come from the soluble dopamine-β-oxidase of the storage vesicles and none from the particle-bound (49). These results suggest that release, in the adrenal gland at least, occurs by exocytosis. More complicated is the question of whether the entire content of a storage granule is discharged during the process or whether compartmentalization exists in the granule such that only a representative portion of the total granule content is released. On the basis of experiments on the adrenal gland, it has been suggested that the entire granular contents are released (49–52). However, in the case of the adrenergic neurone it has been calculated that only a small percent of the NE content of one granule corresponds to a single quantal packet (53), indicating that the quantum in this case may constitute only a small representative fraction of a granule, perhaps the single "functional" compartment of a granule. Nevertheless, it would appear that at least a partial exocytosis occurs in the case of neuronal NE release.

Regardless of whether amine granule contents are released fully or only in part following neurogenic stimulation, the amine granules would seem to be reutilized, since the turnover of NE in the adrenergic neurone is far more rapid than is the life span of the granule. Other evidence that the vesicle is not lost in its entirety may be found in experiments in vivo in which persistently bound reserpine, presumably bound to the granular membrane, is not affected by treatments such as insulin hypoglycemia, which cause intense neurogenic stimulation (54).

The evidence for compartmentalization of NE pools within a granule is in line with the concept of a "functional" or "available" NE pool that is more readily released by a nerve impulse or by indirectly acting agents such as amphetamine. There exists evidence that under certain circumstances newly synthesized or newly stored NE is more readily released by nerve stimulation than is older stored NE both in peripheral organs and in brain (55–58). For example, Kopin et al (56) showed that rapid nerve stimulation of spleen perfused with labeled tyrosine resulted in the appearance of labeled NE in the perfusate of higher specific activity than that retained in the organ. Schildkraut et al (58) showed recently that either spontaneously or after electroshock, a higher percent of intracisternally administered NE is converted to normetanephrine shortly after injection of the NE than at later times. Furthermore the marked ability of the tyrosine hydroxylase inhibitor, α-methyltyrosine, to inhibit amphetamine-induced locomotor activity and stereotypy in the rat, even without complete depletion of brain catecholamine stores (1, 59, 60), supports the hypothesis that newly formed NE is

uniquely important in neurotransmission. The inability of reserpine to inhibit amphetamine's central effects even in the face of severe catecholamine depletion from brain is considered to be further proof that the main catecholamine store is of secondary importance. While there is thus evidence that NE in nerve terminals may be present in more than a single pool, no picture of a small labile NE pool together with a semi-inert storage pool consistently applicable to all situations is yet available. There remain contradictory aspects. Thus recent studies (61) have led to the conclusion that during nerve stimulation it is the re-uptake of released NE, rather than newly synthesized NE, which is the major mechanism of the maintenance of neuronal NE concentrations, such re-use indicating that stored NE plays a major role in nerve function. Furthermore, if reserpine's central action generally lasts for less than two days, and this action is correlated with inhibition of amine uptake as described above and, perhaps more importantly, with inhibition of NE synthesis due to inhibition of uptake of DA into dopamine-β-oxidase-containing granules (62), then it is difficult to understand why reserpine does not act like α-methyltyrosine to inhibit amphetamine's central action since both drugs act, albeit by different means, to prevent NE synthesis and, presumably, to diminish a functional NE pool. It is possible, of course, that the action of amphetamine is largely related to DA, whose synthesis would not be directly altered by reserpine, but there is no evidence that only this amine is involved in amphetamine's central actions although recent findings suggest that after reserpine administration, DA may play the major role (63). Furthermore, reserpine itself may inhibit DA synthesis in DA neurones (64). That amphetamine's mechanism of central action may be more complex than solely via release of only newly synthesized catecholamines is apparent in studies in which low doses of α-methyltyrosine, sufficient to inhibit the locomotor stimulation induced by 2 to 4 mg/kg amphetamine in the rat, were, however, much less able to block the stimulation induced by only 1 mg/kg amphetamine (65).

In the adrenal medulla, where two catecholamine pools seem to exist, it has been suggested that the two pools correspond to two populations of amine granules (66).

Storage specificity of amine granules.—The remarkable specificity of the storage function of amine granules, as compared with the relative nonspecificity of the granular uptake function and, especially, of the neurone membrane amine pump (see below), may be seen in the rigorous structural requirements for storage of false adrenergic transmitters and displacement by them of NE from its storage site (40). Required for storage in adrenergic amine granules are phenylethylamines with a beta-hydroxyl group and at least one phenolic group. The molecule also must be of the correct optical configuration corresponding to that of *l*-NE. An example of storage structural specificity may be seen in the finding that of the four isomers of metaraminol, only the *l*-erythro (1R, 2S) isomer displaces NE and is stored as

a false transmitter (67, 68). The other three isomers, although taken up by the less selective membrane amine pump (39), do not displace NE from the storage granule and are not themselves stored. It would seem that the storage function, as opposed to the transport function of the amine granule or the membrane pump, is the absolute determinant of the nature of the amine stored such that NE, DA, and 5HT are stored exclusively in their respective neurones despite the marked similarities in neuronal transport systems. That there may, however, be transient storage of a spurious amine, presumably at a site not within a main storage pool, but available for release, may be seen in brain slice experiments where, after 6-hydroxydopamine treatment, DA formed from administered dopa, but not tyrosine, is released by electrical stimulation of the slice, suggesting the possibility that after dopa, DA may be temporarily stored in, perhaps, 5HT neurones in a releasable form (69). Furthermore, under certain conditions, 5HT may be taken up and bound in brain catecholamine neurones (70). It is conceivable that such temporary storage of a spurious substance may explain in part some of the findings described above which have been interpreted as evidence for multiple amine pools.

The Neurone Membrane Amine Pump

Perhaps more fully understood than are the transport and storage mechanisms of the amine granule is the neurone membrane amine transport system commonly referred to as the membrane amine pump. In the case of the adrenergic neurone, this system is responsible for the rapid uptake of NE in the vicinity of the neurone whether by virtue of its prior release from the nerve terminal or following injection.

The term amine pump may be a misnomer, since amine uptake across the neurone membrane occurs, largely at least, by virtue of the inward and downhill movement of Na^+ from high extracellular concentrations to low intracellular concentrations. Amine transport at the neurone membrane thus seems to be active in the sense that the cardiac glycoside-sensitive Na^+ pump forms the Na^+ concentration gradient required for the Na^+-dependent amine transfer system. It should be pointed out, however, that it has not been possible to promote membrane amine transport inward or outward, in the presence of a cardiac glycoside even in the face of a Na^+-gradient (71–73), suggesting that the Na^+-K^+ activated ATPase may also be involved in the function of the amine pump for purposes other than maintaining the Na^+-pump. It has been suggested that ouabain may inhibit amine transport by a mechanism not involving the Na^+-K^+-activated ATPase (71, 74).

The main adrenergic neurone membrane amine pump is Na^+-dependent, is inhibited by ouabain, cocaine, and tricyclic antidepressants, is generally insensitive to reserpine, and is relatively nonspecific in that it facilitates the inward transfer of many amines and fails to distinguish qualitatively between a number of stereoisomers of NE or its congeners (75–79), whereas the amine storage granules show a marked specificity as described above.

The inhibition by cocaine and tricyclic antidepressants is competitive in nature (80), and while secondary amine type tricyclic antidepressants such as desipramine seem to be more potent than tertiary amine types such as imipramine on the adrenergic neurone pump, recent evidence suggests that the converse exists in the case of the membrane amine pump of 5HT neurones (81, 82). Thus imipramine is more potent than desipramine in inhibiting the uptake of 5HT on administration of 5-hydroxytryptophan. Interestingly, the tricyclic antidepressants have little or no effect on amine uptake by central DA neurones (see below).

Although there is some evidence that tricyclic antidepressants in high concentration may have an intraneuronal action on adrenergic storage granules to effect amine release or to block intraneuronal amine binding (83), it is generally believed that such action is, at most, minor as compared with the potent action of these drugs on the neurone membrane pump.

It is generally accepted that the membrane amine pump is of primary importance in terminating the action of released or injected NE, and blockade of the amine pump greatly potentiates injected NE. Such a functional role for the amine pump may not occur in every situation. It has been shown, for example, that blockade of the adrenergic membrane pump of the Auerbach's plexus-longitudinal smooth muscle preparation of the guinea pig does not result in a potentiation of the action of NE even though there is present a powerful amine pump that appears to be entirely similar to the amine pump found in other preparations (84). This failure to potentiate has been attributed to the greater size of the synaptic cleft in this system, and it has been estimated, accordingly, that the effective radius of the adrenergic membrane amine pump may be about 600–800Å. Thus the synaptic cleft of the adrenergic neurone-intestinal muscle cell is about 1000–3000Å, and no potentiation results upon blockade of this system, whereas in the nictitating membrane, an organ exhibiting considerable supersensitivity after blockade of the membrane pump, the width of the synaptic cleft appears to be closer to 500Å, well within the estimated effective radius of the pump.

As mentioned above, the membrane amine pump shows an absolute Na^+ dependency. The system also requires the presence of K^+ although other ions such as Rb^+ or Cs^+ can substitute (73). The K^+ dependency appears to be derived from a requirement for this ion in the action of the sodium pump. Excess K^+ also can inhibit monoamine uptake by the adrenergic neurone, and kinetic studies suggest that this comes about by competition of excess K^+ for Na^+ in the coupled Na^+-amine carrier (85).

It has been assumed (86) that the Na^+-dependent amine pump is of the allosteric type found most commonly in the Na^+-dependent transport of sugars and amino acids (87, 88), that is, a system in which affinity of the carrier for the amine is high on the outside of the cell but low on the inside due to the influence of the markedly different Na^+ concentrations on carrier configuration. This does not seem to be the case in those adrenergic systems analyzed, however (38, 88a). Thus, in preparations of rabbit heart or guinea pig small intestine, alteration of the medium Na^+ concentration

changes maximal velocity of the amine pump, but does not affect Km, indicating that the system is of a coupled or stoichiometric type in which, presumably, a ternary system of Na^+, amine, and carrier passes inward across the membrane.

Similar studies have revealed the presence of a second Na^+-dependent amine pump of high amine selectivity, which is also reserpine sensitive (38, 39, 88a). Thus, plotting the rate of accumulation by rabbit heart slices of the four isomers of metaraminol against various Na^+ concentrations revealed that *d*-erythrometaraminol (1S, 2R) and the two threo isomers (1S, 2S; 1R, 2R) are taken up in a monophasic fashion, whereas *l*-erythro-metaraminol (1R, 2S) shows a definite biphasic curve. Treatment with reserpine, but not guanethidine, converts this biphasic response to a monophasic one, but does not affect movement of the other three isomers. Thus, after reserpine treatment, all the isomers are taken up in a qualitatively similar fashion. The optically specific and reserpine-sensitive amine uptake remains Na^+ dependent, but requires only a low Na^+ concentration of 20 to 30 mM. That a different system is operant in the case of the specific amine pump is suggested by the finding that in rabbit heart the ED_{50} of desipramine is higher than that required to block the nonspecific membrane pump.

The significance of these studies is not entirely clear since the requirement for only a low Na^+ concentration such as would obtain intracellularly suggests that the system could operate intraneuronally, but the nature of the experimental design would suggest that amine uptake at the level of the neurone membrane is being altered by the absolute removal of Na^+ or by the presence of reserpine at a low Na^+ concentration. It may be that there exist amine storage particles in or in close proximity to the neurone membrane such that amines pass essentially directly into or onto amine granules. It is tempting to think that such could represent a functional amine pool separate from the main granule storage pool. The short-lived action of reserpine on the optically specific uptake system and the concept of compartmentalization as described above are consistent with this possibility.

The importance of the membrane amine pump as a major factor responsible for termination of the action of norepinephrine is accepted by most investigators although this opinion is not unanimous (89). It is interesting to speculate that the amine pump may be operant between nerve impulses but be turned off during depolarization so as to allow transmitter diffusion across the synapse. Such an inhibition of the amine pump could come about by an alteration of the local ionic environment, possibly due to the influence of calcium ions (90) or possibly by the inhibitory effect of the transiently high potassium concentration in the immediate vicinity of the nerve terminal, an inhibition which would dissipate as K^+ levels at the outside of the neurone return to normal during repolarization.

Amine Transport and Storage in Dopamine Neurones and in Platelets

As stated above, the current evidence is that the transport and storage systems for the various biogenic monoamines are remarkably similar. Thus,

like the adrenergic neurone, striatal dopamine neurones seem to possess a neurone membrane amine pump and an intraneuronal storage mechanism as revealed by histochemical fluorescence techniques and by biochemical investigations (91–93). Like its adrenergic counterpart, the striatal dopamine membrane pump is Na^+-dependent and ouabain-sensitive (93). It differs from the adrenergic neurone in that desipramine, a potent competitive inhibitor of the adrenergic amine pump, is almost without effect on its dopaminergic counterpart (91–94). The dopamine neurone amine pump also differs from the adrenergic system in that, compared with the latter, the dopaminergic amine pump shows even less sterospecificy (94). Thus, uptake by synaptosomes of rat cortex or hypothalamus shows a preference for *l*-NE over *d*, and *d*-amphetamine is reported to be a more potent inhibitor of this amine pump than is *l*-amphetamine. In striatal synaptosomes, however, no optical specificity was noted in the case of NE uptake, and *l*-amphetamine was as active as *d* as an inhibitor. It is interesting to note that the membrane pumps of both systems are inhibited by a variety of antiparkinsonism drugs, the antihistamine type as well as the anticholinergic type, and it has been suggested that such actions on striatal dopamine transport may play a role in their antiparkinsonism action (95). Intraneuronal uptake and/or storage in both adrenergic and dopaminergic systems are similar in that both are highly reserpine sensitive, but the systems obviously differ in their most crucial sense, the stereospecificity of the storage system. Comment with regard to other drug actions on dopamine neurones will be found in a later section.

In platelets, 5HT uptake and storage mechanisms also appear similar to the adrenergic system. Thus, 5HT is localized in platelet granules along with ATP, and granular uptake and storage are reserpine sensitive, while the platelet membrane amine pump is Na^+-dependent and ouabain-sensitive (*27*, 96–100). The major feature of the platelet system is its specificity for 5HT storage, illustrating again the ultimate specificity of the storage system.

Drugs Affecting Amine Transport and Storage Systems

Reserpine, as described above, acts primarily on amine granule uptake and storage functions, the former being reversibly inhibited by reserpine, while the latter is irreversibly inhibited in association with irreversible reserpine binding; the drug also inhibits a highly selective neuronal uptake system unmasked at low sodium concentrations. In peripheral organs, persistently bound reserpine appears to localize in adrenergic neurones (48, 54), presumably mainly at the membrane of amine granules (54), although very shortly after administration the drug may be localized subcellularly simply according to the lipid content of cellular constituents (101). In rabbit platelets, the drug has been found to be associated with 5HT granules (*27*). In the brain also, reserpine presumably localizes in monoamine neurones, but a study of the disposition of labeled reserpine in brain did not reveal a re-

gional distribution consistent with the concentration of any one or a combination of the three brain monoamines (102). It has been suggested that there may exist monoamine neurones in the brain that are relatively deficient in their total of stored amine. Such a possibility is also indicated by the finding that NE is taken up as effectively by slices from NE-deficient brain regions as by slices from NE-rich brain areas (103).

6-Hydoxydopamine.—A remarkable tool for research on the adrenergic neurone is realized in 6-hydroxydopamine, which, in sufficient dosage, leads to selective destruction of adrenergic nerve endings (104–108). Initial experiments on NE depletion following 6-hydroxydopamine treatment led to the suggestion that the compound depleted via the false transmitter route or via damage to intraneuronal amine storage granules. More recent work, however, has established that granular uptake of 6-hydroxydopamine is not necessary for its action in destroying adrenergic nerve terminals, but uptake into the neurone via the amine pump is required (104, 108). Thus reserpine, which inhibits uptake by the granules, does not inhibit 6-hydroxydopamine-induced chemical sympathectomy, but imipramine, a membrane pump inhibitor, blocks this action. Thus it appears that the selective action of this compound results from its rapid accumulation in adrenergic nerve terminals followed, provided there is sufficient concentration of the drug, by destruction of the terminals. Not all adrenergic neurones are affected similarly, the sensitivity depending on blood supply and the local density of adrenergic innervation (107). The effect of 6-hydroxydopamine is slowly reversible, NE concentrations returning to normal in the cat about 14 weeks after drug administration. As might be expected, adrenergic nerve function recovers more rapidly (106).

Recently an explanation of the biochemical action of 6-hydroxydopamine has come forth (109) in studies demonstrating that oxidation products of this substance, which form rapidly at physiological pH, combine covalently with nucleophilic groups to effect irreversible alteration of protein structure. Thus treatment of serum albumin with labeled 6-hydroxydopamine at pH 7.4 resulted in covalent bonding of radioactivity with the protein, a binding which was markedly inhibited either by prevention of 6-hydroxydopamine oxidation or by prior masking of nucleophilic groups by acetylation. An alternative proposal to explain the destructive action of the drug on adrenergic neurones is that hydrogen peroxide generated by the reaction of 6-hydroxydopamine with molecular oxygen may be the damaging agent (110). In either case the physiological specificity on nerve terminals of this nonspecific reaction is explainable by the selective localization of 6-hydroxydopamine in adrenergic nerve terminals.

Given peripherally, 6-hydroxydopamine does not affect central neurones, due to the drug's inability to cross the blood-brain barrier. When the drug is given intracisternally, however, central NE neurones are affected similarly to peripheral neurones (105). Surprisingly, 6-hydroxydopamine has consid-

erably less effect on striatal DA neurones and no effect on 5HT neurones (105, 108). It is not now possible to explain this selectivity for NE neurones, especially in light of the current view that the membrane pump of the various monamine neurones is relatively nonselective and that intraneuronal granular uptake of 6-hydroxydopamine is not a requirement for its action on adrenergic nerve terminals.

Adrenergic neurone blocking agents.—These compounds are defined as drugs that, without depletion of the neurotransmitter, act on the postganglionic sympathetic neurone to inhibit the ability of a nerve impulse from releasing NE (111). These drugs, which do not act centrally as they enter the brain only slightly, if at all, include the prototype drug bretylium as well as debrisoquin, bethanadine, and guanisoquin. The action of these drugs has been aptly described as akin to local anesthetic action at the adrenergic neurone, and indeed a local anesthetic action of some of these agents has been demonstrated. The specificity of action of bretylium on peripheral adrenergic function has been attributed to the specific localization of the drug at the adrenergic neurone.

More recently it was found that although these agents at high concentration in vitro act as inhibitors of the adrenergic membrane pump, at low concentrations they actually promote the retention in neurones of amines that are MAO substrates (112). As all of the compounds mentioned above are only weak to moderately strong MAO inhibitors, it was suggested that the drugs must accumulate specifically in the adrenergic neurone in sufficient concentration to act as functionally specific adrenergic neurone MAO inhibitors (112, 113). Studies on the disposition of debrisoquin indicate that sufficient drug remains in adrenergically innervated tissues to inhibit neurone MAO during the antihypertensive activity of the drug even after frank neuronal blocking action has ceased, suggesting that specific neuronal MAO blockade may play a role in the antihypertensive action of these compounds (114). Recent studies in man with antihypertensive doses of debrisoquin showed conclusively that the drug inhibits adrenergic neuronal MAO but not kidney or intestine MAO (115). Thus the drug caused an increase in urinary excretion of methylated NE metabolites, a decrease in VMA excretion and increased tyramine responses. There was, however, no enhancement of tryptamine excretion, a measure of kidney MAO, nor did a sampling of intestinal mucosa show MAO blockade in this tissue.

More recently it was demonstrated that the neurone blocking action of bretylium is associated with a lowering of the amount of NE found in the soluble fraction of a homogenate of spleen, a lowering which, along with neurone blockade, is blocked or reversed by *d*-amphetamine, suggesting that in the presence of bretylium, a functional NE pool cannot be replenished from the main store (116). In the case of debrisoquin, however, evidence has been presented that this drug may interfere with NE granular storage, thus allowing an increase in cytoplasmic amine which is protected from the action of MAO by the enzyme-blocking action of the drug (117).

Guanethidine.—This antihypertensive drug combines the adrenergic neuronal blocking action of bretylium with the depleting action of reserpine. In high concentration, the drug also inhibits the membrane amine pump, although this action would not be seen after usual antihypertensive dosage. Guanethidine, like the neuronal blocking agents, gains entry into the adrenergic neurone by the membrane pump. Guanethidine has no MAO inhibitory action, and thus even though it accumulates in the neurone, it does not block MAO. It is interesting to speculate that if guanethidine were an MAO inhibitor it might retain antihypertensive activity via MAO inhibition. Guanethidine, given peripherally, has no direct central action as it does not cross the blood-brain barrier. After injection into the brain, however, the drug lowers brain NE (118).

An interesting example of drug interaction may be seen in man in the action of tricyclic antidepressants or amphetamine, both of which act on the membrane pump to inhibit the antihypertensive action of guanethidine by blocking accumulation of this drug in the neurone (119).

False transmitters.—The subject of false transmitters was reviewed extensively in 1968 by Kopin (4). Most of the investigation at that time had concerned itself with false transmitters in the peripheral adrenergic nervous system. More recently it has been found that a false adrenergic transmitter is formed in certain species after amphetamine administration. Thus, in the rat, p-hydroxynorephedrine is formed by parahydroxylation of amphetamine followed by beta hydroxylation. This compound (the *l* isomer) has the necessary structural characteristics for storage in the adrenergic amine storage granules, and is associated with persistent NE depletion following administration of amphetamine to the rat (120, 121).

Recent evidence suggests that, just as metaraminol and α-methylnorepinephrine serve as false transmitters in the adrenergic neurone, α-methyl-m-tyramine (and presumably α-methyldopamine) may act as false dopaminergic transmitters. Thus after α-methyl-m-tyrosine administration, α-methyl-m-tyramine selectively accumulates in the striatum of rat or rabbit, while its β-hydroxylated product, metaraminol, accumulates in the hypothalamus in association with lowering of DA and NE levels respectively (122). While in the rat, at least, much of the striatal dopamine lowering after α-methyl-m-tyrosine administration may be a consequence of synthesis inhibition (123), nevertheless the marked selectivity of localization of α-methyl-m-tyramine in the striatum, a prolonged localization in the case of the rabbit, argues for a false transmitter role of this substance. α-Methyl-m-tyramine is released in vivo from the rat striatum by amphetamine and by amfonelic acid (124), the latter compound being a marked CNS stimulant that causes amphetamine-like central stimulation but does not share amphetamine's cardiovascular actions (125, 126).

Lithium.—The observation that lithium is of benefit in the treatment of manic patients has naturally led to investigation of the possible interaction of lithium ions with amine transport and storage mechanisms as well as

with amine metabolism. Recent thorough reviews of the pharmacology of lithium have been presented by Davis & Fann (127) and by Gershon (128). With specific regard to interactions with amine transport and storage mechanisms, perhaps the most relevant findings are that Li^+, even though it cannot substitute for Na^+ in the functioning of the membrane pump, nevertheless appears to enhance the rate of NE uptake into isolated synaptosomes (129) or of 5HT into platelets (130). Treatment with Li^+, however, also has been reported to enhance (131) or inhibit (132) NE release from brain. Clearly, no coherent picture emerges at the present time as to a clinically relevant interaction of Li^+ with amine transport and storage systems in the brain. It is interesting to note that another alkali metal ion, Rb^+, has a number of biochemical effects opposite those of Li^+ and also seems to elicit contrasting behavioral actions (133, 134).

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