PHYSIOLOGIC AND PHARMACOLOGIC CONSIDERATIONS OF BIOGENIC AMINES IN THE NERVOUS SYSTEM[1,2]

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In little more than a decade, pharmacologic investigations of the metabolism and disposition of neurohumors in the brain have become one of the most important and productive approaches to the problem of linking brain chemistry and function. Research activity that began with investigations of the effects of a single drug, reserpine, upon levels of serotonin and norepinephrine in whole brain (1–5) has flourished into studies of a broad spectrum of compounds capable of influencing all aspects of the "life cycle" of biogenic amines in brain and, for that matter, in other tissues. The fact that a drug like reserpine alters mood, affect, and behavior in man, and also changes the capacity of brain to store its biogenic amines has an obviously compelling quality which invites speculation [c.f. review (6)] on the role of these amines in certain aspects of brain function. It is now recognized that a variety of psycho-active drugs can evoke changes in brain amines (7). From a patho-physiological standpoint (8), special significance is attached to brain amines with regard to causal or contributory relationship of the altered brain chemistry to clinically encountered states of brain dysfunction [e.g. phenylketonuria (8, 9)].

This is a review of the effect of chemical agents on biogenic amines with special reference to brain. In view of the explosion of interest, data, and reviews (6, 7, 10–35) in this area, it was essential for us to be selective at the expense of being completely inclusive. Accordingly, the biogenic amines which we have included are the catecholamines (NE and DA) and serotonin (5-HT). Acetylcholine, which differs from the above amines in many characteristics, has been excluded, as have the polypeptides and neuro-active amino acids. A recent review on acetycholine and central cholinergic mechanisms is available (36). In the case of histamine, relatively little exploration in the nervous system has been done (37). With the advent of more suitable

[1] The survey of the literature pertaining to this review was completed in August, 1967.

[2] The following abbreviations are used: αMMT (alpha methyl-*m*-tyrosine), αMPT (alpha methyl-*p*-tyrosine), COMT (catechol-O-methyltransferase), CNS (central nervous system), CPZ (chlorpromazine), DA (DOPAmine), DMI (desmethylimipramine), DOPA (dihydroxyphenylalanine), 5HIAA (5-hydroxyindoleacetic acid), 5-HT (serotonin), 5-HTP (5-hydroxytryptophan), MAO (monoamine oxidase), NE (norepinephrine), *p*CPA (*p*-Cl phenylalanine).

analytical methods for histamine (38, 39) involving purification on ion-exchange columns (38), interesting data have begun to appear (40, 41).

In order to comprehend the scope of drugs capable of altering biogenic amines and the possible sites and mechanisms of their actions, it is necessary to review our contemporary knowledge encompassing the chemical steps from the synthesis of an amine to its release and the dissipation of its effect. Very briefly, the following stages are thought to constitute the life cycle of a biogenic amine in brain (12–14, 19, 22–24).

1. Active transport of the precursor amino acid (e.g. tyrosine and tryptophan) across the blood brain barrier and presumably across the neuronal cell membrane, catalyzed by appropriate permeases.
2. Sequential biosynthesis of the amine in the neuron by hydroxylation and decarboxylation and subsequent β-oxidation (in the case of DOPA-mine).
3. Uptake of the amine by a membrane-bound granule, and storage by association with some material, which serves as the binding substance. The amine in this form is inactive and exists in dynamic equilibrium with other pools of amine in the neuron that have not yet been morphologically defined.
4. Physiologic release of the amine into an "active" form free to combine with proteins (such as monoamine oxidase (MAO)) of special affinity for the amine, or to diffuse out of the cell.
5. Interaction of the amine with a specific receptor, initiating a physiologic response.
6. Dissociation from the receptor site and termination of the effect by either enzymatic destruction, re-uptake of the amine by the cell, diffusion away from the area, or combination with non-specific protein.

Within the context of these six steps, a drug may have multiple sites of action and the agents mentioned in this review will be discussed with reference to these sites. Since a wide variety of chemical agents influence monoamines in the nervous system, it was necessary to limit our attention to those compounds with a somewhat clearly defined effect on monoamine metabolism, and to those drugs of major importance, such as reserpine, amphetamine, chlorpromazine, etc., for which a large body of data has accumulated with respect to their interaction with monoamines in the nervous system.

Transport of Precursor Amino Acids to Sites of the Biosynthetic Enzymes

While it is generally agreed that the hydroxylation steps in the synthesis of 5-HT from tryptophan and NE from tyrosine are rate-limiting (*vide infra*), in order that these steps may take place, the appropriate amino acids must move from the circulation to the intracellular sites of the enzymes. There is also general agreement that amino acids are transported across certain anatomic barriers in various tissues including brain by catalytic mechanisms or permeases which are rapid, selective, and stereospecific (42).

There is evidence that these permeases are quite non-specific and that certain amino acids may be grouped according to the way they are handled by a single transport system (43). The significance of this finding is that within groups of amino acids each individual amino acid can compete with all the others for a given permease. This may find pathophysiologic importance in those metabolic disorders which lead to aminoacidemias and resulting imbalances in circulating amino acids. It is conceivable that such conditions as phenylketonuria, histidinemia, "maple sugar urine" disease, and branched chain aminoaciduria (44) may be associated with altered monoamine levels in brain by virtue of competition with appropriate precursor amino acids at the blood-brain barrier, or by an elevation of the blood levels of appropriate precursor amino acids presented to the transport system. In the former case, brain monoamine levels would tend to decrease, while in the latter they would increase.

However, there is no unanimous agreement that high blood levels of phenylalanine lower 5-HT in brain simply by antagonism of the transport of tryptophan and 5-hydroxytryptophan. An earlier explanation was based on the demonstrated inhibition of 5-hydroxytryptophan decarboxylase *in vivo* by the keto-acid metabolites of phenylalanine, such as phenylpyruvic acid (45). Sandler and his co-workers (46) later confirmed this inhibition by studies *in vivo*. More recently, Grahame-Smith & Maloney (47) demonstrated that phenylalanine, itself, inhibits tryptophan hydroxylation in homogenates of whole brain. Since Nakamura et al. (48) had shown that partially purified tryptophan hydroxylase from brain is not inhibited by phenylalanine, Grahame-Smith & Maloney (47) suggested that the inhibition they observed occurs through inhibition of tryptophan transfer across the membrane of the nerve-ending particle, thus reducing net hydroxylation but not affecting the mitochondrial or soluble enzyme. A clear demonstration that high circulating levels of phenylalanine can reduce the uptake of 5-hydroxytryptophan-1-C^{14} into brain *in vivo* has already been presented (49)

By increasing levels of certain amino acids in the diet, and, therefore, in the circulation, experimental models of some of the above-mentioned metabolic disorders have been studied, and altered monoamine levels in brain have been correlated with deficits in "learning" or in "learned performance." Considerable evidence has been accumulated to demonstrate, for example, an impairment of maze performance subsequent to the administration of excessive dietary phenylalanine (49–54).

Behavioral effects have been ascribed to other amino acids when present in high concentration in blood. Leucine has been associated with a severe degree of mental retardation in humans (55), and reversible changes in behavior have been noted in normal subjects after oral administration of high levels of L-tryptophan (56). Whether the well established close correlation between altered brain monoamines and altered behavior in these aminoacidemias is a cause and effect relationship must still be ascertained. Data supporting this relationship are impressive. Phenylalanine (50) and leucine

(57) have both been shown to reduce 5-HT levels in brain, while tryptophan increases the brain content of this amine (58). McKean et al. (51) have shown recently that a good correlation exists between quality of maze performance and brain content of 5-HT when the amine is altered by the above three amino acids. In this work, phenylalanine and leucine were associated with poor maze performance, while tryptophan resulted in maze performance superior to controls and reversed the behavioral deficit produced by the other two amino acids.

To date, no chemical compounds classifiable as drugs other than amino acids have been discovered which inhibit the transport of amino acid precursors of amines into the nervous system (an example of such a drug effect is hemicholinium which inhibits access of choline to choline acetylase in nerves, thereby reducing acetylcholine synthesis). A large number of potent psychotropic drugs were shown to be without influence upon the transport of 5-hydroxytryptophan, which is believed to be handled by the same system that transports tryptophan, phenylalanine, leucine, isoleucine, proline and, possibly, alanine (59, 60). It is probable, however, that some drugs will be found to inhibit the amino acid permeases. At present, the most predictable structures to have such an action would be analogues of the amino acids. In this category, the tryptophan and phenylalanine hydroxylase inhibitor, *p*-chlorophenylalanine, and the phenylalanine hydroxylase inhibitor, *p*-fluorophenylalanine, should be examined. Jequier et al. (61) have concluded from a detailed study that inhibition of tryptophan hydroxylase by *p*-chlorophenylalanine can be correlated with, and is assumed to be responsible for, depletion of brain 5-HT (*vide infra*). On the other hand, Koe & Weissman (62) have shown that *p*-chlorophenylalanine reduces the increase in 5-HT after loading with 5-hydroxytryptophan, and since these investigators have demonstrated that *p*-chlorophenylalanine does not affect the decarboxylating enzyme, this effect can be interpreted as a suppression of entry of 5-HTP to the metabolic site. Parenthetically, *p*-chlorophenylalanine has been proven to produce the most authentic chemical model of phenylketonuria in rats, since it causes marked elevation of blood and tissue phenylalanine without an increase in tyrosine (63).

Biosynthesis of Amines in the Nervous System

Tyrosine hydroxylase.—First demonstrated in extracts of brain (64, 65) and adrenal medulla (64), this enzyme, which is stereo-specific for L-tyrosine was shown to be associated with particles which sediment with mitochondria (66). A soluble enzyme has been prepared from beef adrenals and purified 400 times (34). The hydroxylase activity in homogenates of bovine splenic nerve does not seem to be particle-bound either (67). The purified hydroxylase requires a tetrahydropteridine co-factor, probably dihydrobiopterin (34), which also functions as a co-factor for phenylalanine hydroxylase (68). This enzyme does not oxidize tyramine, *m*-tyrosine or tryptophan and is inhibited, competitively by many analogues of tyrosine. Perhaps

the best known of these is α-methyl-*p*-tyrosine, which has been shown to inhibit the enzyme *in vivo* and lead to a reduction in brain catecholamines (69). Reduction in catecholamine synthesis by as much as 70 per cent has been achieved in man with the oral administration of α-methyl-*p*-tyrosine (70, 71). Significant reduction in blood pressure and improvement of the general clinical state have been achieved in patients with pheochromocytoma, but not in patients with essential hypertension (70, 71). In animal experimentation, α-methyl-*p*-tyrosine has proved to be a very useful pharmacological tool for the selective depletion of brain catecholamines. Through its use, it has been possible, for example, to provide evidence that the central action of amphetamine-like stimulants requires unimpaired biosynthesis of catecholamines in brain [(72–77); *vide infra*].

Inhibition of tyrosine hydroxylase is clearly the most effective means of blocking norepinephrine synthesis, since it is now well-established that this enzyme is rate-limiting in the biosynthesis of catecholamines. In a recent careful analysis of this question, Udenfriend et al. (78) compared inhibitors of tyrosine hydroxylase, DOPA decarboxylase and DOPAmine-β-oxidase with respect to conversion of tyrosine-^{14}C and DOPA-^{3}H to norepinephrine *in vivo*. The tyrosine hydroxylase inhibitors, α-methyl-*p*-tyrosine and α-methylphenylalanine, were found to be most effective in blocking formation of NE from tyrosine. Lowering of NE levels in various tissues of the guinea pig by α-methyl-*p*-tyrosine was found to be directly related to the degree of inhibition of tyrosine hydroxylase. In view of this, it is perhaps surprising that α-methyl-*p*-tyrosine failed to control the blood pressure of patients with essential hypertension (70, 71). This may indicate that essential hypertension is more related to increased sensitivity of vascular adrenergic receptors than to the size of stores of endogenous NE, if indeed the adrenergic system is involved at all.

Presumably because of the difficulty in solubilizing this amino acid analogue, nephrotoxicity has been encountered after the administration of large doses of α-methyl-*p*-tyrosine (79). It was, therefore, of great interest to find that the readily soluble methyl ester of DL-α-methyltyrosine [H-44/68] also blocked catecholamine biosynthesis (80). The sedation seen with H-44/68 was reversed with administration of small doses of L-DOPA (80), and the effect could be correlated with a return of histochemically demonstrable fluorescence in the catecholamine neurons. Disappearance of catecholamines after H-44/68 was accelerated by such psychotropic drugs as haloperidol and chlorpromazine, a fact which is interpreted to mean that these drugs activate central adrenergic neurons (81). The selective decrease of NE and DA content of the brains of mice and rats induced by H-44/68 is accompanied by a lowered conditioned avoidance response in trained animals (80). This type of alteration in the conditioned avoidance response seems to be a common feature in the action of these drugs, since it was shown recently than an analogous tyrosine hydroxylase inhibitor 3, α-dimethyltyrosine methyl ester (H-59/64) produced similar effects (74, 75).

Carlsson, Corrodi and their colleagues have also developed a series of α-substituted DOPAcetamides as potential inhibitors of catechol-O-methyl transferase and the α-propyl-derivative (H-22/54) appeared to be the most potent (82). However, in mice treated with an MAO inhibitor (nialamide) H-22/54 completely blocked the CNS stimulation usually seen after MAO-inhibition, and the usual elevation in 5-HT levels was not seen. Since decarboxylation of aromatic L-amino acids is not inhibited by H-22/54 and the DOPAcetamides do not release biogenic amines, it was assumed that both tryptophan and tyrosine hydroxylase were blocked. Subsequent work has established that H-22/54, like certain catecholamines, inhibits tyrosine hydroxylase by competing with the pteridine cofactor (83).

Another interesting series of tyrosine hydroxylase inhibitors is a group of iodinated tyrosine derivatives. The most potent inhibitor yet found is α-methyl-3-iodotyrosine, which has a $K_i = 10^{-7} M$ (34). In addition, the normal intermediates of thyroglobulin metabolism, 3-iodotyrosine and 3,5-diiodotyrosine, are also extremely potent inhibitors of the hydroxylase (84, 85). Goldstein et al. (86) showed that 3-iodotyrosine can, in fact, cause considerable reduction in NE content of brain, heart, salivary, and adrenal glands of the rat. In view of these observations, it is tempting to speculate on a possible thyroid-sympathetic nervous system interaction (33).

Since the requirements for tyrosine hydroxylase and the technique for its assay have only recently become available, the tempo of activity with respect to new inhibitors will increase (87, 88). The most recent series of potent inhibitors consists of phenylcarbonyl derivatives containing catechol or triphenolic ring systems (89).

Tryptophan hydroxylase.—Until 1965, little was known of the enzyme that catalyzes the hydroxylation of tryptophan in normal mammalian tissues. Renson et al. (90) had suggested that although phenylalanine hydroxylase of liver is capable of hydroxylating tryptophan, this enzyme is not responsible for the bulk of 5-hydroxyindoles produced *in vivo*. In 1965, Lovenberg et al. (91) succeeded in demonstrating tyrptophan hydroxylase activity in cell-free extracts of murine mastocytoma cells, and showed that the enzyme has an absolute requirement for reduced pteridine and ferrous ion.

Despite the demonstration of its presence in peripheral tissues, some question was raised about the importance of tryptophan hydroxylase for the biosynthesis of 5-HT in brain. Grahame-Smith (92) reported that brain tissue contains tryptophan hydroxylase but at a very low level of activity. Gal et al. (93) also suggested that the rate of tryptophan hydroxylation in brain is too low to account for its 5-HT content. However, with the development of a sensitive radio-assay (94) for tryptophan hydroxylase activity, Jequier et al. (61) were able to show a considerably higher rate of hydroxylation in rat brainstem (ca. 1 μg 5-HTP formed per gram brainstem per hr), which appears sufficient to account for 5-HT levels normally found in brain.

Without question, the most interesting inhibitor of tryptophan hydroxylase now available is *p*-chlorophenylalanine (PCPA). In a relatively com-

plete pharmacological study of this compound, Koe & Weissman (62) found that it is a specific depletor of brain 5-HT in mice, rats, and dogs. These investigators showed that PCPA did not inhibit MAO or 5-HTP decarboxylase *in vitro*, nor did it affect these enzymes in rat tissues *in vivo*. In contrast, it did inhibit liver tryptophan hydroxylase *in vitro* and strongly suppressed tryptophan and phenylalanine hydroxylating activity in rat tissues *in vivo*. These results suggested that PCPA causes 5-HT depletion by inhibiting its biosynthesis at the tryptophan hydroxylase step. However, the possibility of some blockade by PCPA of tryptophan or 5-HTP uptake by brain was not eliminated and was indeed, suggested by some of the data (*vide supra*, section on Uptake of Precursors). The relatively slow rate of depletion suggested further that an active metabolite of PCPA, possibly *p*-chlorophenylpyruvic acid, might be responsible for the effects of 5-HT metabolism.

In a more recent and detailed analysis of the mechanism of action of PCPA, Jequier et al. (61) concluded that inhibition of tryptophan hydroxylase is the major cause of the 5-HT depletion. Their data indicate that tryptophan hydroxylase is the rate-limiting step in the biosynthesis of 5-HT; furthermore, the relatively high K_m observed for tryptophan ($3 \times 10^{-4} M$) suggests that the enzyme may not be fully saturated with substrate normally and that the overall rate of 5-HT synthesis may be partially dependent upon availability of the substrate. This is also indicated by the recent finding (95) that loading doses of tryptophan in rats result in elevated levels of brain 5-HT. Jequier et al. (61) have made an additional interesting observation that while PCPA is a competitive inhibitor of rat brain tryptophan hydroxylase *in vitro*, it causes an almost complete loss of tryptophan hydroxylase activity in rat brain *in vivo* due to an irreversible inactivation of the enzyme. These investigators suggest that a metabolite of PCPA, formed outside the brain, is responsible for the irreversible inhibition seen *in vivo*. Characteristic in the depletion of brain 5-HT by PCPA is the relatively slow return of amine levels to normal. In view of the current evidence that PCPA inhibition of the hydroxylase is irreversible, this slow recovery is apparently due to the time required for synthesis of new enzyme protein.

The availability of a selective depletor of brain 5-HT like PCPA held great promise as a tool that might provide insight into the behavioral systems which involve 5-HT. However, with brain 5-HT levels at around 10 per cent of normal after PCPA, rats displayed few apparent signs of gross behavioral change, apart from increased irritability on handling. In addition, reserpine and tetrabenazine were able to elicit their typical gross behavioral effects in such rats. In human beings, PCPA was also markedly devoid of effects on behavior (96) in the dosage used. One explanation for these findings other than the unlikely possibility that 5-HT plays no role in brain function, is that the last 10 per cent of the 5-HT store remaining after PCPA represents the pool critical for the behavioral mechanisms in which 5-HT is engaged (97–99). On the other hand, Tenen (100) recently described effects of PCPA which pointed to increased sensitivity to pain (electric shock). Perhaps as a result of this, the drug allowed a faster acquisition of a conditioned avoidance

response if a low intensity of current was used. Eventually, other types of psychophysiological testing may provide clues to 5-HT involved brain function.

DOPA-5-HTP decarboxylase.—This enzyme, as DOPA decarboxylase was first discovered by Holtz (101). Like other L-amino acid decarboxylases, it was found chiefly in the cytoplasmic portion of various cells by numerous investigators; but recently Udenfriend (34) pointed out that when the homogenization of the tissues is carried out with care, over 60 per cent of the decarboxylase activity is found associated with particles sedimenting with mitochondria. Much information is available on the substrate specificity of DOPA decarboxylase (102); and because of the relative lack of specificity of this enzyme, it was not surprising when it was shown to be identical to 5-HTP decarboxylase (103). In keeping with the observation of Udenfriend (34) of the subcellular distribution of the enzyme, Rodriquez de Lores Arnaiz and De Robertis (104) have reported that much of the 5-HTP decarboxylase in brain is found within the synaptosome fraction, from which it can be released with further disruption of membranes.

Since DOPA decarboxylase was the first of the three enzymes in the biosynthesis of catecholamines to have been studied, it was also the first for which potent inhibitors were developed (105, 106). For example, α-methyl-DOPA inhibits the enzyme in animals *in vivo* (107), lowers blood pressure in man and causes a decrease in levels of NE in brain and sympathetically innervated tissues (108). The fall in NE was at first assumed to be the result of inhibition of the decarboxylase, but this was later shown to be an erroneous assumption (109). Rather, the mechanism is now known to involve the decarboxylation of α-methylDOPA itself to form α-methyl-DOPAmine, which is then hydroxylated by DOPAmine-β-oxidase, yielding α-methylnorepinephrine (110–112). This substituted NE, despite some slight activity, acts chiefly as a false transmitter and leads eventually to transmitter failure. Support for this mechanism comes from the finding that even more potent inhibitors of L-amino acid decarboxylase have little effect upon endogenous stores of NE or on blood pressure, if they are not themselves decarboxylated and converted to amines (34).

The decarboxylase is also inhibited by decaborane, which *in vivo* causes a 60 per cent depletion of DA and a 25-fold increase in DOPA (113). Decaborane is a strong reducing substance which might reduce the enzyme and cause disappearance of the co-factor, pyridoxal, in much the same manner as sodium borohydride leads to inhibition of glutamic decarboxylase (114).

Other inhibitors of the decarboxylase include α-methyl-DL-DOPAhydrazine and 3-hydroxy-4-bromobenzyloxyamine (NSD-1055), which is also a DOPAmine-β-oxidase inhibitor. However, since the decarboxylase is not rate-limiting either in the case of 5-HT or catecholamine synthesis, the inhibitors of the enzyme are not expected to have any practical significance, unless, of course, the inhibitor, like α-methylDOPA, is decarboxylated itself to a product that can deplete tissue stores of the amine by displacement.

DOPAmine-β-oxidase (hydroxylase).—This enzyme, catalyzing the final step in the biosynthesis of NE was first isolated from the adrenal medulla in 1960 (115, 116), Recent work (117) has indicated that the enzyme is present in catecholamine-containing granules of the adrenal medulla. It has also been shown to be present in brain (118). This is not a highly specific enzyme, but can oxidize almost any phenylethylamine derivative to the corresponding ethanolamine; and it has an affinity for certain phenylethylamine isosteres, which proved to be inhibitors rather than substrates (119). Among the potent inhibitors of DOPAmine-β-oxidase are benzylhydrazine and benzyloxyamine; but even when the enzyme was inhibited more than 90 per cent, levels of endogenous NE were not markedly reduced (120). This would again point to the fact that DOPAmine-β-oxidase is not rate limiting in the biosynthesis of catecholamines, as it was long believed to be.

One of the most interesting inhibitors of DOPAmine-β-oxidase is the alcohol deterrent drug, diethyldithiocarbamate [disulfiram (121)] which was first shown to inhibit the conversion of DOPAmine-^{14}C to NE-^{14}C *in vivo* by Goldstein et al. (122). Disulfiram has been shown by both histochemical and biochemical means to reduce tissue catecholamines (123). In addition, it was demonstrated recently that this inhibitor caused considerable depletion of NE in the spinal cord rostral to a transection in the mid-thoracic region, but not caudal to the lesion (124). The same results were seen after a decarboxylase inhibitor, seryl-trihydroxybenzylhydrazine (RO4-4602). Similar results were reported earlier (125), with the tyrosine hydroxylase inhibitor. H-44/68 (*vide supra*). These findings raise the interesting question of whether nerve impulses stimulate synthesis in the brain as they do in the periphery (126).

In the brain, disulfiram causes a decrease in NE content and an increase in DOPAmine stores (127). This should make disulfiram a useful tool in separating behavioral effects of NE and DOPAmine. This finding of a 40 to 50 per cent decrease in brain NE by disulfiram is at odds with earlier results (34). Goldstein & Nakajima (127) suggested that the discrepancy is due to the fact that disulfiram is not effective in the guinea pig, the species used by the former investigators. It has also been reported that 5-HT, tryptamine and histamine are inhibitors of DOPAmine-β-oxidase, but the physiological significance of this is dubious since the amount necessary in each case was $2\times10^{-3}M$ (128).

Feedback-inhibition of endogenous biosynthesis of monoamines.—The view that synthesis of monoamines in the neuron proceeds at a constant "supramaximal rate," independent of nerve activity was initially proposed (129, 130); but there is increasing evidence that NE synthesis at least may be controlled by variations in the content of NE in some critical pool in the tissue (131–133). This latter concept is supported by the evidence of Nagatsu et al. (64) that on incubation with different tyrosine hydroxylase preparations *in vitro*, DL-NE, in a concentration of $2\times10^{-4}M$, caused a 25 to 50 per cent inhibition of the ring-hydroxylation of tyrosine in the formation of NE. More recently, Stjärne et al. (134) provided better evidence for possible physiolo-

gic significance of such synthesis-inhibition by demonstrating that the synthesis of NE from tyrosine in a fraction from an homogenate of bovine splenic nerves can be 85 per cent inhibited by NE concentrations as low as $1.2 \times 10^{-5} M$. These investigators also found that the inhibition operated not only at the first step in the synthetic sequence, but at the step of the β-hydroxylation of DA as well. These findings provide an example of regulation of the synthesis of a neurotransmitter by the classical principal of feedback control of the rate-limiting step in the biosynthetic pathway, by variations in the accumulation of the end-product.

Other examples of control of the biosynthesis of monoamines by products and substrates along the biosynthetic pathway are available. It is interesting, for example, that DOPA inhibits decarboxylation of 5-HTP per se (135) and through the formation of its metabolite, DA (136) and NE (137). Modifications of these products and substrates may well provide effective inhibitors. For example, α-methyl-5-HTP has recently been reported to be a tyrosine hydroxylase inhibitor (138); and the enzyme inhibitory properties of α-methyl-DOPA (139) and α-methyl-tyrosine have already been mentioned in this review.

Uptake and Storage of Monoamines

Previous reviews (140–143) have indicated the degree of insight into the neuronal storage of central amines which was elicited by the techniques of differential centrifugation for separation of subcellular particles. This approach provided the first evidence beyond the dissimilarities (144, 145) in regional amine levels that the monoamines were indeed stored in neuronal elements and not in perivascular nerves. Immediately following the centrifugal analyses came the disclosures of two techniques which more or less completely proved the existence of intra-neuronal storage: the development of the fluorescent histochemical technique (146–148) and the correlation of decreased amine levels with the destruction of specific neuroanatomical pathways (149, 150). Under a wide variety of experimental conditions, both the histochemical and the anatomico-chemical techniques have been applied by several laboratories to the end that complete systems of catecholamine and serotonin-containing neurons have been mapped out and studied (151–153).

Although there is some question as to whether the results of the anatomico-chemical studies can, in all cases, be interpreted as loss of storage due to destruction of the storing neurons, or to the loss of a transsynaptic trophic (149, 150, 154) influence, the fluorescent histochemical method has been widely applied to the study of the storage mechanisms of the central monoamine neurons (123–125, 155–161). With this technique, it has been possible to observe differences among various portions of different neuronal systems in response to reserpine and other depletor drugs (155), ethanol (124), amphetamine (158), chlorpromazine (156), synthesis inhibition (123–125), monoamine oxidase inhibition, plus precursor loading, (157, 158), and a

variety of combinations of these treatments including the effects of electrical and behavioral stimulation (159–161). Thus, in terms of data production relating to the monoamine systems of the CNS, fluorescence histochemistry has proven itself to be invaluable, particularly with respect to the establishment of one of the crucial bits of data required for the identification of a neurotransmitter, namely, its occurrence within particular nerve endings in a pathway.

There is a tendency, however, to expect the technique to provide certain data which may be beyond the realm of its capabilities, i.e. to reveal points about the release and binding of the monoamines in relation to other facets of transmitter function. Among the shortcomings of the technique which have been cited (162) are the quantitative estimation of content (163) without corresponding micro-chemical assays, and a relative uncertainty in the discrimination of catechols from indoles (162), or norepinephrine from DA (162–165) when each is known to be present in a region. These shortcomings have been partially corrected by the use of thinner tissue sections (166) and by the use of microspectrophotometry (163, 165, 167–169), although a great deal of data published prior to these sophistications in technique remains to be confirmed.

We would prefer to discuss subcellular correlates of the storage process with information provided by the structural tool capable of doing so: electron microscopy. However, despite an abundance of applied effort such information is only recently becoming interpretable. In the peripheral sympathetic nervous system, fine structural analyses have provided considerable information on the identification of 400 to 600Å granular synaptic vesicles as the storage organelles of norepinephrine [c.f. review (170)]. This information covers four general areas. Such granular vesicles are present in virtually all sympathetically innervated structures (170, 171). The granular contents of these synaptic vesicles can be correlated with the fluorescence histochemical reactivity and biochemical measurement of norephinephrine content (164, 165, 167) after depletion (164, 165, 172). The granular contents can be restored after depletion by incubation with norepinephrine *in vitro* (164) or by the injection of rather large amounts *in vivo* (173, 174). By the use of electron microscopic autoradiography, the labelling of catecholamine stores with radioactive amines (175) or precursors (176) makes possible the observation of grains over nerve endings or axons containing granular vesicles. Lastly, the nerves containing the granular vesicles disappear when sympathetic innervation is destroyed (177). Such data strongly indicate that granular synaptic vesicles represent norepinephrine-containing neural processes and that the granular material is itself indicative of the presence of the monoamine. The latter point is strengthened by the results of electron-microscopic histochemical experiments on adrenal medullary chromaffin granules (178, 179), which indicate that norepinephrine-storing granules are made electron opaque by the fixatives glutaraldehyde and osmium tetroxide commonly used for electron microscopy. However, it has also been estab-

lished that there are at least two types of granular synaptic vesicles in the peripheral nervous system (170) and only the 400 to 600Å electron dense vesicle shows the proper tissue distribution and reactivity in depletion and repletion experiments (164, 170, 172–174).

Based on this moderately successful application of fine structural analysis to the peripheral nervous system, similar experimental approaches have been applied to the identification of the monoamine storing particles of the CNS. Such studies have consisted almost entirely of pharmacological depletion studies (179–191) and autoradiographic localizations of the amines or their precursors injected into the cerebral ventricular system (192–195). Furthermore, these attempts to uncover the storage organelles in the CNS have concentrated on the relevance of a synaptic vesicle of 800 to 1200Å diameter with variable degrees of internal electron density in electron micrographs (170). Such large granular vesicles are seen together with the more typical 400 to 600Å synaptic vesicles in the peripheral sympathetic nerves but are also seen in preganglionic cholinergic nerve endings where their significance is unknown (170). Until recently (171), however, the large granular vesicles were the only consistent form of granular synaptic vesicle observed in the brain. These large granular vesicles have been estimated in various regions of the rat brain (182, 183) in which they appear to correlate in frequency with the levels of norepinephrine and serotonin, but not of DOPAmine. The large granular vesicles were also present in almost all of the nerve endings and axons over which autoradiographic grains were found after labeling stores with H^3-norepinephrine (192–195), H^3-DOPA (193) or H^3-5-HT (194). The electron density of the granular contents of the large vesicles has been reported both to change (180, 181, 185, 186, 188, 189), and to resist change (182–184), in response to pharmacological treatments which raise or lower the amine levels. A recent extensive semi-quantitative report failed to find any change in electron-opacity in these granular vesicles after a variety of pharmacological manipulations (182) and suggests that the granularity may be due only in part to possible storage of amines. Only preliminary observations have as yet been reported on the results of destruction of monoamine pathways in the brain as observed at a fine structural level (196); although, here again, the large granular vesicles were seen in the degenerating nerves.

The main problems with the acceptance of the large granular vesicles as storage organelles of the monoamines in the CNS has been their apparent lack of observable change with altered amine levels and the failure to observe the same type of small granular vesicle which does appear to be the storage vesicle in the peripheral nervous system. Recently (197–199), such small granular vesicles have been reported in the CNS with the use of a more severe method of tissue oxidation (171). With $KMnO_4$ fixation, small granular vesicles are observed in endings which also appear to be associated with larger vesicles. Thus, regional quantifications (182, 183) and autoradiographic localizations (192–195) may require reinterpretation to include the possibility that the fixatives used in those studies did not fully reveal all the

material capable of producing the appearance of vesicles with granular contents. Further evidence is needed to identify the granular contents with the actual presence of the amines and to determine whether or not the serotonin-containing nerves are more like norepinephrine- or DOPAmine-containing nerves, but at least the cause of some of the confusion concerning the large granular vesicles may now be resolved. The large granular vesicles do possess a matrix which will react with phosphotungstic acid (200), an electron-microscopic reagent which is regarded as reacting with proteins containing basic amino acids. Moreover, the matrix of the large vesicles appears to be dissolved or decreased by exposure to the more potent oxidant ($KMnO_4$) used in the demonstration of the small granular vesicles, which is also a chemical property of proteins (201). Thus, the main source of the electron opacity of the large granular vesicles could be a protein binding substance (200). Further analysis of the nature of the intravesicular amine-binding matrix is clearly warranted (202).

In addition to the problems concerning the identification of the one or more intracellular storage particles is the clarification of the number and interrelationship of the chemical "pools" of stored amine. Based on the centrifugal analyses defining "soluble" and "particle-bound amine" [cf. (34)], the concept of vesicular and extra-vesicular amine storage has been repeatedly proposed (15, 165, 203), with the exact proportion in each compartment depending to some extent on reluctance to accept possible artifactitious results due to the homogenization process (204) and the analytical tools used (34, 205). Furthermore, the various neuronal monoamine containing neurons do not undergo the depletion and repletion of storage pools at the same rate when surveyed by the light microscopic histochemical approach (152, 153, 155).

Beyond the concept of the vesicular and extravesicular pools, there is also partial evidence which may be interpreted as favoring the existence of pools representing storage of recently synthesized amine, recently incorporated amine, and more or less tightly bound amine (205). However, since many of these experiments have failed to take into consideration the regional and intraneuronal variations in storage kinetics, the existence of one or more such storage subcompartments is not yet fully convincing. However, it is clear that animals, chronically depleted of either catecholamines (72–75) or serotonin (62), may have only subtle behavioral dysfunctions, indicating that the great majority of the stored amine can be eliminated without incurring gross malfunction (97–99). The failure to observe changes in the large granular vesicles could then be interpreted as a representation of the small amount of amine which always seems to be measurable no matter how severe the depletion.

Release of, Response to, and Reuptake of Monoamines

After biosynthesis and storage, the next stages in the life cycle of biogenic amines represent three dynamic aspects of central monoamine synapses: release, response, and reuptake. These "three R's" underlie the initiation,

elicitation, and termination of the amine-response. The study of these functions, therefore, is intensely pursued by pharmacologists both for insight into the transmission process and for elucidation of the mechanisms of action of psychotropic and neurotropic drugs [cf. reviews (6, 22, 23, 206)].

The release of amine in the strictest physiologic interpretation denotes the release of transmitter into the diffusable volume of the synaptic cleft, in response to stimulation and depolarization of the nerve terminal. No microchemical method for studying excitation-secretion coupling succinctly in the CNS has yet been devised. Nevertheless, by imposing less stringent criteria, experimental studies on the release of monoamines have been reported. Studies performed *in vitro* have shown that both NE (207, 208) and 5-HT (209) can be recovered from the incubation fluid surrounding isolated spinal cords which have been subjected to vigorous tetanic stimulation. When brain slices of olfactory bulb or hypothalamus had accumulated H^3NE, the rate of efflux was significantly increased by more physiologic parameters of stimulation (210, 211). This latter effect is also observed when depolarization is induced by high K^+ and is inhibited when Ca^{++} is removed from the media. Such ionic influences tend to discount the significance of possible physical artifacts which might occur in an *in vitro* release system.

Experiments which demonstrate monoamine release *in vivo* generally depend upon depletion of fluorescent nerve terminals (161, 209) or whole brain amine levels (159, 160) as an index of the effect. Prolonged and vigorous stimulation of the amygdala results in generally decreased telencephalic NE levels (159), a result which is facilitated by prior inhibition of tyrosine hydroxylase (160). These data must be interpreted as a polysynaptic effect of the stimulation, since no monoamine-containing pathways are known to arise in the amygdala (151). Stimulation of the monoamine-containing cell bodies of the caudal medulla and brainstem (161) results in lowered NE levels in spinal cord and diminished fluorescence of both NE- and 5-HT-containing nerve terminals (161). This effect is also enhanced by blockade of the hydroxylating enzymes (161). The magnitudes of the electrical stimulation applied in such experiments are far above physiologic standards. The resultant prolonged depolarization might lead to lowered intraneuronal amine content through accelerated catabolism without release from the neuron ever having occurred. Milder degrees of stimulation applied *in vivo* to the serotonin-containing neurons (212) of the dorsal raphe nucleus result not only in lowered 5-HT levels in the forebrain distribution of these nerve endings (153, 213) but in elevation of the 5-HIAA levels of an even greater magnitude (212). Here, it is certain that the stimulation has caused greater catabolism by MAO, but whether the amine mobilized from the storage sites was catabolized before or after neuronal release will require better localization of MAO activity in the cellular environment of these nerve endings. That some 5-HT was released from the neuron is strongly suggested by the recent observations (214, 215) that hyperthermia and altered responsiveness to sensory stimulation accompany the electrical stimulation producing the

chemical changes. Elevated 5-HT levels in ventricular perfusates have previously been observed with induced hyperthermia (128, 216). Lowered 5-HT levels resulting from lesions (219), depletion (220), or synthesis inhibition (62, 100) have been reported to elevate the sensitivity to painful stimuli.

When cannulae of the push-pull type are inserted into the brain parenchyma to aspirate cellular fluids, the release of either endogenous or radioactive amines has been reported following electrical (221, 222) stimulation or drug treatments (223). These cannulae, however, seem likely to lead to sufficient tissue damage to make the significance of such types of release somewhat dubious. Cannulae inserted into the ventricular cavities would be less likely to injure the underlying tissues; with this system release of DA and its catabolite, homovanillic acid, after very low levels of electrical stimulation to the substantia nigra have been noted (224). Here, the assumption must be made that excess transmitter which is liberated from the endings can successfully migrate through the brain into the ventricular fluids.

These physiologic methods for studying release, although still somewhat gross and indirect, nevertheless represent increasing sophistication in research on release. Although biochemical methods for the study of drug-induced release are more widely applied, they are equally indirect (15). If it can be shown by appropriate controls that synthesis is not impaired nor catabolic enzyme activity enhanced, then the estimation of decreased amine levels and increased metabolites could be taken as evidence of release. To be of physiologic significance, however, the release should be onto receptor sites. For the catecholamines, biochemical evidence arising largely from the peripheral sympathetic nervous system relates catabolites of the enzyme catechol-O-methyl-transferase (23, 225) to extraneuronal (226) amine metabolism, and products of MAO activity to intraneuronal degradation. For serotonin, nearly all catabolism occurs via MAO [however, see (227–229)] so that release of 5-HT onto receptors is difficult to ascertain biochemically. Further evidence that COMT is mainly extraneuronal within the CNS are the observations that the ratio of H^3-NE:H^3-normetanephrine are higher when the H^3NE is synthesized from labeled precursors *in vivo*, than when the H^3NE is accumulated after intraventricular injection (230). In the latter case, the amine is thought to migrate extracellularly where it should have prolonged exposure to COMT (231).

If the level of normetanephrine were an accurate reflection of amine released onto receptor sites in the CNS, increased levels of this metabolite after drug treatment would only indicate unequivocal evidence of release when the reuptake process was unimpaired. Thus, the increased normetanephrine seen after desmethylimipramine (230, 232) is not taken as evidence of increased release while that seen after amphetamine is (233). The fact that Li^{++} treatment (234, 235) decreases formation of normetanephrine (232), while metabolic turnover (236) is doubled (237), may be taken to indicate that there is decreased release. On the other hand, the increased normetanephrine level after amphetamine must be interpreted in the light of the ef-

fective competitive inhibition by this drug for the deamination of NE by MAO, when the latter enzyme is assayed with NE as the substrate at concentrations similar to endogenous tissue levels (238). If amphetamine were an effective competitor for MAO, more of the NE released from storage sites by the drug would be available for reaction with COMT. One of the newer approaches to the study of the mechanism of action of LSD (239) has also utilized measurements of increased 5-HT levels and decreased 5-HIAA levels (240, 241) to indicate decreased serotonin release.

Release of monoamines by reserpine and amphetamine.—New insight into the mechanism by which amphetamine and reserpine evoke release of monoamines with opposite behavioral effects has recently been provided, largely through the experiments of Glowinski and his colleagues [cf. review (19, 230, 231, 242–248)]. When H^3NE is injected into a lateral cerebral ventricle, it is taken up and accumulated by the brain and disappears in a multiphasic fashion (231), dropping more rapidly during the first four hours as the labeled amine mixes with the endogenous stores (230). Similar but less complete information is available for the uptake accumulation and disappearance of radioactive 5-HT (194, 249, 250). By four hours after injection, the remaining H^3NE in the brain is largely in nerve endings, as shown by density gradient analysis and by electron microscopic autoradiography (192–195).

Reserpine given after the labeling with H^3NE reduces both the endogenous and exogenous NE levels, although it is interesting that soon after labeling, relatively less of the labeled amine is released by the drug (230, 245). When the labeled homogenized brains are subjected to a single high speed centrifugation, reserpine appears to have its effect earliest on the supernatant fraction (246); such an effect was reported earlier for 5-HT release (251) by reserpine. The reserpine treatment also results in a greater proportion of labeled deaminated catabolites (251). When the reserpine treatment precedes the labeling, a small amount of H^3NE can still be accumulated in a tissue site where it is resistant to the further depleting effect of additional reserpine (245). However, the ability to accumulate H^3NE from ventricular injections returns within 24 hours after reserpine treatment and is back to normal levels long before endogenous amine levels have been reestablished (245). A similar time course has been reported for the inability to accumulate exogenous 5-HT after reserpine treatment, as estimated by a non-isotopic technique (252). From fluorescence histochemical studies, this lag period between the recovery from behavioral depression and the recovery of normal intraneuronal distribution of the fluorescent products can be ascribed to the time required for the axonal migration of amine synthesized in cell bodies (153, 253). The reserpine-induced behavioral depression correlates temporally with the period of reduced uptake (245, 246) and the submaximal chronic depletion of brain amines with reserpine is relatively asymptomatic (97–99) after the initial period of effect.

Amphetamine also results in the reduction of H^3NE levels whether given before or after the labeled amine (230, 238), although greater reduction is ob-

served when the drug is given after an initial uptake has occurred (19, 238). This effect of amphetamine is accompanied by increased relative levels of H^3-normetanephrine, interpreted (238) as release beyond MAO sites (*vide supra*). The behavioral excitation of the drug (254–256) is accompanied by an effect on the subcellular level indicating that the drug is acting on unidentified structures storing amine in the high-speed particulate fraction (246). By fluorescent histochemical analysis, the release of amine by amphetamine is noted mainly in cortex and in both NE and DA neurons (158); biochemically the findings in cortex are described as normal after H^3NE labeling (243, 247, 248) as is the level accumulated in the DA-rich areas of the brain (247, 248). Of further interest are the observations that although the uptake of 5-HT and DA are normal after amphetamine (158), the amount of NE retained after synthesis from labeled precursors is reduced. The effect of amphetamine in reducing the accumulation of NE can be demonstrated in brain slices as well as *in vivo* (158). It is also significant to the understanding of the amphetamine response that amphetamine-induced excitation is blocked by inhibition of tyrosine hydoxylase with α-methyl-tyrosine (72–77, 257–260). This effect is not due simply to the sedation accompanying α-methyl-tyrosine (258–260) or the resultant lowering of NE content, since equal depletion of brain levels with either reserpine (72, 73, 76) tetrabenazine (257) or α-methyl-*m*-tyrosine (76, 261) does not inhibit the amphetamine response. While these interactions with amphetamine appear to implicate NE almost exclusively, a simple analogue of amphetamine, 4-Cl-amphetamine (262–264), results in 5-HT depletion accompanied by lowered 5-HIAA levels, occurring presumably without inhibition of tryptophan hydroxylation (262, 263). Another amphetamine analogue, prenylamine, exerts an effect primarily on the DA content of the striatum (265).

The above results permit the following scheme. Reserpine which reduces amine storage presumably by acting on the storage particles, releases amines earliest from unidentified structural elements present in the supernatant fraction, and does so with increased catabolism by MAO. Recovery from reserpine-induced behavioral and autonomic symptoms correlates with recovery of the accumulation process but not with the slower recovery of endogenous amine levels. Reserpine does not appear to affect either the synthetic enzymes (19, 245) or uptake of amine into the neuronal cytoplasm (19, 155, 157, 158, 203). Overall synthesis could be impaired by accelerated catabolism of unbound DA before the latter is converted to NE (266). Amphetamine is thought to result in excitation, presumably by release of catecholamines; this latter effect is more pronounced on the elements present in the particulate fractions. The release induced by amphetamine is followed by increased amounts of normetanephrine, which may stem either from increased catabolism of amine at receptors or from competitive inhibition for MAO with the NE. The excitation produced behaviorally is prevented by blockade of NE synthesis but not by direct depletion of storage (*vide supra*). In short, reserpine affects primarily a surplus reserve pool of stored amine,

and its symptoms may be related to the time during which the nerve endings are unable to maintain the smaller functional pool by the reuptake process and possibly by reduced biosynthesis. Amphetamine releases amine to a different catabolic fate than does reserpine and from sites more immediately associated with synthesis and response.

Effects of monoamines at receptor sites.—The interpretation of such drug effects would be considerably simplified with better understanding of the true functional response of neurones to naturally released monoamines, if their suspected transmitter roles (30) are indeed real. A true skeptic might even wonder whether the effects of a drug, such as reserpine, on the monoamine storage process, and possibly on behavior, is the cause or effect of a larger biochemical action, such as alteration of brain amino acid and protein metabolism (267, 268).

Functional responsiveness of neurons has been pursued biochemically only to a minor degree by the anatomical and subcellular localization of drug binding by isolated receptors (269, 270) for radioactive drugs and for LSD (271) and psilocybin (272). Much more data are available from electrophysiological analysis of the effects of monoamines applied by microelectrophoresis (273) on the activity of single neurons.

Although many of these data have recently been reviewed (30–32), some generalizations and some specific points are worthy of re-emphasis. Early attempts to characterize the influence of monoamines on the activity of single neural units largely underestimated this ability (273–276). Presumably, these results were due in part to the use of anesthetized animals for the testing, although certain other technical factors may also have been contributory (277). At any rate, the recent reinvestigations of monoamine responsiveness (mainly in unanesthetized animals) have shown that neuronal activity in the brainstem (278–280), spinal cord (277, 281–287), cortex (288–293), and lateral geniculate (294) can be influenced by these substances. Analyses of these areas and in several others including olfactory bulb (295), caudate nucleus (296–298), amygdala (299), hippocampus (300–303), and septum (304) confirm earlier results obtained in hypothalamus (305) indicating that the monoamines produce mainly but not exclusively (278–280, 293) decreased spontaneous activity, interpreted in some instances as hyperpolarization (287, 298).

To be functionally meaningful, monoamine responsiveness might be restricted to those neuroanatomical areas in which monoamine nerve terminals are found. Since such information can be provided by the extensive literature based on the fluorescence histochemical method (151–153), it is possible to test this possibility. In the hypothalamus (305), septum (304), caudate (296–298), brainstem (278–280), and spinal cord (281–287), the responses which have been seen might be expected to be reproduced physiologically by natural activation of the many monoamine-containing nerve terminals found in these regions. However, the frequency with which monoamine responsiveness can be seen in cortex (288–293), olfactory bulb (295,

306), lateral geniculate (294), cerebellum (307), amygdala (299), and hippocampus (308) is difficult to correlate with the relative scarcity of monoamine nerve terminals found in these structures.

It is not helpful, then, to realize that the major share of pharmacological investigation carried out with the microclectrophoretic technique has been performed in precisely these areas of lower amine content of nerve endings. In the olfactory bulb (295, 309), NE has been proposed as the mediator of a recurrent inhibitory synaptic pathway which is demonstrated by the facts that NE is itself a depressant of spontaneous activity, that its effects and that of the synaptic inhibition are both blocked by dibenamine (310) and by LSD and BOL (309), and that the synaptic inhibition is also reduced after depletion of NE with either reserpine or α-methyl-*m*-tyrosine (295, 309). NE-fluorescent nerve endings are found to be restricted to that layer of the bulb in which synaptic inhibition is most easily demonstrated (311), although neurons throughout the bulb are capable of responding to the amine (306). It is of interest that the olfactory bulb can be shown to be capable of taking up NE (312) and of releasing it *in vitro* (210, 211).

Alpha-receptor blocking agents are also reported to block the responsiveness to NE manifested by neurons in the caudate nucleus (288) and spinal cord (277, 281), two areas in which there are high frequencies of catecholamine-containing nerve endings, but in which there has been no extensive correlation of these responses with particular synaptic pathways. The inhibition produced by NE in spinal cord on Renshaw cells (281, 286, 287) is probably distinct from the strychnine-sensitive type of presynaptic inhibition (284). In the caudate, the neuronal response is identical (i.e. depression of activity) with that resulting from stimulation of the substantia nigra (297), where the DA-containing nerve endings are said to arise (313–315). The release of DA into the ventricular space after nigral stimulation (224) has already been mentioned.

Occasional examples (278, 280, 289, 293) of neurons excited by either the catecholamines or 5-HT appear to be the exception rather than the rule. That these findings are more than somewhat unique is suggested by the fact that the neurons exicted by NE can be blocked by chlorpromazine in the brain stem (280) and by the β-adrenergic blocking agent, dichloroisoproteronol, in the cerebellum (307); β-receptor type response may also be elicited under certain conditions by DA in the caudate (316). However, the excitation seen by NE in the cerebellum must be interpreted in view of the low amine content and scarcity of catecholamine nerve endings seen here (153). On the other hand, the cerebellum does take up H^3NE (243), and the regional turnover and subcellular distribution of the amine in this structure and its responsiveness to drugs (238, 247, 248) may be indicative of this distinctive type of response on nerve activity.

The lack of good pharmacological blocking agents with exclusive effects on responses to 5-HT has hampered relation of these responses to synaptic pathways. LSD or derivatives of it have only slight (239, 293, 295) effects

on this responsiveness. Characterization of the 5-HT receptor (in an area of low 5-HT content, the geniculate) has shown (276) that the indole hydroxyl is essential to the depressant effect and that O-methylation of the hydroxyl or alkylation of the ethylamine-N reduces potency. The scant molecular characterization of the catecholamine receptor is the observation that D-NE is as effective as L-NE in depressing activity of single units (285). Theoretical approaches to the construction of dose-response curves for microelectrophoretically applied substances (317, 318) have not yet been applied experimentally.

Since the major qualitative type of response to the monoamines is depression of spontaneous, synaptically induced, or artificially activated neuronal activity, it may be profitable to reconsider briefly the interpretation of certain behaviorally-manifested drug effects. For example, psychotomimetic drugs and the stimulant amphetamine have at best weakly-evoked depressant effects in areas where either catecholamines or 5-HT are potent depressants (289, 293, 295, 319). Thus, drug-induced behavioral stimulation may well be a form of disinhibition in which neurons tonically inhibiting behavior are now themselves strongly inhibited. Microelectrophoretic data also help to add another veil to the already murky view of the etiology of reserpine-induced depression (1–3, 16, 18). Recent evidence interpreted as favoring release of 5-HT as the important factor (252) was countered by a report of successful production of the complete reserpine syndrome in animals already severely depleted of serotonin by inhibition of tryptophan hydroxylase (62). Points used previously to implicate catecholamine release as the most important factor in the reserpine-induced behavior were the observations that large doses of DOPA would transiently reverse overt behavioral sequelae of reserpine treatment, while 5-HTP would not (2, 320, 321). However, these latter observations may well need reinterpretation in light of the demonstration that DOPA applied by microelectrophoresis appears to be generally excitatory on cortical neurons (289) [an effect which may explain previously observed EEG effects (322)], while 5-HTP applied by microelectrophoresis has no effect (289). These results urgently need confirmation.

Further clarification of the mechanism by which the monoamines and biochemically related drugs influence neuronal functions awaits not only the identification of the synapses at which the monoamines affect transmission, but more extensive delineation of the physiologic roles performed by the multineuronal circuits in which the monoaminergic synapses are links. At present, there are tempting, but incomplete data relating the importance of monoamines to the control of central temperature regulation (216–218, 323–328), estrus cycling (329), release of pituitary hormones [cf. (330)], and to the onset of sleep (331, 332). The latter effect is most exciting: depletion of serotonin by synthesis inhibition (331) or by destruction of 5-HT-containing neurons (332) is reported to decrease significantly the frequency of the rapid eyeball movement (REM) stage of sleep in cats.

Reuptake of monoamines and the effect of drugs thereon.—Electrophysiologic analysis of the importance of the reuptake process for the termination of monoamine transmitter effects is also in its earliest stages. In general the microelectrophoretic studies cited above have indicated that the direct application of monoamines produces depressant effects on neuronal activity which are of considerably longer duration than the current used to apply them. Such a system would seem to be well suited for the testing of drugs on the reuptake process, but has yet to be exploited. However, while there is little knowledge of the relative importance of reuptake for the conservation of amine and temporal dispersion of the response to released monoamine in the brain as compared to the peripheral nervous system (22, 23), there are extensive data relating to the uptake process in the brain. Uptake and concentration of monoamines by brain minces (333–335), brain slices (210, 211, 336, 337), nerve-ending rich fractions (338–342), and by the brain *in vivo* (230, 231, 242, 248, 343–345) have been extensively reported and reviewed (19, 22). The process is energy and temperature-dependent (22, 23, 211), ion-sensitive (23, 211, 339, 342), and can be blocked by the ATPase inhibitor, ouabain (338). When studied *in vivo* (19) accumulation of labeled or exogenous amine is roughly proportional to the endogenous amine content of various brain regions. The ability of perivascular cells and choroid plexus to take up exogenous amines is real (193, 343) and is likely to have an influence on purely biochemical estimations of drug effects on the distribution and metabolism of exogenously administered amines. However, regional brain differences in the selectivity of the uptake process (242–248) and on the rates of metabolic turnover (346, 347) and amine binding (337) have made clear the significance of the neuronal uptake process in the brain.

The drugs most commonly employed for the study of uptake in the brain are the anti-depressant, desmethylimipramine (DMI) and the tranquilizer, chlorpromazine (CPZ). Under certain experimental conditions (19, 156, 231, 232), both drugs appear to be able to decrease the initial uptake and to prolong the disappearance of labeled amine. The drugs can also influence the extent to which brain slices take up or release NE when stimulated (210, 211). Decreased uptake is associated with relative increase in normetanephrine (19, 231). While the central effects of either CPZ or DMI on behavior and monoamine metabolism are without overt effect (348) on the levels of either NE or 5-HT, CPZ can lower striatal DA content (349) and DMI has been reported to prevent the rise in C^{14}5-HIAA levels seen after ventricular perfusion of C^{14}5-HT (250). The fact that both CPZ (81, 350) and DMI (132, 133) have been reported to increase the synthesis rate or turnover (236) of NE and yet do not result in elevated levels, might be explained by the postulated failure to reaccumulate amine released during functional activity (19, 211, 235, 244). Whether this effect is the cause or result of the synthesis increase (350, 133) remains to be determined.

The effects of DMI appear to be limited to NE metabolism since the drug does not retard the uptake of either 5-HT (344) or DA (238, 243) in

nerve endings. On the other hand, CPZ treatment results in enhanced fluorescence of diencephalic DA-containing neurons (156). Despite the wide variety of biochemical effects attributed to CPZ, it is also possible that the tranquilization produced by this drug may be explainable fully on the basis of its physiologic actions in the reticular formation (280), where it is known to elevate the threshold for arousal by sensory stimulation. In this system, CPZ blocks neurons excited by NE and potentiates the inhibitory response of NE on the other responsive cells possibly by inhibiting reuptake (232). The hypothermia seen after CPZ treatment may also be a result of pharmacological interference with catecholamine response in the hypothalamus.

It should be clear that despite the concentrated effort of many investigators, there is a paucity of knowledge of the mode of action of monoamines in the brain and the effects elicited by drugs through or exclusive of monoamine function at the cellular level. Further study of the "3 R's" is clearly indicated.

Enzymatic Biotransformation of Biogenic Amines

The biological destruction of 5-HT is catalyzed largely by MAO, and of catecholamines, by MAO and catechol-O-methyltransferase (COMT). A large number of drugs, capable of inhibiting MAO, are available; and there are a few classes of compounds known to inhibit COMT. Because of their potential clinical usefulness and their importance as experimental tools, these chemical compounds have been the subject of numerous reviews, so that further review here seems superfluous. The reader is directed to references (21, 24, 27, 28, 351, 352) for MAO inhibitors and to (225) for COMT inhibitors. In relation to the section above on feedback inhibition by endogenous products and substrates, some of the acid degradation products of epinephrine and NE have been found to inhibit COMT (353).

Summary

Several broad generalizations seem to emerge from consideration of the foregoing information.

(*a*) This is a burgeoning field in which fundamental biochemical and physiological information is at a stage where it is now possible to formulate first approximations of the mechanism of pertinent drug action.

(*b*) While it is possible to pin-point the action of a number of compounds that alter the metabolism of biogenic amines, the precise mechanism of action of the important drugs that influence the amines (e.g. reserpine, amphetamine, chlorpromazine, imipramine) is still unknown. A partial exception to this is α-methyl-DOPA, the mechanism of which seems to rest upon its decarboxylation and β-hydroxylation to form a "false transmitter."

(*c*) A truly satisfactory understanding of the mechanism of action of any agent which alters the metabolism or receptor interaction of the amines in

brain must await the demonstration of the physiological functions subserved by these amines.

(*d*) There is a growing body of evidence that the catecholamines at least exist in neurons in certain functionally and pharmacologically defined pools; but these pools have not yet been identified morphologically. The above survey of the literature has indicated to us a need for more investigations attempting to correlate changes in the subcellular and cytochemical localization of amines with pharmacologically induced and physiological activity.

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