EFFECT OF DRUGS ON AMINES IN THE CNS[1] 6510

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Important aspects of the effects of drugs on various stages of the life cycle of biogenic amines in brain including the active transport of the precursor amino acids across the blood-brain barrier, sequential biosynthesis, storage of monoamines in neuronal synaptic structures, release of monoamines, interaction of the amines with receptor sites, and termination of the effects by either enzymatic destruction, reuptake mechanisms, or diffusion, have been reviewed extensively in previous volumes of the *Annual Review of Pharmacology* (1–5) and at length in other reviews or monographs (6–17). For these reasons and because of space limitations, we restrict ourselves to a discussion of selective recent data which either support or change interpretations on the mechanism of action of drugs on amines, particularly catechol- and indolealkyl-amines in brain. Histamine has been excluded since its neuronal function in the CNS and its correlation to behavior, as modified by drugs, is still little explored. It is likely that, with the use of new analytical methods for the determination of histamine, this amine will also become a candidate for the regulation of neuronal function. A recent comprehensive review on this topic is available (18).

General Considerations on the Effect of Drugs on Biogenic Amines and on Turnover Rates in Particular

In recent years it has become increasingly clear that changes in the turnover rate of biogenic amines provide a better indication of the functional state of populations of neurons than do changes in their tissue levels. Many drugs which fail to alter the tissue levels of monoamines still cause significant alterations in the rate at which the amine stores are renewed, that is, the turnover rate. An instructive example of the complete lack of correlation between the turnover and the static level of NE has been presented by Kopin et al (19). These investigators found that 10 minutes after the administration of octopamine, the activity of tyrosine hydroxylase in heart

[1] Abbreviations used in this review are: NE (norepinephrine), 5HT (5-hydroxytryptamine), 5HTP (5-hydroxytryptophan), 5HIAA (5-hydroxyindole acetic acid), MAO (monoamine oxidase), DMI (desmethylimipramine), DOPA (3,4-dihydroxyphenylalanine).

was decreased whereas 5 hours after the administration of the drug the activity of the enzyme was increased. However, the endogenous levels of NE were reduced to comparable values at both of these times. These results also emphasize the importance of temporal studies for a proper interpretation of the action of drugs. Moreover, it has been shown that chronic administration of nicotine increased the turnover rate of NE in brain while not affecting the concentration of the endogenous catecholamine (20). With regard to serotonergic neurons, the action of LSD illustrates the lack of correlation between the tissue level of 5HT and its turnover. Thus, the hallucinogen increases the level of 5HT in brain (21) but actually decreases its turnover rate (*22, 23*).

A number of methods have been developed to estimate the rate of turnover of amine stores in the peripheral and the central nervous system. These methods have been extensively and authoritatively reviewed by Costa (24) and Costa & Neff (25) and the interested reader is referred to these reviews. We will only briefly point out various conceptual assumptions and limitations of these procedures, which should be considered for a proper interpretation of data on changes of turnover rates of amines by drugs. Almost all of the methods are based on the assumption that a steady-state condition exists between the rate of synthesis and the rate of metabolism of the amines. If a drug or an experimental situation alters the level of the amine, new steady-state conditions must therefore be established and maintained throughout the turnover experiment.

Estimation of the turnover rate of biogenic amines by isotopic methods. —These methods involve either the administration of tracer doses of the labeled amine or the labeling of amine stores by the administration of precursor amino acids. When labeled amines are used to determine turnover, the major assumption is made that the labeled substance mixes uniformly with endogenous amines in a single homogeneous pool. In order to assure this, it is critical that tracer doses of the labeled amine are administered. Neff et al (26), for example, have shown that after administering tracer doses of H^3-NE, the specific activity of heart NE declined exponentially in a monophasic manner, whereas labeling with larger nontracer doses caused a biphasic exponential decline of the specific activity of NE. In the brain, turnover rates of NE have been computed from the decline of NE after the intraventricular injection of the radioactive amine (27). Though many problems remain to be resolved for general acceptance of this method (e.g., nonspecific uptake and binding of the labeled amine, multiphasic decline of the radioactive NE, effect of drugs on these processes), many of the results discussed in this review on the effect of drugs on turnover rate of catecholamines in brain have been obtained by using this method. In the case of precursors, it is assumed that the newly formed amines mix instantaneously with those of the endogenous stores and turn over at a rate identical to that of the latter. However, some of the newly synthesized amines may never be

stored, but may be preferentially released, as has been demonstrated by Kopin et al (28) on stimulation of the splenic nerve. Boullin et al (29) have presented additional evidence that nerve impulses do not release NE from the total store but from a limited pool. Thus, the pool containing the recently synthesized NE rather than the less dynamic storage pool may be the significant one to estimate the actual rate of synthesis of NE. In this regard, Sedvall et al (30) presented evidence that the rate of synthesis of heart NE may far exceed the previous estimates of the turnover of this amine. Since the precursor method requires intravenous infusion and therefore a considerable confinement and limitation of movement of the animal, the question arises as to how such immobilization stress, which in itself alters turnover (31), influences the results. Neff et al (32) have demonstrated, however, that the turnover rate as calculated by this isotopic method agrees well with that obtained by a nonisotopic method.

In order to study the turnover of cerebral 5HT, brain stores have been labeled with radioactive 5HT following the intravenous infusion of labeled tryptophan (33, 34) and the intracisternal administration of tryptophan-H^3 (35). In general, similar assumptions have been made in deriving the kinetic model as were made for the estimation of the turnover of catecholamines. The validity of the use of the rate of decline of labeled 5HT following its intraventricular administration in tracer amounts has not been unequivocally established as a method for estimating the turnover of endogenous 5HT stores in brain.

Estimation of the turnover rate of biogenic amines by nonisotopic methods.—The nonisotopic methods which have been most widely used to determine turnover of biogenic amines include the measurement of the rate of decline of tissue NE after inhibition of its synthesis by tyrosine hydroxylase inhibitors (36, 37), the accumulation of 5HT or the decline of 5HIAA after inhibition of MAO (38), and the accumulation of 5HIAA after the administration of probenecid (39). Since only competitive inhibitors of tyrosine hydroxylase, such as α-methyltyrosine (which competes for the substrate) and derivatives of dopacetamide (which compete with the cofactor) are available, it is imperative that high tissue levels of the inhibitors are maintained to assure complete inhibition throughout the course of the experiment. Moreover, various test situations and drugs may alter the metabolism and distribution of the blocking agent and hence lead to erroneous conclusions. When synthesis and turnover rates of 5HT in brain are estimated by employing nonisotopic methods, the following assumptions have to be made: (*a*) oxidative deamination of 5HT is the major metabolic pathway for 5HT in brain; (*b*) the MAO-inhibitor used blocks MAO exclusively and does not exert other effects on the life cycle of 5HT; (*c*) negligible amounts of 5HT are lost from brain by diffusion.

Finally, it must be emphasized that conclusions on the influence of drugs on turnover of amines that are based on only one time for estimating a rate

constant may be erroneous. For example, Nybäck & Sedvall (40) concluded on the basis of measurements at an early time after the administration of the labeled tyrosine that chlorpromazine selectively increased the turnover of dopamine without affecting that of NE. However, at later times the neuroleptic caused a similar alteration in the turnover of NE and dopamine (41). Moreover, results which are based only on one method for the estimation of turnover may well be subject to extensive revision.

Biochemical Pharmacology of Amphetamine and Amphetamine Derivatives

Amphetamine.—While it has long been accepted that the peripheral sympathomimetic action of amphetamine is mediated through catecholamines (42–44), only recent experimental data indicated that many of its central actions, including locomotor stimulation and stereotyped behavior, might also be mediated through catecholamines in brain (45–49). Moreover, studies with tyrosine hydroxylase inhibitors have led to the view that amphetamine is an indirectly acting sympathomimetic amine whose central actions require an uninterrupted synthesis of catecholamines (50–52). Thus, the locomotor stimulation, stereotyped behavior, anorexigenic effects, and stimulation of nondiscriminated avoidance behavior evoked by amphetamine are blocked by low doses of α-methyltyrosine (α-MT) which do not alter the locomotor activity of control animals and do not exert central adrenergic blocking properties. Moreover, doses of α-MT which clearly block the central action of amphetamine do not interfere with either the metabolism of amphetamine or its entry into the brain (53, 54), and its locking action can be restored by the administration of 1-DOPA (50, 51, 55). Amphetamine has been shown to release preferentially H^3-catecholamines newly synthesized from H^3-tyrosine in brain slices (56). Moreover, in moderate pharmacological doses it does not significantly increase the release of stored H^3-NE into the perfusate from the hypothalamus in vivo (57).

The persistence of the central action of amphetamine in reserpinized animals can no longer be interpreted to indicate that amphetamine is a directly acting sympathomimetic in the CNS, as the administration of reserpine does not impair the activity of enzymes involved in the synthesis of catecholamines in brain (58, 59) and actually increases the turnover rate of brain catecholamines (60, 61). The reported enhancement of some of the central actions of amphetamine in animals treated acutely or chronically with reserpine (62, 63) is clearly not the consequence of an impaired metabolism of amphetamine (54, 64, 65) and might in part be the consequence of an enhanced receptor
(66).

Rapid stimulation of peripheral sympathetic nerves results in a selective release of newly synthesized NE, indicating that mobilization of stored NE plays a minor role in the maintenance of transmitter release (28). It is tempting to speculate that the NE released by rapid peripheral sympathetic

nerve stimulation and that released by amphetamine in the CNS may arise from the same "functional" pool within the neuron. Moreover, low doses of amphetamine which fail to alter the steady state levels of endogenous catecholamines, have been reported to increase the turnover rate of NE (67) and dopamine (68) in brain. Assuming that neuronal activity parallels turnover of monoamines, these effects rather than the decrease of tissue concentrations of catecholamines, may be responsible for the central actions of amphetamine. The susceptibility of the actions of amphetamine to blockade of the synthesis of catecholamines by tyrosine hydroxylase inhibitors is in accord with this view.

In species in which hydroxylation of the aromatic ring of amphetamine is a major metabolic pathway (69, 70), the in vivo formation of the "false transmitter," p-hydrozynorephedrine (71), may be responsible for some of the pharmacological actions of amphetamine. Groppetti & Costa (72) have shown that, in rats, the persistence of NE depletion in heart and brain after the administration of amphetamine is related to the presence of p-hydroxynorephedrine. However, the formation of this metabolite is not required for the primary release of NE (73, 74, and unpublished observations from this laboratory) or for most of the pharmacological effects of amphetamine. For example, the l-isomer of amphetamine is not a substrate of dopamine β-hydroxylase and is not converted to p-hydroxynorephedrine in vivo (75), yet it causes the release of NE in a number of tissues. Moreover, locomotor stimulation, stereotyped behavior, piloerection, and hyperthermia are still present and can even be enhanced and prolonged when the formation of p-hydroxynorephedrine is decreased or prevented by tricyclic antidepressants such as DMI (64, 68, 72, 74, 76). In contrast, when the formation of the hydroxymetabolites is blocked, the cardiovascular effects of amphetamine are diminished (77 and unpublished observations from this laboratory). This obvious difference in the action of amphetamine on central and certain peripheral adrenergic neurons poses a fascinating problem for further exploration. The observation that a phenolic group is a structural requirement of NE depletion by intracisternally administered phenylethylamines (78) is not necessarily at variance with the above view since nonphenolic phenylethylamine derivatives may not reach and maintain depleting levels in brain after their intracisternal administration. The accumulation of p-hydroxynorephedrine in adrenergic neurons after chronic treatment with amphetamine may be involved in the development of tolerance to some of the effects of amphetamine (79, 80).

Since tyrosine hydroxylase inhibitors affect the synthesis of both NE and dopamine and block both the locomotor activity and the stereotyped behavior elicited by amphetamine, it is not possible to determine whether a particular effect is attributable to an interaction with NE or dopamine or both. Recent data suggest that the stereotyped activity elicited by amphetamine depends on the availability of dopamine (47, 81), whereas NE appears to be required for other forms of activity, e.g., locomotor stimulation

and aggression. Inhibitors of dopamine β-hydroxylase, such as diethyldithiocarbamate and disulfiram, decrease both spontaneous and amphetamine induced locomotor activity, but do not inhibit and actually may enhance the amphetamine-induced stereotyped activity (47, 82–85). Taylor & Snyder (86) demonstrated that d-amphetamine is a more potent inhibitor of catecholamine uptake by NE neurons in the brain than is l-amphetamine, whereas the two isomers are equally active in inhibiting catecholamine uptake by the dopamine neurons of the corpus striatum. These results of in vivo experiments agree with earlier in vitro studies on the inhibition of uptake of catecholamines by the two isomers into synaptosomes from striatal and nonstriatal brain regions (87, 88). d-Amphetamine is ten times more potent than l-amphetamine in enhancing locomotor activity, while it is only twice as potent in eliciting the typical compulsive gnawing behavior (86). These data lend further support to the view that the locomotor stimulation induced by amphetamine involves central NE neurons while dopamine neurons play an important role in the production of stereotyped behavior. The results described above, as well as other studies (46, 89), suggest that an inhibition of uptake of catecholamines by amphetamine may also be an important mechanism in the central action of this drug. In addition, direct release (45, 48, 49) and inhibition of MAO (46) have been demonstrated. While Glowinski et al (46) concluded that the action of amphetamine on oxidative deamination was primarily due to direct inhibition of MAO, recent studies by Rutledge (90) provided evidence that the inhibition of the oxidative deamination of NE by amphetamine is primarily an indirect effect which is the result of an inhibition of the neuronal uptake of NE. This conclusion is based on results demonstrating that pretreatment of rabbits with amphetamine causes an inhibition of the oxidative deamination of NE of isolated rat cortex slices and synaptosomes, but not in preparations without an intact neuronal membrane. Interestingly, these effects of amphetamine on oxidative deamination are strikingly similar to those produced by tricyclic antidepressants (46, 91–93) which also inhibit the uptake of amines through the neuronal membrane and do not inhibit MAO (94–96).

Halogenated amphetamine derivatives.—Since the initial report of Pletscher et al (97), many investigators have confirmed that the chlorinated amphetamines cause a selective and long-lasting depletion of cerebral 5HT (98–104). The simultaneous decrease of 5HT and its major metabolite, 5HIAA, caused by these drugs suggests that an inhibition of the synthesis of the amine may be involved. Since tryptophan hydroxylase is the rate-limiting enzyme in the synthesis of 5HT, an inhibition of this enzyme would seem most likely. However, chlorinated amphetamines failed to inhibit the hydroxylation of tryptophan by enzyme preparations isolated from rat liver and from bacteria (97, 98). Moreover, the chlorinated amphetamines have been shown to cause a release of 5HT from platelets and also to inhibit the metabolism of the released amine (100). Other investigations provided evi-

dence that p-chloroamphetamine reversibly inhibits MAO isolated from rat brain (101, 105). MAO-inhibition could explain the decrease in cerebral 5HIAA. However, if the chlorinated amphetamines cause a release of cerebral 5HT, as well as inhibit its metabolism, it would be difficult to explain the marked decline in the level of cerebral 5HT, unless either the amine is metabolized through an unknown alternate metabolic pathway or its synthesis is interrupted. Van Praag and associates have presented evidence that the former possibility is highly unlikely (106, 107). Recent studies in vivo have implicated an inhibition of cerebral tryptophan hydroxylase in the prolonged depletion of cerebral 5HT and 5HIAA by p-chloroamphetamine (108). This interpretation is based on a number of in vivo measurements of the effect of p-chloroamphetamine on the turnover of cerebral 5HT, the synthesis of 5HT from labeled precursors, and the inability of the drug to release labeled 5HT. Recent unpublished results from our laboratory have indicated that the activity of cerebral tryptophan hydroxylase measured in vitro is inhibited after the intraperitoneal administration of p-chloroamphetamine, although the drug does not inhibit tryptophan hydroxylase when added to homogenates of rat brain in vitro. p-Chloromethamphetamine also does not inhibit brain tryptophan hydroxylase in vitro (109). These data indicate that the chlorinated amphetamines might selectively inhibit cerebral tryptophan hydroxylase in vivo and suggest that the sensitivity of the peripheral and central hydroxylase to this inhibitor differs considerably. This interpretation agrees with other investigations demonstrating that cerebral and hepatic hydroxylases have different properties with regard to substrate specificity (110). Moreover, α-methyl-p-chlorophenylalanine has been reported to inhibit the hydroxylation of tryptophan by the hepatic hydroxylase, but this compound is inactive as an inhibitor of cerebral tryptophan hydroxylase, while p-chlorophenylalanine inhibits both enzymes (111). Another halogenated derivative of amphetamine, fenfluramine, has been shown to cause a long-lasting depletion of both 5HT and 5HIAA in brain (112). Like p-chloroamphetamine, fenfluramine does not inhibit the hydroxylation of tryptophan by hepatic preparations (113). It will be of interest to determine if this drug also selectively inhibits cerebral tryptophan hydroxylase.

The chlorinated derivatives of amphetamine elicit central and peripheral pharmacological actions which are similar to those of the parent compounds (101, 114). The behavioral stimulation caused by the chlorinated amphetamines is not related to the alteration in the levels of 5HT in brain (98, 101, 114). The initial excitatory action of p-chloroamphetamine, like that of amphetamine, is, however, temporally related to its effect on cerebral metabolism of NE (89). The initial marked alteration of the metabolism of brain NE complicates the use of p-chloroamphetamine as a tool to study serotonergic mechanisms at early times after the administration of the drug.

Although the metabolism of p-chloroamphetamine has not yet been determined, one would predict that oxidative deamination would be the primary route as the presence of the chlorine group in the aromatic ring would

tend to block p-hydroxylation. Although the metabolites were not identified, Miller et al (115) have demonstrated that there are species differences in the rate of metabolism of p-chloroamphetamine which may explain the differences in intensity and duration of depletion of cerebral 5HT among various species.

Newer Aspects of the Biochemical Pharmacology of Tricyclic Antidepressants

Currently held views on the mechanism of action of antidepressant drugs of the imipramine class have been reviewed in last year's *Annual Review of Pharmacology* by Himwich & Alpers (5). The inhibitory action of tricyclics on the uptake of NE in peripheral and central noradrenergic neurons is well established and generally, secondary amines have been found to be more potent than the corresponding tertiary amines in blocking the uptake of NE through the neuronal membrane (3, 116–120). Recent studies provided evidence that DMI-like drugs exert a similar blocking action on NE reuptake in brain in vivo (121).

Although results obtained with isolated nerve granules of bovine splenic nerves (122) indicated that it is unlikely that reasonable pharmacological doses of DMI impair the "granule amine pump," recent studies using brain slices indicate that DMI may also act on the intraneuronal concentrating mechanism of storage vesicles. When slices of various brain areas (except caudate) were incubated with H^3-tyramine, it was found that DMI given in vivo or added in vitro inhibited the synthesis of H^3-octopamine but not the uptake, retention, and oxidative deamination of H^3-tyramine (123). Since DMI does not inhibit dopamine-β-hydroxylase, these data indicate that the drug prevented the uptake of H^3-tyramine into the storage granules where it is converted to H^3-octopamine. Leitz (124) studied the effect of DMI on metaraminol induced release of NE from heart slices and found that DMI reduced the release of NE to a much greater extent than it blocked the uptake of metaraminol. Since the release of NE and the uptake of metaraminol were stoichiometrically related, the results indicate that DMI not only reduced the uptake of metaraminol but acted within the neuron to prevent metaraminol from releasing NE. However, the finding that DMI blocks the depletion of heart NE by amphetamine (125), without affecting the accumulation of the latter, does not necessarily reflect an intraneuronal action of DMI since DMI-like drugs inhibit the metabolic conversion of amphetamine to p-hydroxyamphetamine and consequently the formation of p-hydroxynorephedrine. These phenolic derivatives of amphetamine appear to be responsible for the prolonged depletion of NE from peripheral storage sites. Moreover, DMI does not block the effect of amphetamine on the decline of the level of NE in brain (74 and unpublished observations from this laboratory).

Interesting differences have recently been pointed out between the effect

of a single dose and long term administration of imipramine on the turnover of NE in brain (126). A single dose of the antidepressant slowed, while chronic administration of the drug increased, the decline of H^3-NE in brain. However, since the level of tritiated NE in brain was measured at one time only, it is not possible to compute the rate of turnover of the endogenous amine. In rats, the chronic administration of imipramine leads to an accumulation of its secondary amine analog, DMI (127), which is known to be a more potent blocker of the membrane transport mechanism for NE. Thus, differences in the duration of drug administration and in the methods utilized for the estimation of turnover may account for some of the discrepancies in the findings of various studies of the effect of tricyclic antidepressants on the turnover of NE in brain (126, 128).

During the last few years, attention has focused mainly on adrenergic mechanisms involved in the pharmacological action of tricyclic antidepressants, and these and other studies have generated the catecholamine hypothesis of affective disorders (129). So great was the momentum for general acceptance of this heuristic hypothesis that other possible interpretations have been obscured. Only recently, it has been demonstrated that the amine uptake mechanism for 5HT in 5HT neurons could be blocked by tertiary amines of tricyclic antidepressants (imipramine, chlorimipramine, and amitriptyline) while secondary amines (DMI, nortriptyline, and protriptyline) were less active (130–132). This greater potency of the tertiary amines compared to the secondary analogs is interesting because this order of activity is the inverse of that found for the inhibition of the uptake of (133, 134). The blockade of the reuptake of 5HT by tertiary amines of tricyclic antidepressants may cause an increased amount of 5HT at its receptor sites. Negative feedback mechanisms might then cause the impulse frequency of the 5HT neurons to decrease, which might explain the decrease in turnover of cerebral 5HT following the administration of these drugs (135–138). The effects of tertiary amines of tricyclic antidepressants on 5HT neurons are provocative and cannot be ignored. The question arises whether the clinical antidepressant activity of these drugs is more closely related to their effects on 5HT or on NE neurons. At present, we would side with the view expressed by Carlsson et al (130) that with respect to psychomotor activation or increase in drive, secondary amines appear in general to be more potent than tertiary amines, suggesting that NE neurons are predominantly involved. As regards brightening of mood in depressed patients, tertiary amines appear to be superior, suggesting involvement of 5HT neurons.

A new tricyclic antidepressant drug, iprindole, is of interest as this drug displays pharmacological (139, 140) and clinical (141) profiles similar to those of imipramine-like drugs. However, unlike the imipramine-like antidepressants, this drug does not block the neuronal uptake of NE or alter the metabolism of intraventricularly administered tritiated NE (142). Iprindole

may well constitute an important tool for further studies on the mechanism of the therapeutic action of this particular group of drugs. Since iprindole is a tertiary amine, it will be interesting to ascertain whether or not this drug interferes with serotonergic mechanisms in brain.

Drug Interactions which Involve Biogenic Amines

Tricyclic antidepressants which block the uptake of NE into central noradrenergic neurons (118, 134) enhance and prolong various behavioral effects elicited by amphetamine (96, 139, 143–146). These properties of imipramine-like drugs have been used to formulate or support various hypotheses on the mode of their antidepressant action, and the potentiation of various central actions of amphetamine has been widely employed as a screen for potential antidepressants. Sulser et al (76) first demonstrated that the potent action of DMI in enhancing and prolonging the locomotor stimulation of amphetamine is associated with a striking and sustained increase in the concentration of d-amphetamine in brain due to an inhibition of the metabolism of amphetamine. A number of investigators have since confirmed these data and provided convincing evidence that this action of DMI-like drugs is the consequence of an inhibition of p-hydroxylation of amphetamine (64, 72, 74, 147–149). It could still be argued, however, that tricyclic antidepressants enhance the action of amphetamine by blocking the reuptake of catecholamines which are released by increased amounts of amphetamine onto adrenergic receptor sites. Studies on the pharmacology of a new antidepressant drug, iprindole, have weakened this argument. This drug shares with imipramine-like antidepressants the ability both to potentiate and prolong many central actions of amphetamine (139, 140) and to inhibit markedly the metabolism of amphetamine (140). This inhibition of the metabolism of amphetamine by iprindole appears to be the sole factor involved in the interaction of the two drugs, as iprindole does not block the neuronal uptake of NE or alter the metabolism of intraventricularly administered H^3-NE (142). Small doses of chlorpromazine, though usually reducing the intensity of amphetamine-induced locomotor stimulation, have been reported to prolong many effects elicited by amphetamine (146, 150–152). This action of chlorpromazine is also associated with a marked and prolonged elevation of the level of amphetamine in brain caused by an inhibition of the metabolism of amphetamine by chlorpromazine (153). High doses of chlorpromazine, however, block the action of amphetamine on locomotor stimulation despite the increased level of amphetamine in brain. These results have since been confirmed and extended (64, 154, 155). The observable pharmacological effects of the chlorpromazine-amphetamine combination appear to depend on the relative potency of chlorpromazine in blocking p-hydroxylation of amphetamine (potentiation and prolongation), in inhibiting the reuptake of the catecholamines (156) released by amphetamine (potentiation), and in blocking adrenergic receptor sites (blockade). Such studies emphasize the importance of metabolic considerations in the

proper interpretation of drug interaction studies. It is noteworthy that chlorprothixene, chlordiazepoxide, diazepam, nialamid, and cocaine have also been found to inhibit p-hydroxylation of amphetamine in vivo (64).

Compounds which alter the level of either catechol- or indolealkylamines have been widely used as tools to ascertain the role of biogenic amines in the pharmacological action of various drugs. While we agree with Weiss & Laties (157) that the establishment of dose-response curves for amine depletors is imperative in biochemical pharmacology, they misinterpreted our data on the relative role of storage and synthesis of brain NE in the psychomotor stimulation evoked by amphetamine or by the DMI-tetrabenazine combination (53). The doses of α-MT (50 mg/kg) and α-MMT (500 mg/kg) have been deliberately chosen and are based on an analysis of their effect on both the rate and the degree of depletion of NE in brain. The purpose of the study was to demonstrate that the failure of either d-amphetamine or of the DMI-tetrabenazine combination to elicit behavioral stimulation can not be correlated with the absolute level of NE in brain, and that the mechanism by which the reduction in the level of catecholamines is brought about, is critical.

Zhelyaskov et al (158) have demonstrated that α-methyl-5-hydroxytryptophan (α-M5HTP) inhibits tyrosine hydroxylase in vitro by a mechanism that is not competitive with the substrate, and that the drug depletes brain and heart NE in vivo. If the reduction of brain NE were the exclusive consequence of the inhibition of tyrosine hydroxylase, one might expect that α-M5HTP should block the central action of amphetamine. However, α-M5HTP is ineffective in antagonizing the stimulatory effect of amphetamine (159) indicating that either the hypothesis of the critical role of the availability of newly synthesized catecholamines in the central actions of amphetamine has to be revised or α-M5HTP decreases the level of catecholamines in vivo by a mechanism other than inhibition of tyrosine hydroxylase. Dominic & Moore (159) demonstrated that α-MT (50 mg/kg) and α-M5HTP (200 mg/kg) produce a similar reduction of NE in brain but that the latter drug is much less effective in reducing the level of dopamine. Since the turnover rate of dopamine in brain is greater than that of NE (36), an inhibition of tyrosine hydroxylase in vivo by α-M5HTP appears to be an unlikely mechanism. The decarboxylation product of α-M5HTP, α-methyl-5HT, does not inhibit tyrosine hydroxylase (158) but rapidly depletes myocardial NE (160, 161). The decline of cerebral catecholamines by α-M5HTP is probably the consequence of its decarboxylated product, α-methyl-5HT.

The use of reserpine and of tyrosine hydroxylase inhibitors as tools furnished data supporting the view that the central stimulatory action of p-chloroamphetamine is mediated through the relase of stored catecholamines, whereas the storage of catecholamines appears not to be as essential for the central stimulatory action of amphetamine as long as the synthesis of catecholamines is not impaired. Thus, low doses of the tyrosine hydroxylase in-

hibitor, α-MT, blocked both the psychomotor stimulation and the stereotyped behavior elicited by amphetamine (50–53, 55, 159) but very high doses of α-MT are required to antagonize the action of p-chloroamphetamine (114, 162, and unpublished observations from this laboratory). On the other hand, reserpine which impairs the storage but not the synthesis of catecholamines (58), blocked the central action of p-chloroamphetamine but not that of amphetamine (162 and unpublished observations from this laboratory).

Since the initial description by Koe & Weissman (163) of a marked, long lasting and selective depletion of cerebral 5HT by p-chlorophenylalanine (PCPA), this drug has been widely used as a tool to study the role of serotonergic mechanisms in the action of drugs. However, this compound appears not to exert a selective effect on brain 5HT. Thus, following the administration of PCPA, a simultaneous decrease in cerebral NE (164, 165) and a decrease in tyrosine hydroxylase (166) have been reported. Although it has been clearly demonstrated that the decrease in cerebral 5HT is the result of an inhibition of tryptophan hydroxylase (167), the mechanism of the prolonged inhibition of the enzyme is not yet understood (168–170).

The failure of PCPA either to antagonize or shorten the sedative action of reserpine (163) suggests a dissociation between the latter and the biosynthesis of 5HT. The observation appears inconsistent with the view that the sedative action of reserpine and of related substances is mediated by a flow of free 5HT onto receptors (171). The possibility still remains, however, that PCPA might not act uniformly at all sites of 5HT synthesis in brain.

Brain 5HT has been implicated in the mechanism of tolerance to morphine and physical dependence. Thus, during tolerance development to morphine, Way et al (172) found an increase in the turnover of 5HT in the brain of mice. Moreover, tryptophan hydroxylase has been implicated in this mechanism because pretreatment with PCPA reduced both the development of tolerance to morphine and the development of physical dependence. Although confirmation of this action of PCPA on the development of tolerance to morphine in other species is mandatory and the role of an increased synthesis of catecholamines in brain has also to be considered (173), the results suggest that an increased synthesis of biogenic amines in brain may be associated with basic mechanisms involved in the development of tolerance to morphine.

Other Psychotropic Drugs that Affect the Availability of Amines in the CNS

Reserpine-like drugs.—Reserpine and related natural and semisynthetic analogs, certain benzoquinolizines, and ring-substituted aralkylamines are potent depleters of catechol- and indolealkyl-amines because of their interference with the intraneuronal storage of amines (174). Reserpine-like

drugs do not directly inhibit any enzymes involved in the synthesis of catecholamines and 5HT (58, 59). However, reserpine appears to inhibit the uptake of dopamine into the site where dopamine-β-hydroxylase is located, thus impairing the synthesis of NE from dopamine indirectly (59). Recent studies, utilizing a push-pull cannula to measure release of neurohumors into the perfusate from the hypothalamus in vivo, have strengthened the view that most of the NE released by reserpine is deaminated in the nerve endings by MAO (121). However, a small amount of the amine appears to escape deamination after its release by reserpine from intraneuronal storage sites and enter the perfusate. It is tempting to speculate that the amount of NE found in the perfusate after reserpine may indeed have been available at central adrenergic receptor sites. This could explain the finding that central sympathetic outflow is not appreciably reduced after reserpine administration despite a marked depletion of the levels of NE in brain tissue (175–177).

After a large dose of reserpine, adrenergic function recovers centrally (178, 179) and peripherally (180, 181) at a time when the levels of monoamines are still very low. The functional recovery is associated with the ability to take up and store exogenous amines (171, 182–185). Recent studies by Andén & Lundborg (186) have shown that axotomy markedly inhibited the recovery of the uptake-storage mechanism for NE in the caudal spinal cord after reserpine treatment, suggesting that formation and downward transport of new granules from the cell bodies to the nerve terminals are the most important factors in the recovery from reserpine. Studies on the recovery of NE in adrenergic axons of the rat sciatic nerve after high doses of reserpine indicated an increased synthesis and rate of downward transport of amine storage granules during the third to fifth day after reserpine (187).

Although under normal conditions, reserpine does not significantly alter amine transport through the neuronal membrane, Sugrue & Shore (188, 189) called attention to the existence in heart slices of a second Na^+ dependent, optically specific, reserpine-sensitive amine carrier mechanism, which is separate and distinct from the main reserpine-insensitive, relatively nonspecific membrane transport system. The findings that the reserpine-sensitive carrier operates at concentrations of sodium similar to those existing intracellularly are of great interest. Thus, the possibility exists that this carrier system operates at both the neuronal membrane and the intraneuronal storage granule where amine transport would be dependent upon low Na^+ existing intraneuronally.

Anti-Parkinson drugs.—The neuronal uptake of catecholamines in the striatum differs from other areas in the brain with regard to its resistance to inhibition by DMI (190) and its lack of stereospecificity for the uptake of d- and l-NE (87). The latter observation is of teleological interest since the neurons of the striatum contain mainly dopamine which has no stereoisom-

ers. Recently, it was found that a variety of anti-Parkinsonian drugs (benztropine, trihexyphenidyl, diphenhydramine, orphenadrine, phenindamine, diethazine) are potent noncompetitive inhibitors of dopamine uptake into synaptosomes isolated from rat corpus striatum (88) while d- and l-amphetamine are potent competitive inhibitors of the uptake of dopamine by the striatal transport mechanism (87). Inhibition of dopamine uptake by drugs may potentiate the synaptic action of this catecholamine in a manner analogous to the potentiation of NE caused by inhibitors of its neuronal reuptake. The potentiation by anti-Parkinson drugs of the actions of dopamine released at striatal synapses may be related to their therapeutic efficacy. Other potent inhibitors of striatal dopamine uptake, such as l-amphetamine, are currently being clinically tested as potential anti-Parkinson drugs (Snyder, personal communication).

MAO-inhibitors and chlorpromazine-like drugs.—The excitation which occurs in various species after the administration of MAO inhibitors is known to be associated with increased levels of NE in brain (191, 192). However, a measurement of the level of NE in brain cannot provide information concerning its actual availability at central adrenergic receptor sites. Experiments with a push-pull cannula have provided data suggesting that MAO inhibition leads to an increase in extraneuronal NE in brain (193). This increase, as measured in the perfusate from the hypothalamus, may be the consequence of the rise in intraneuronal amines leading to the often suggested "spill-over" from completely filled intraneuronal stores. The increased availability of both intra- and extraneuronal NE may be responsible for the reduced rate of the synthesis of NE after blockade of MAO, as a consequence of feed-back inhibition of tyrosine hydroxylase (194). After the administration of MAO inhibitors to cats, the concentration of 5HT in the effluent from the perfused cerebral ventricles increased (195). Thus, MAO inhibition might also lead to a "spill over" of 5HT from filled intraneuronal 5HT storage sites. This "spill over" may explain the inhibition of the activity of single 5HT containing neurons in the midbrain raphe nuclei following the administration of pargyline, and the absence of its inhibition after pretreatment with p-chlorophenylalanine (196).

The increased rate of synthesis of dopamine in the nigro-neostriatal pathway after the administration of phenothiazines, butyrophenones, and thioxanthenes can be interpreted as a reflex compensation resulting from blockade of dopaminergic receptor sites (197–201). The findings of a rather selective increase in the synthesis of dopamine from tyrosine-C^{14} in the striatum after the administration of chlorpromazine-like drugs, has led to the conclusion that part of the neuroleptic action and particularly the extrapyramidal side effects could be related to the blockade of dopaminergic receptors in the nigro-striatal pathway (200, 202).

Using the increase in the concentration of homovanillic acid in the cerebrospinal fluid as an index of the increased turnover of dopamine following

the administration of chlorpromazine-like drugs to cats, the correlation between the incidence of extrapyramidal side effects in man and the acceleration of the turnover of dopamine in brain is excellent, even when various phenothiazines and haloperidol were compared on a quantitative basis (203). There exist, however, marked species differences in the action of these drugs on the metabolism of dopamine in brain (204).

Miscellaneous

The marked reduction of catecholamines in the CNS brought about by α-methylmetatyrosine (α-MMT) has been explained by stoichiometric replacement of the normally occurring amines by its decarboxylation products (205–208). Recent results provided evidence, however, that α-MMT may also inhibit the synthesis of catecholamines in brain by diminishing the conversion of tyrosine to DOPA (78). The reduction in the levels of catecholamines in brain caused by α-methyltyrosine (α-MT) is the consequence of a competitive inhibition of tyrosine hydroxylase (209). Moreover, the formation of α-methyl-catecholamines (α-methyl-dopamine and α-methyl-NE) from α-MT occurs to a significant extent in vivo and their presence in the CNS has been demonstrated (210). The accumulation of these metabolites may play an important role in the development of tolerance to the behavioral depressant effects of α-MT after its chronic administration (211) and might be in part responsible for the persistence of the depletion of cerebral NE. These possibilities should be considered for a proper interpretation of the drug's action following its acute or chronic administration.

The l-isomer of α-methyltryptophan has been reported to decrease simultaneously cerebral 5HT and 5HIAA. This effect appears to be the consequence of a reduced supply of tryptophan to the brain presumably resulting from an accelerated degradation of the amino acid by hepatic tryptophan pyrrolase (212).

γ-Hydroxybutyrate produces a selective dose-dependent increase of dopamine in brain of normal as well as reserpinized rats (213–215). This effect is not caused by its intermediate, γ-butyrolactone, and is not due to an inhibition of either catechol-O-methyltransferase or MAO (214, 215). The mechanism of this selective action of the drug on the accumulation of dopamine in brain is puzzling and further investigations are necessary to determine the physiological significance of these findings.

Potent selective inhibitors of DOPA decarboxylase in peripheral tissues have proved to be valuable tools in dissociating central and peripheral actions of l-DOPA (216) and α-methyl-DOPA (217). Recently, Henning & Rubenson (218) demonstrated that l-DOPA produced a centrally mediated hypotensive action in rats pretreated with α-hydrazino-β-(3,4-dihydroxyphenyl) propionic acid (MK 485), a decarboxylase inhibitor with minimal central action (219, 220). Systemic administration of l-DOPA without the peripheral decarboxylase inhibitor resulted in a pronounced increase in mean arterial blood pressure, presumably due to the peripheral action of the

decarboxylation products of the amino acid. Interestingly, inhibition of dopamine-β-hydroxylase by a disulfiram-like drug abolished the fall in blood pressure which occurred after MK-485 and l-DOPA. Although neither enzyme activities nor levels of catecholamines in brain have been determined, the results point to the importance of central noradrenergic mechanisms in the hypotensive response to l-DOPA. The use of predominantly peripheral decarboxylase inhibitors such as RO4–4602 [N-(DL-seryl)-N′-(2,3,4-trihydroxybenzyl) hydrazine] or MK-485 [α-hydrazino-β-dihydroxyphenyl) propionic acid] may aid in protecting the orally administered l-DOPA from extensive decarboxylation in the periphery and thus allow the accumulation of higher levels of dopamine in brain after the administration of smaller oral doses (221–223).

Two new potent inhibitors of dopamine-β-hydroxylase have been recently described: 1-phenyl-3-(2-thiazolyl)-2-thiourea (U-14,624) (224) and Fla-63 (225). The former compound selectively decreases NE in the brain without altering the levels of the catecholamine in heart (226).

The catecholamine depleting properties of 6-hydroxydopamine were originally described by Porter et al (227). Recent investigations of the biochemical and morphological effects of 6-hydroxydopamine have shown that this compound causes irreversible damage of peripheral sympathetic nerve terminals, resulting in a selective, long lasting depletion of NE (228–231). The events leading to the degeneration of adrenergic nerve terminals are not yet understood. Although the initial decline in the levels of NE is related to a displacement of the neurohumor from intraneuronal storage sites by 6-hydroxydopamine, intraneuronal binding of the drug is not essential for the degenerative process (232–234). The intraventricular administration of 6-hydroxydopamine causes a long-lasting reduction of cerebral NE and dopamine which suggests a similar destructive effect on central adrenergic neurons (235–239). Since the drug does not affect the brain concentration of 5HT, the selective "chemical sympathectomy" produced by this compound should prove to be a useful tool in the investigations of central adrenergic mechanisms. More data on the mechanism of the biochemical and ultrastructural effects elicited by the drug are, however, still needed to use it effectively.

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