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METABOLISM OF NOREPINEPHRINE IN THE CENTRAL NERVOUS SYSTEM

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I. INTRODUCTION

The uneven regional distribution of norepinephrine and the striking effects on its concentrations in the mammalian brain by certain drugs that affect behavior were reported more than 10 years ago by Vogt. These early observations stimulated great interest in the biochemistry and pharmacology of central catecholamines. While knowledge about the biochemical pharmacology and metabolism of catecholamines in the peripheral sympathetic system has accumulated rapidly, progress in brain research has been limited by such difficulties as the blood-brain barrier for catecholamines.

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In the past 3 or 4 years, new experimental approaches have brought striking advances in our understanding of central amines. New histochemical techniques permit the direct visualization of central monoamine-containing neurons. The newer centrifugal fractionation techniques have greatly aided the study of the intracellular localization of catecholamines and the enzymes involved in their metabolism. Radioactive tracer techniques, which had been developed for the study of peripheral catecholamine metabolism, have been applied to the study of the central nervous system. The availability of many new drugs that affect brain norepinephrine has contributed greatly to our understanding of norepinephrine metabolism in the brain.

The present review will concentrate on norepinephrine metabolism in the mammalian brain. A great deal of information is available about the metabolism of brain norepinephrine. This is largely due to the fact that information gained, and techniques developed in the study of the peripheral metabolism of norepinephrine have been applied successfully to the study of this amine in the brain. The biochemistry of central norepinephrine and the effects of drugs on its metabolism are discussed. In the pharmacological survey, some aspects of the mechanisms of drug action are pointed out, but the contributions of drug studies to the present understanding of the various aspects of central norepinephrine metabolism will be emphasized. Several reviews are available which deal with various aspects of catecholamine metabolism in the brain, or related subjects (32, 100, 155, 159, 230, 266). The present review includes literature available up to April, 1966.

II. DISTRIBUTION

A. Gross anatomical distribution

The presence of an adrenergic substance "sympathin" in extracts of mammalian brain was first described by von Euler in 1946 (90). A few years later, Holtz (156) confirmed this observation in brain and spinal cord and showed that norepinephrine concentrations far exceed those of epinephrine. The presence in brain tissue of dopamine, the precursor of norepinephrine, was suspected in 1957 by Montagu (224), demonstrated that year by Weil-Malherbe and Bone (304), and confirmed by Carlsson *et al.* (57).

Until 1954 it was generally suspected that brain norepinephrine may be located mainly in blood vessels. In that year, however, Vogt measured catecholamines of cat and dog brain by bioassay and first demonstrated their relatively high concentrations in specific anatomical areas. This regional localization supported the concept, now widely accepted, that catecholamines are located in central neurons and may play a role in their function.

Whole brain concentrations of norepinephrine have been reported in several species (28, 110, 134, 224, 257, 277), ranging from about 0.10 to 0.50 μg/g, with 0.21 μg/g found in human brain (224). Brain norepinephrine concentration appears to increase with age, but a difference in concentration between newborn and adult animals is found only in those species which are neurologically relatively immature at birth, such as the rat and rabbit (25, 172). There is some

uncertainty about the presence of epinephrine in the mammalian brain. In early studies, its concentration was estimated to range from 5 to 25% of norepinephrine levels in several species (224, 302). Recent studies with sensitive and specific methods suggest that epinephrine levels may be considerably lower (137, 277, 305) (see Section IIIA)

The pattern of distribution of norepinephrine first described by Vogt in 1954 (302) for the dog and cat brain is generally similar in other species. The highest concentrations are in the hypothalamus, with a range of 1 to 3 μg/g in man (25, 26, 270), rat (120), cat (28, 197), and monkey (257). Other regions can be ranked as follows: midbrain and pons, medulla oblongata and striatum, followed by a group of structures of very low concentration, including hippocampus, cerebral cortex, cerebellum (25, 28, 120, 197, 257, 270, 302), and spinal cord (195, 201). In the corpus striatum, and particularly in the caudate nucleus, where norepinephrine concentration is relatively low (275), dopamine is found in very high concentrations (3 to 8 μg/g) (28, 159). There is generally more norepinephrine in gray than in white matter (89). Since various dissection procedures were used, it is not possible to describe more precisely the regional distributions of endogenous catecholamines in the central nervous system.

B. Systems of norepinephrine neurons

Since 1962, fluorescence microscopy has been used to study the localization of catecholamines at the cellular level (95, 96). This technique is based on the formation of specific fluorescent derivatives of biogenic amines in the presence of formaldehyde. Monoamine-containing neurons in brain tissue were first described by Carlsson *et al.* (47, 48). The distinction between catecholamines and serotonin has been based on differences in the colors of their fluorescent products (95). In the presence of formaldehyde, norepinephrine and dopamine are converted to isoquinolines with very similar fluorescence spectra. A recent report suggests that it may be possible to distinguish between them by another histochemical reaction (61), but at present, complex pharmacological methods are used to differentiate norepinephrine and dopamine. After treatment of animals with α-methyl-*m*-tyrosine (α-methyl-*m*-hydroxyphenylalanine) (102), neurons that contain mainly dopamine recover their fluorescence faster than norepinephrine-containing neurons. This appears to be due to the persistence of nonfluorescent metaraminol, formed by decarboxylation and β-hydroxylation of α-methyl-*m*-tyrosine in norepinephrine-containing neurons (3, 102). After treatment with the tyrosine analogue, metatyrosine (*m*-hydroxyphenylalanine), fluorescence disappears selectively from catecholamine neurons, but not from serotonin-containing neurons (102). Treatment with α-methyl dopa, followed by reserpine, leads to the disappearance of the fluorescent α-methyl dopamine from dopamine-containing neurons; but in norepinephrine-containing neurons, fluorescence does not disappear because the α-methylnorepinephrine formed is relatively resistant to depletion by reserpine (45).

In many areas of the brain fine nerve fibers with extensive terminal arborizations and "varicosities" containing large amounts of catecholamines or sero-

tonin (102, 103) have been demonstrated with these techniques. These fibers resemble sympathetic nerve fibers of the periphery (104). The norepinephrine terminals are widely distributed, from the spinal cord to the cerebral cortex, but are most abundant in the hypothalamus (47), olfactory bulb (78), retina (202), median eminence (101), limbic system, and many nuclei of the cranial nerves (70, 73, 74, 104). The distribution of these fibers in the spinal cord has also been described (46, 74, 75). Dopamine terminals are found mainly in the neostriatum, olfactory tuberculum, and nucleus acumbens (45). Certain areas, especially the lower brain stem and parts of the hypothalamus, appear to contain collections of cell bodies with lower concentrations of catecholamine or serotonin (73). Norepinephrine-containing cell bodies tend to be concentrated specifically in the ventral brain stem (73).

Several kinds of evidence support the conclusion that amines are contained within specific neurons in the central nervous system. The experimental approaches used include axotomy, inhibition of the catabolism of amines, and the creation of specific lesions. After axotomy, catecholamines and serotonin accumulate at the section, proximal to the cell body, particularly after the use of monoamine oxidase (MAO) inhibitors (72). This phenomenon and the retrograde cell body reaction after axotomy have been useful in mapping the distribution of specific systems of central amine-containing neurons.

Chemical and histochemical techniques have been used in animals to relate the disappearance of neuronal amines to the effects of lesions. Gross mesencephalic and specific hypothalamic lesions lead to a fall in catecholamine and serotonin concentrations or fluorescence in the brain (79, 147, 148). Lesions have been used to demonstrate a nigro-striatal system of dopamine-containing neurons (6, 104, 250). Thoracic cord section was found to decrease spinal cord norepinephrine (201), and subsequently several norepinephrine-containing bulbospinal systems were described (74). In addition, ascending systems containing norepinephrine neurons extend from the lower brain stem to the hypothalamus, preoptic area, limbic system, and cortex (5, 79, 104). Precisely localized lesions in the median forebrain bundle of the lateral hypothalamus, and in the dorsomedial brain stem tegmentum significantly decreased both norepinephrine and serotonin in the whole brain, whereas lesions in the ventrolateral tegmentum decreased norepinephrine only (149). Median forebrain bundle lesions in the cat reduced norepinephrine concentrations, mainly in the telencephalon (150). Since there is no known direct neural pathway between these structures, the depletion cannot result from direct destruction of monoamine-containing neurons. It is suggested that in contrast to the peripheral sympathetic system, trans-synaptic decreases of norepinephrine may be possible in the brain (150). However, cortical lesions did not produce changes in brain norepinephrine concentration (1).

Biochemical (117, 120) and electron microscopic radioautographic (30, 185) evidence indicates that radioactive norepinephrine, administered intraventricularly, labels the endogenous stores of catecholamines. The localization of carbon-14 by radioautography after the intraventricular administration of ^{14}C-norepinephrine has been used to study the distribution of catecholamine-

containing structures in the brain, and the interrelationships of catecholamine-containing nuclei and tracts (260). Catecholamine-containing systems are localized in the brain stem, hypothalamus, limbic system, and other subcortical structures, in general agreement with the conclusions of previous chemical and histochemical studies.

C. Intraneuronal localization

The intracellular localization of norepinephrine has been studied with centrifugation and electron microscopy. In 1957, Weil-Malherbe and Bone isolated from brain homogenates a particulate fraction rich in norepinephrine (305). This finding has been confirmed by other investigators (27, 134). Crúsciel (69) found that norepinephrine is concentrated in a particulate fraction in the dog hypothalamus. This fraction, described by Whittaker (317), contains "pinched off nerve endings" or "synaptosomes" (86, 131, 317), and also contains acetylcholine and serotonin (146, 217, 218). In rat brain stem (186) and bovine hypothalamus (212) preparations, 60% of the total norepinephrine was in a cell fraction containing synaptosomes and mitochondria. After further purification, 50% of the norepinephrine in this fraction was confined to synaptosomes (212). Regions of the rat brain with high concentrations of endogenous norepinephrine, such as hypothalamus and medulla oblongata, have relatively higher proportions of this amine localized in particulate fractions than structures with low concentrations of endogenous norepinephrine, such as cerebellum and cerebral cortex (121).

Rat brain synaptosomes can be disrupted by osmotic or sonic shock to liberate free vesicles which contain norepinephrine in amounts, varying with the vigor of the disruption procedure (84, 214). These liberated vesicles resemble synaptic vesicles seen in intact tissues by electron microscopy, although they do not exhibit the sedimentation characteristics of splenic nerve particle preparations (214). "Dense core" vesicles, somewhat larger than those found in peripheral adrenergic nerve endings, are found in the anterior hypothalamus of the rat (242), but these granulated vesicles are rare compared to the simple type of vesicle commonly found in the brain (84, 214). It has been suggested that the frequency of the granulated vesicles, as estimated from electron micrographs of tissue sections (162) or of subcellular fractions of specific brain regions (85), may be correlated with the concentration of endogenous norepinephrine in the hypothalamus. Precipitates of amines within synaptic vesicles have recently been shown with a histochemical method combined with electron microscopy (318). With these methods one is not yet able to distinguish endogenous norepinephrine from dopamine and serotonin.

Histochemical findings indicate that norepinephrine is highly localized at axonal varicosities (48), which probably correspond to the synaptosomes containing norepinephrine that are obtained by subcellular fractionation. Normally there is relatively little norepinephrine in cell bodies and proximal axons (48), although the amount varies from cell to cell and region to region. There tends to be a region of moderate norepinephrine concentration around the nuclei of certain neurons (73).

In general, it appears established that central norepinephrine is largely contained in a particulate component of the neuron. The origin of the norepinephrine found in the soluble subcellular fraction of brain homogenates and its possible functional significance as a site of storage are subjects of controversy. The existence of an unbound form of intraneuronal norepinephrine is not yet clear.

III. METABOLISM

A. Synthesis

The entry into the mammalian brain of norepinephrine (308) and its immediate precursor, dopamine (220), is severely limited by a blood-brain barrier, which appears to be fully developed at birth in the rat (118). Nevertheless, these amines are present in the brain in significant concentration; this suggests that they are synthesized locally in brain tissue. Precursors and enzymes for norepinephrine synthesis, first shown in the peripheral sympathetic system, are present in the brain. In addition to its precursor function, dopamine may also be a central neurotransmitter in certain neurons. Phenylalanine and tyrosine are normal constituents of brain, present in a free form in low concentrations (294). Dopa (dihydroxyphenylalanine) is readily decarboxylated to dopamine, and it has been difficult to measure its concentration in brain tissue (9, 49, 224, 270). Peripherally administered tyrosine (59) and dopa (49, 111) enter the brain (37, 299). These precursors are actively concentrated in brain slices *in vitro* (139, 319).

The enzymes necessary to convert tyrosine to norepinephrine are present in the central nervous system. Tyrosine hydroxylase activity has recently been measured in homogenates of brain stem (235) and of caudate nucleus (16). The conversion of tyrosine to dopa is the rate-limiting step in the synthesis of norepinephrine in the peripheral sympathetic system, and it is likely to be rate-limiting in the brain as well (187, 289). In addition, nonspecific decarboxylation by which dopamine is formed from dopa (157) and several other amines from amino acids (192) occurs throughout the brain and the regional distribution of the decarboxylase involved generally follows that of catecholamines (28, 31, 182). The hydroxylation of dopamine (173) is the last step required for norepinephrine synthesis, and several phenylethylamine derivatives appear to be hydroxylated by the same enzyme (66). Activity of dopamine β-hydroxylase has been detected (296) in the hypothalamus and, somewhat surprisingly, in the caudate nucleus, where very little norepinephrine is found. The methods currently used to assay this enzyme have been criticized (65, 296) as lacking specificity and sensitivity, but a new method of assay (65) may clarify its central distribution. Although dopamine neurons predominate in the caudate nucleus, there may be a small proportion of norepinephrine-containing neurons in this region which contain dopamine β-hydroxylase. Radioactive norepinephrine was not formed *in vitro* from labeled precursors in caudate tissue (208), but has recently been found in the caudate nucleus of the cat (198) and the basal ganglia of the rat (120) after the administration of labeled precursors directly into the brain *in vivo*.

The peripheral administration of catecholamine precursors can influence the formation and concentration of dopamine and norepinephrine in the brain. The

administration of amino acids that compete with tyrosine for uptake in brain (59) decreases brain catecholamine concentration (133). The administration of dopa is followed by a marked increase in dopamine concentration in brain tissue (28, 42, 120, 197, 307), while brain norepinephrine concentration appears to be much less increased (42, 307). Some elevation of norepinephrine has been found, particularly in areas of the brain with very low endogenous norepinephrine concentration, such as cerebellum and cerebral cortex (197). The effect of dopa to increase the concentration of dopamine much more than norepinephrine may be partly explained by the presence of decarboxylase activity in sites lacking dopamine β-hydroxylase activity, possibly in serotonin neurons, for example.

More direct demonstration of the formation of catecholamines from their precursors in brain tissue has been achieved by the use of radioactive precursors. Thus, radioactive dopamine and norepinephrine have been found in slices of whole brain (207) and slices of specific areas of brain (208) after incubation with ^{14}C-tyrosine (207). Labeled dopa, dopamine and norepinephrine have been found in the brain after the introduction *in vivo* of ^{14}C-tyrosine into specific nuclei of the cat brain (198) or into the lateral ventricle of the rat brain (122). Similar results were obtained after the peripheral administration of ^{14}C-tyrosine (37, 299). Labeled norepinephrine was also found in brain after the peripheral administration of ^{3}H-dopa (120), or ^{14}C-dopamine (260).

Thus, a pathway for the synthesis of norepinephrine from tyrosine, similar to that present in the peripheral sympathetic system, has been demonstrated in the brain. Alternative enzymatic steps for the synthesis of central norepinephrine may exist. For example, it has been suggested that the brain contains an enzyme that transaminates aromatic amino acids (99); since this reaction seems to be reversible, dopa might form in the brain by transamination of 3,4-dihydroxyphenylpyruvate (109).

The presence of epinephrine in mammalian brain tissue is uncertain, and activity of the methyl transferase necessary to form epinephrine from norepinephrine has been very difficult to detect in brain tissue *in vitro* (10). However, there is some evidence that very small amounts of radioactive epinephrine and its O-methylated derivative, metanephrine, can be synthesized in brain *in vivo* after the injection of ^{14}C-tyrosine or ^{3}H-norepinephrine into the brain stem of the cat (196), or of ^{14}C-dopamine into the lateral ventricle of the rat (220).

The intracellular localization of norepinephrine synthesis in the brain is now under study. After depletion with reserpine or α-methyl-*m*-tyrosine (76), catecholamine fluorescence reappears first in the cell body, and after axotomy it accumulates proximally within cell bodies and axons (72). These observations have been taken to mean that norepinephrine is synthesized in cell bodies and may be transported in a particle-bound form to the nerve endings (72, 76). Two enzymes needed for norepinephrine synthesis, tyrosine hydroxylase (16, 235) and and dopamine-β-hydroxylase (296), have been found highly localized in a crude subcellular fraction that contains mitochondria and synaptosomes. Tyrosine hydroxylase activity (194) and the activity of nonspecific amino acid decarboxylase (262) were retained in a purified brain synaptosomal subfraction that ap-

parently represents nerve terminals. These enzymes appear to synthesize norepinephrine at nerve terminals, since radioactive dopamine and norepinephrine, which form rapidly *in vivo*, can be isolated in a purified synaptosomal fraction of rat hypothalamus after the intraventricular introduction of labeled dopa (121).

B. Uptake and storage

Exogenous catecholamines accumulate in catecholamine-containing neurons by a relatively specific but complex phenomenon commonly called "uptake," which includes at least 2 stages. We use the term "uptake" to denote the transfer of catecholamines across cell membranes into the intraneuronal space by active transport (82, 164). A second process includes intraneuronal redistribution and retention of amines in specific intracellular storage sites.

The accumulation of exogenous norepinephrine administered into the peripheral circulation occurs only to a small extent in the brain (308, 314), and only in areas where the blood-brain barrier for catecholamines is minimal, such as the area postrema and parts of the hypothalamus (106, 308). The problem of the blood-brain barrier has been circumvented by *in vitro* studies and by the direct introduction of radioactive catecholamines into the brain.

Initially, Dengler and his co-workers showed that slices of brain accumulate tritiated norepinephrine to levels up to 5 times those of the incubating medium (82) if the concentration of the amine in the medium is small. Their evidence favored the existence of a saturable and active process of norepinephrine accumulation, which could be inhibited by ouabain (G-strophanthin) or metabolic inhibitors. They could not find a similar concentrating mechanism for dopamine in slices of cortex or even caudate nucleus, possibly because of the use of relatively large amounts of ^{3}H-dopamine of low specific activity (82). Recently the cellular accumulation of exogenous norepinephrine in brain slices was shown histochemically in animals pretreated both with reserpine, to deplete endogenous stores of brain catecholamines, and with a MAO inhibitor, to prevent the destruction of accumulated exogenous catecholamines (143). High concentrations of fluorescent material were found in axons and synaptic terminals.

Incubation of minced brain with radioactive norepinephrine, followed by homogenization and subcellular fractionation in a continuous sucrose density gradient, revealed an accumulation of the radioactive amine in a synaptosomal fraction (254). The subcellular distribution of the radioactive amine paralleled that of endogenous norepinephrine (254). The accumulation of exogenous catecholamines in a particulate fraction has also been observed after the incubation of labeled amines with rat brain homogenates (222) or guinea pig brain stem particles (163), and the incubation of unlabeled norepinephrine with particulate fractions of rat (212, 221) or beef brain (212). Exogenous norepinephrine (163, 212, 221) and dopamine (163) accumulated above the concentration of amines in the incubating medium. The effect was greater at 37° C than at 0 to 4° C (163). Temperature-dependent accumulation of exogenous norepinephrine also occurred in a purified synaptosomal fraction and in isolated vesicles prepared by the disruption of synaptosomes (212). In one study with crude particulate preparations, the accu-

mulation of norepinephrine *in vitro* was not prevented by metabolic inhibitors (221). In nerve granules prepared from peripheral sympathetic neurons, ATP and Mg^{++} stimulated the accumulation of exogenous norepinephrine (92). In a crude particulate preparation of guinea pig brain stem, ATP failed to exert a significant effect on the accumulation of ^{14}C-norepinephrine (163). The importance of ATP and Mg^{++} in these processes is not yet clear.

It is likely that an active transport process at the neuronal membrane and binding by intracellular particles are both involved in the accumulation of norepinephrine in nerve terminals. However, the mechanism of binding at the vesicular level has not been elucidated. Active membrane transport or a trapping process, or both, could be involved (212).

Studies have shown that radioactive catecholamines introduced directly into the cerebrospinal fluid accumulate in brain (203, 219, 220). After the injection of a small amount of radioactive norepinephrine (123) or dopamine (120) into the lateral ventricle of the rat, the amines were detected in brain tissue even 1 or 2 days after the injection. The degree of accumulation of ^{3}H-norepinephrine or ^{3}H-dopamine paralleled the concentration of endogenous catecholamines in various parts of the brain (120), with the greatest accumulations in the hypothalamus and the least in the cerebral cortex and in the cerebellum. Since high concentrations of radioactive norepinephrine were found in the corpus striatum, which has a high concentration of dopamine but very little norepinephrine, it appears that exogenous norepinephrine may accumulate in dopamine-containing neurons as well as norepinephrine neurons (120). After administration of ^{3}H-dopamine, the greatest accumulation of this amine occurred in the corpus striatum, where dopamine neurons predominate. In addition, there was a marked accumulation of endogenously formed radioactive norepinephrine in various areas, especially in the hypothalamus and medulla oblongata.

After intraventricular administration of ^{3}H-norepinephrine, the amine accumulated largely in a synaptosomal fraction of whole brain (124, 281), hypothalamus, or caudate nucleus (121). The subcellular distribution of the accumulated ^{3}H-norepinephrine was similar to that of the endogenous amine (124). Similarly, after its intraventricular administration, ^{3}H-dopamine was found largely in a synaptosomal fraction of whole brain (281). In 2 recent electron microscopic autoradiographic studies, after the intraventricular administration of ^{3}H-norepinephrine, photographic grains were located mainly over nerve endings and axons containing large dense synaptic vesicles (30, 185).

Thus, it is apparent from studies *in vitro* and *in vivo* that norepinephrine is actively accumulated and retained in brain tissue. In addition, it is likely that active accumulation of dopamine occurs. The uptake mechanism may be an important central process for the removal of extraneuronal norepinephrine physiologically released, as it is in the peripheral sympathetic system. It is difficult to study this reuptake process directly in the central nervous system. However, it has been found that drugs known to inhibit the uptake of exogenous norepinephrine decrease the accumulation of ^{3}H-norepinephrine endogenously formed in the brain from ^{3}H-dopamine (119). If the labeled norepinephrine is formed in-

traneuronally, and physiologically released, its decreased accumulation may then reflect a blockade of the reuptake process.

C. Depletion and release

Many investigators have studied the disappearance of norepinephrine from the central nervous system, but there is only indirect evidence of its release by neuronal activity. Disappearance of radioactive norepinephrine, either taken up directly (123, 167) or formed endogenously from labeled precursors (37, 167, 299), has been studied to determine norepinephrine turnover rates. These studies indicate the disappearance of a major part of radioactive norepinephrine from whole brain with a half-life of 3 to 4 hours, followed by a slower phase with a half-life of 17 to 18 hours (37, 123, 167, 299). This pattern of disappearance, even of endogenously formed norepinephrine, suggests that norepinephrine is stored mainly in a form with a rapid turnover, and to a lesser extent in a more tightly bound form with a slower turnover.

Chemical (138) and histochemical assays (105) revealed a fall in norepinephrine concentration in various brain regions after prolonged and stressful stimulation of the amygdaloid body. After the inhibition of norepinephrine synthesis, vigorous stimulation of the medulla oblongata hastened the disappearance of fluorescence from spinal nerve terminals (77). Although there is evidence for the central release of acetylcholine (200, 223) and dopamine (200), the direct release of norepinephrine from central neurons by nerve stimulation *in vivo* has not been demonstrated, as it has in the peripheral sympathetic nervous system. After incubation of exogenous norepinephrine with crude brain particles (221), purified synaptosomes, or vesicles from disrupted synaptosomes (212), spontaneous release of the accumulated amine was found to occur by a temperature-dependent process. Release of norepinephrine *in vitro*, associated with increased turnover, has been detected chemically after prolonged electrical stimulation of spinal cord preparations (4). Radioactive norepinephrine, accumulated or endogenously formed in brain slices or fragments, was released into a perfusing medium by electrical stimuli of low voltage and brief duration (177). Stimulation increased the release of radioactive norepinephrine into the medium several-fold above spontaneous efflux. This release could be reproduced with increased concentrations of potassium, which depolarizes membranes, and inhibited by reduced concentrations of calcium.

D. Catabolism

Oxidative deamination and O-methylation, the 2 enzymatic pathways of catecholamine metabolism in the peripheral sympathetic system (12), also occur in the central nervous system (123, 203, 206). The presence of MAO in the brain has long been known (29). Its activity is found widely and rather evenly distributed in the central nervous system, with somewhat greater activity in the hypothalamus (31). Catechol-O-methyltransferase (COMT) is also generally distributed in brain tissue (13), and its cofactor, S-adenosylmethionine, is formed in the brain (13, 19). MAO is present in brain mitochondria (308), mainly at

synaptic nerve endings (261). The precise localization of COMT, a soluble enzyme (13), at synapses has not been determined. Some COMT activity is associated with the synaptosomal fraction, and is made soluble by osmotic shock (2). It is frequently suggested that in the brain, as in the peripheral sympathetic system, COMT functions extraneuronally, but this hypothesis requires further investigation.

Endogenous noremetanephrine (3-methoxy-4-hydroxyphenylethanolamine) (11, 140, 210) and 3,4-dihydroxymandelic acid (210, 269) have been detected chemically in the brain. Several metabolites known to occur in the peripheral sympathetic system were found in the brain after the introduction of labeled norepinephrine into the cerebrospinal fluid. They include normetanephrine, 3,4-dihydroxymandelic acid, 3,4-dihydroxyphenylglycol, 3-methoxy-4-hydroxyphenylglycol, and 3-methoxy-4-hydroxymandelic acid (123, 203, 219). Radioactive normetanephrine and 3-methoxy-4-hydroxphenylglycol were found in highest concentrations (123, 203). Since relatively little 3-methoxy-4-hydroxymandelic acid was found, there may be a reductase in the brain to convert to a glycol the intermediary aldehyde formed by oxidative deamination. Regional differences in the relative concentrations of endogenous normetanephrine and dihydroxymandelic acid were found in rabbit brain (210). From these data it was concluded that there are regional differences in the relative importance of MAO and COMT. However, a tracer study revealed no major regional differences in the relative amounts of methylated and deaminated radioactive metabolites formed from ^{3}H-norepinephrine in the rat brain (120). The degree of accumulation of metabolites is not necessarily an index of the amount formed since they may undergo subsequent metabolism and removal from the brain at different rates. For example, normetanephrine has difficulty passing the "brain-blood" barrier (123, 193) and must first be deaminated.

There is evidence that COMT is important in the extraneuronal metabolism of norepinephrine in the peripheral sympathetic system (179). The evidence for this in the central nervous system is only indirect. After the administration of dopa, a deaminated metabolite of dopamine, dihydroxphenylacetic acid, appeared in the brain before its O-methylated, deaminated derivative, homovanillic acid; this suggested that deamination occurs near the intraneuronal site of formation of dopamine (49). The ratio of radioactive normetanephrine to ^{3}H-norepinephrine was higher after the administration of exogenous norepinephrine than after the formation of ^{3}H-norepinephrine intraneuronally from labeled precursors (122). These findings indicate that exogenous free norepinephrine is attacked by COMT more readily than endogenously formed bound norepinephrine, and are consistent with the conclusion that COMT is very effective in metabolizing extraneuronal norepinephrine. Since MAO is localized in mitochondria (31, 238), and since inhibiting it leads to a marked rise in norepinephrine concentration (24, 68, 132, 243, 287) and fluorescence (106, 142) in central neurons, it is likely that MAO is important in the intraneuronal metabolism of norepinephrine in the brain. It is clear that central norepinephrine is metabolized by both deamination and O-methylation. The cellular localization of these proc-

esses and the importance of each in the initial metabolism of norepinephrine is not yet clear.

E. Turnover

Turnover studies give only an overall estimation of the rate of formation of norepinephrine, which equals its rate of disappearance. The disappearance of norepinephrine from the brain appears to be almost entirely due to enzymatic degradation since unmetabolized brain norepinephrine does not readily reach the circulation (123). The physiologically important processes of release and reuptake of norepinephrine cannot yet be measured directly. An estimate of the rate of formation of norepinephrine was made by observing the restitution of norepinephrine stores after depletion with a short-acting reserpine-like compound. After 8 hours, 60% of the depleted norepinephrine had reappeared (286). A half-life of norepinephrine turnover of 3 to 4 hours has been estimated by measuring the change in specific radioactivity of norepinephrine in whole brain after the peripheral administration of labeled precursors (37, 299), or the direct administration of a small amount of ^{3}H-norepinephrine into the cerebrospinal fluid (123, 167). Similar values for the half-life of norepinephrine turnover were estimated from the disappearance of endogenous norepinephrine following the inhibition of norepinephrine synthesis (64). In addition, there is a much smaller amount of central norepinephrine with a slower turnover (37, 123). The significance of this apparent division of norepinephrine into 2 states is not yet clear.

Different turnover rates of norepinephrine occur in several regions of the rat brain. They were estimated by following the changes in specific radioactivity of norepinephrine introduced directly into the brain, or formed from labeled precursors, or, alternatively, by measuring the disappearance rate of endogenous norepinephrine during the inhibition of tyrosine hydroxylase by α-methyl-p-tyrosine (167), which prevents synthesis of norepinephrine (289). There appears to be an inverse relationship between turnover rate and endogenous concentration of norepinephrine in various parts of the brain (167). In the cerebellum, for example, norepinephrine has a turnover rate about twice that of the hypothalamus. Since the concentration of norepinephrine in the hypothalamus is 10 times higher than in the cerebellum, the amount of norepinephrine synthesized in the hypothalamus per unit time is about 5 times greater (167). In the peripheral sympathetic system norepinephrine turnover is faster in cell bodies than in nerve endings (98). A similar difference in turnover rates within neurons may also occur in the central nervous system.

At present there is little information about the physiological regulation of norepinephrine synthesis in the central nervous system. It is probable that tyrosine hydroxylase is the rate-limiting enzyme. Since norepinephrine can inhibit tyrosine hydroxylase *in vitro* (235), and since norepinephrine turnover is decreased in the presence of a MAO inhibitor (64), it has been suggested that norepinephrine may serve as a modulator of its own synthesis by exerting a negative feedback influence at this enzymatic step (64). Neuronal activity in the brain may also influence the synthesis of norepinephrine. In the peripheral sympathetic

system postganglionic stimulation increases (310) and decentralization decreases (232) the rate of synthesis of norepinephrine by influencing the conversion of tyrosine to dopa. In the brain, neural activity induced by released norepinephrine, or by drugs, may also exert a positive feedback influence on norepinephrine synthesis.

Several concepts about central norepinephrine-containing neurons are firmly based on experimental evidence. Norepinephrine is located intraneuronally, mainly in nerve terminals, and is stored largely by intracellular vesicles in a bound form protected from enzymatic attack. Enzymes necessary for its synthesis from tyrosine are located in central neurons. In addition, the enzymes MAO and COMT, important for the chemical inactivation of catecholamines, are found in the brain. MAO is partly intraneuronal, while COMT may be mainly extraneuronal. There is some evidence that electrical stimulation and membrane depolarization can release norepinephrine in the central nervous system. Finally, a neuronal uptake mechanism for the removal of extraneuronal norepinephrine has been demonstrated. These facts are consistent with the widely held idea that norepinephrine contained in specific neurons acts as a central neurotransmitter. The overall metabolism of norepinephrine in such neurons includes several processes: synthesis and storage, direct intraneuronal metabolism or release, and removal of released norepinephrine by reuptake, extraneuronal metabolism, or circulation.

F. Effects of stress and environment

Several stressful conditions have been shown to lower brain norepinephrine. These include, anoxia (302), electrical shock (21, 213, 228, 241), cold (213), and prolonged swimming or other forced activity (21, 228). Electrical shock did not alter the subcellular distribution of brain norepinephrine (186). Electroconvulsions or audiogenic seizures (184, 272), and sham-rage induced by amygdaloid stimulation (138) lower brain norepinephrine. Stress due to cold increased brain norepinephrine turnover (130). In hibernation, brain norepinephrine concentration (300) and turnover (87) decreased. Grouping of mice produced variable effects on brain norepinephrine, depending on the number of animals. Grouping for a week lowered norepinephrine (312), but grouping for several months elevated brain norepinephrine above the levels in isolated mice, and increased turnover (313). The significance of these observations is not clear, although they have generally been attributed to increased release of norepinephrine with increased neuronal activity.

IV. ACTION OF DRUGS ON CENTRAL NOREPINEPHRINE

A. Possible sites of action of drugs

The available data allow the construction of a theoretical model of the metabolism of brain norepinephrine at the synaptic cleft. A model must account for the processes of synthesis, storage, release, effects on "receptors," and inactivation

by reuptake or metabolism. These processes are represented schematically in Figure 1. Drugs that affect norepinephrine metabolism may affect any of these processes. Certain aspects of norepinephrine metabolism are not clear, such as the compartmentalization of stored norepinephrine, the process of release, and postsynaptic effects. There is evidence that norepinephrine is stored in more than one form, possibly in different kinds of vesicle, in different states within the vesicles, or partly in an extravesicular state. For simplicity, these subtleties have been omitted from the model, but they will be discussed as required by experimental findings. Nerve terminals probably do release norepinephrine and it probably does have postsynaptic effects, but there is less information about these processes than about other phases of norepinephrine metabolism in central neurons. The present discussion concentrates on those aspects of drug action which are best understood, and on drugs that aid in understanding the metabolism of central norepinephrine.

The model depicts possible sites of drug interaction with norepinephrine metabolism at synapses, where norepinephrine would function if it is a central neurotransmitter. Compounds may have only one action but more often exert complex effects. Furthermore, drugs may act not only at nerve terminals, but also at other portions of the neuron. Centrally acting compounds may affect more than one neurochemical system, and it is dangerous to assume that observed neurological effects are fully explained by the actions of drugs on norepinephrine metabolism alone.

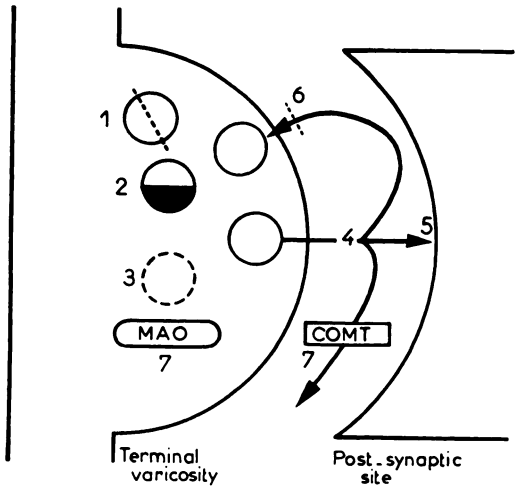

FIG. 1. Possible sites of interaction of drugs with central norepinephrine metabolism. The metabolism of norepinephrine at a synapse in the central nervous system is diagrammed. Some of these processes may also occur in the cell body and axon. Drugs may interfere with norepinephrine synthesis, 1; compete for storage sites, 2; alter the storage sites, 3; influence release, 4; produce or inhibit postsynaptic effects, 5; or interfere with inactivation by reuptake, 6; or enzymatic metabolism, 7;

B. Alteration of synthesis

1. Availability of precursors. The administration of precursors elevates the levels of brain amines (28, 197). The administration of dopa or of 3,4-dihydroxyphenylserine in the presence of MAO inhibition produces mild central excitatory effects. Dopa administration increases dopamine throughout the brain strikingly, but has only a small effect on norepinephrine concentrations (28, 71, 197, 307). Decarboxylation of administered 3,4-dihydroxyphenylserine or 5-hydroxytryptophan results in increases of norepinephrine (43) or serotonin (197), respectively. The decarboxylation of any of these amino acids may occur nonspecifically in neurons that contain dopamine, norepinephrine, or serotonin (108, 192). Thus, it is not clear whether the behavioral effects produced by these precursors are due to the elevation of amine concentrations in specific neuron systems, or throughout all 3 neuron systems.

2. Inhibition of synthetic enzymes. Several compounds lower brain norepinephrine concentrations by interfering with enzymatic synthesis. Although inhibitors of dopa decarboxylase and dopamine β-hydroxylase have been found, inhibitors of tyrosine hydroxylase are more effective in lowering brain norepinephrine levels, probably because this step is rate-limiting in norepinephrine synthesis. The most thoroughly investigated inhibitor of tyrosine hydroxylase is α-methyltyrosine. This agent produces a profound and rapid decrease of brain norepinephrine (289). Its action appears to be limited to a reversible and competitive inhibition of tyrosine hydroxylase (235, 289). Repeated doses reduce brain norepinephrine to undetectable levels, but have no effect on serotonin concentrations. Signs of mild sedation may occur (253, 289, 301). Since the metabolites, α-methyldopamine and α-methylnorepinephrine, are detectable in brain tissue several hours after the administration of α-methyltyrosine, displacement by amine analogs may contribute to the depletion of norepnephrine (202). Other inhibitors of tyrosine hydroxylase have been described, including iodinated derivatives of tyrosine, some of which occur endogenously and are involved in thyroid hormone synthesis (128, 285).

The decarboxylase that converts dopa to dopamine acts on several amino acids, and these may mutually inhibit the decarboxylation of each other (283, 295). α-Methyldopa (Aldomet, α-methyl-3,4-dihydroxy-β-phenylalanine) competitively inhibits the decarboxylation of many amino acids (282, 295) and prevents the hydroxylation of phenylalanine and tryptophan (40). Brain catecholamines are depleted after α-methyldopa, to some extent by decarboxylase inhibition, but, more importantly, by displacement of norepinephrine by α-methylnorepinephrine formed by metabolism of the amino acid (51, 231, 282). Similar mechanisms explain the norepinephrine-depleting action of α-methyl-*m*-tyrosine (α-methyl-*m*-hydroxy-β-phenylalanine) (43). The conclusion that decarboxylase inhibition alone does not deplete norepinephrine is supported by the fact that the certain benzylhydrazines and benzyloxyamines, *e.g.*, 3-hydroxybenzylhydrazine fumarate, which are excellent inhibitors of dopa decarboxylase, do not lower brain norepinephrine concentrations, apparently since decarboxylase activity is present in great excess (34, 43). Other classes of decarboxylase

inhibitors are known. They include the chalcones (60), and agents such as semicarbazide (aminourea or carbamylhydrazide) (295) and various other hydrazine derivatives (252, 295), which affect pyridoxal phosphate, the coenzyme of dopa decarboxylase. Certain hydrazines, such as seryl-trihydroxy-benzylhydrazine, appear to inhibit decarboxylase without interfering with pyridoxal phosphate (39). Seryl-trihydroxy-benzylhydrazine produced little decrease of endogenous brain monoamines, and in contrast to α-methyldopa, did not depress spontaneous locomotor activity (248).

The benzylhydrazine and benzyloxyamine derivatives also inhibit dopamine β-hydroxylase *in vitro* (67). Brain norepinephrine was decreased *in vivo* by a benzylhydrazine derivative, (181), but this effect may partly be due to depletion of stored norepinephrine and not only to inhibition of synthesis (173). The use of benzyloxyamines is limited by their toxicity (237). Disulfiram [Antabuse, bis(diethylthiocarbamyl)disulfide] inhibits dopamine β-hydroxylase (129, 145, 233), and it decreases brain norepinephrine and increases brain dopamine (130, 145). In addition to disulfiram, other chelating agents inhibit the β-hydroxylase *in vitro* (135, 173), and diethyldithiocarbamate can lower brain norepinephrine concentrations (54). Of this class of enzyme inhibitors, disulfiram has been used more frequently in the study of norepinephrine metabolism, but there is a need for more potent and longer lasting inhibitors of dopamine β-hydroxylase.

C. Alteration of retention

1. Displacement by analogs. Several methylated analogs of catecholamine precursors, such as α-methyldopa and α-methyl-*m*-tyrosine, act as inhibitors of synthetic enzymes and induce prolonged decreases of brain norepinephrine (153, 231, 251). Transient reductions of the concentrations of dopamine (153, 231) and serotonin (153, 280, 282) also occur, and are accompanied by signs of decreased central neural activity (282). This sedation does not appear to be correlated with the prolonged decrease of brain norepinephrine levels. The persistent decrease of norepinephrine cannot be due to decarboxylase inhibition alone; since the enzyme inhibition is only short-lasting, α-methyldopa and α-methyl-*m*-tyrosine are quickly decarboxylated, and their products undergo further metabolism without accumulating (3, 43, 298). The generally accepted mechanism for norepinephrine depletion by these drugs is displacement of norepinephrine from neuronal storage sites by the amine analogs, α-methylnorepinephrine and metaraminol, formed endogenously by decarboxylation and β-hydroxylation of α-methyldopa and α-methyl-*m*-tyrosine (3, 43, 51). This interpretation is supported by the observation that these methylated amino acids are without effect on brain norepinephrine when dopa decarboxylase and dopamine β-hydroxylase are inhibited by other agents (108, 282, 298).

Another compound, prenylamine [Segontin, N(1,1-diphenylpropyl(3))-amphetamine], although a structural analog of amphetamine, produces manifestations of decreased central neural activity, while lowering brain catecholamine concentrations, as amphetamine does (170, 273). Prenylamine appears to inhibit the accumulation of exogenous norepinephrine by adrenal medullary granules

(50). Although the mechanism of action of this drug is not well understood, it may act competitively like other structural analogs of norepinephrine, at one or more enzymatic or storage sites (170).

The α-methylated analogs may replace norepinephrine and act as "false" and sometimes less effective neurochemical transmitters in noradrenergic neurons (43, 81). This hypothesis is supported by the finding that endogenous norepinephrine and α-methylnorepinephrine, endogenously formed from α-methyldopa in the heart, were released by sympathetic nerve stimulation in the same proportion as they were found in cardiac tissue (234). In peripheral sympathetically innervated tissues of animals pretreated with a MAO inhibitor, there is an increase of endogenous tyramine and its hydroxylated derivative, octopamine. The latter amine accumulates in nerve endings and is released by nerve stimulation, acting as a false transmitter in competition with norepinephrine (178). This phenomenon helps to explain the hypotensive effects of MAO inhibitors (178). Similarly, after MAO inhibition, endogenous octopamine (171) and ^{3}H-octopamine (281), synthesized endogenously from labeled tyramine, accumulated in the brain. The localization of labeled octopamine largely in a synaptosomal subcellular fraction suggests that this norepinephrine analog may act as a false transmitter in the brain. Furthermore, recent evidence suggests that electrical stimulation of brain slices can release radioactive metaraminol (α-methyloctopamine) and octopamine (177).

2. Alteration of storage sites. Various agents lower brain catecholamine concentrations by means other than interfering with synthesis, or displacement by structural analogs (276). The most familiar drugs of this class are the *Rauwolfia* alkaloids, which produce long-lasting diminutions of brain serotonin (249), dopamine (57), and norepinephrine (35, 58, 158), and induce a characteristic syndrome of central depression. Other compounds that affect the storage of both catecholamines and serotonin include the benzoquinolizines, which are shorter acting and appear to lower amine levels more in the brain than in the periphery

The *Rauwolfia* alkaloids that produce manifestation of decreased central neural activity also produce the greatest decreases of brain amine concentrations. They include reserpine (Serpasil), rescinnamine (Moderil), raunescine, and recanescine (276). The alkaloid reserpine (3,4,5-trimethoxybenzoylmethyl reserpate), which has been studied extensively, appears to exert similar biochemical effects in the peripheral sympathetic system and in the brain. Small doses of reserpine profoundly decrease brain amine concentrations within 1 hour, with a maximal decrease in 3 hours (24). However, in the brain (116, 122), as well as in peripheral sympathetically innervated tissues (97, 168, 274), there appears to be a small store of norepinephrine that is not depleted by further reserpine treatment. In histochemical studies of the effects of reserpine (73, 102, 103), the rates of depletion and recovery of norepinephrine fluorescence among separate systems of neurons, and even within the same system, varied widely. After reserpine administration, cell bodies and nerve terminals lost norepinephrine fluorescence at similar rates. Fluorescence reappeared in cell bodies within 24 to 48 hours, but only after several days in nerve terminals. Chemical estimation of catecholamine

levels revealed a gradual return toward normal values within several weeks (141). After reserpine treatment, "dense core" vesicles were no longer demonstrated in hypothalamic nerve terminals by electron microscopy (144).

The effects of reserpine, which include a variety of neurological and behavioral manifestations, were maximal in 2 to 4 hours, persisted for about 12 to 24 hours, and disappeared within 16 to 48 hours after a single dose of the drug (63, 141). At the time of functional recovery, central amine concentrations had returned only to a small fraction of normal values (141). The behavioral recovery paralleled the appearance of a small store of brain amines, which may be physiologically important (141). Simultaneously, the brain recovered its ability to accumulate small amounts of exogenous norepinephrine, which, in turn, could be depleted by another dose of reserpine or by amphetamine (122). Similarly, the return of sympathetic function after administration of reserpine was correlated with the recovery of the ability of peripheral sympathetically innervated tissue to accumulate small amounts of exogenous norepinephrine (7, 168, 292).

In subcellular distribution studies, the distribution of norepinephrine between particulate material and the supernatant solution is altered by reserpine treatment. Except in one brain study (134), initially, relatively less norepinephrine has been found in soluble supernatant fractions of brain (124, 307) or peripheral tissues (169). A similar pattern of initial depletion of serotonin has also been observed (113). The explanation of these observations awaits further clarification of the cellular localization of amines that appear in the soluble supernatant fraction after homogenization.

Reserpine alters the catabolism of brain norepinephrine. As in the periphery (179), there is a decrease of normetanephrine and an increase of deaminated metabolites in whole brain (116, 140) and in various parts of the brain (119). The altered pattern of metabolites suggests that after reserpine, more norepinephrine is exposed directly to intraneuronal MAO, and that less is physiologically released and methylated extraneuronally to form normetanephrine. Inhibition of MAO partially protects brain amines from the depleting effects of reserpine (33, 197, 243, 284, 307). These results imply that reserpine counteracts a form of intraneuronal storage that normally protects norepinephrine from MAO. It is clear that norepinephrine exists in at least 2 states in intact neurons, and that some norepinephrine found in the supernatant subcellular fraction is rapidly depleted by reserpine. Initially, reserpine might expose to enzymatic destruction a fraction of the intraneuronal amines normally either held loosely by vesicles, or in a protected form in the cytoplasm.

It has also been noted that reserpine in low concentrations prevents the accumulation of radioactive norepinephrine in cerebral slices (82). Similarly, reserpine prevents the accumulation throughout the brain of exogenous norepinephrine administered intraventricularly (116, 119). In peripheral tissues, the reduced accumulation of exogenous norepinephrine after reserpine treatment is due to a failure of retention, and not to a block of the initial uptake across the neuronal membrane (142, 168, 180, 188). The inhibition of MAO allows exogenous norepinephrine to accumulate in the brain *in vivo* (122) and in nerve terminals *in vitro*

(143) in the presence of reserpine. Similarly, α-methylated amines, which are not attacked by MAO, accumulate in central neurons *in vitro* (142) and *in vivo* (45) in the presence of the alkaloid. This accumulation of amines provides evidence that, as in the peripheral sympathetic system, reserpine does not act by preventing uptake at central neuronal membranes.

It is unlikely that reserpine interferes directly with the enzymes that synthesize norepinephrine. Loading doses of dopa reverse the depletion of brain catecholamines (197) and the behavioral syndrome (55) produced by reserpine. This reversal is even more striking in the presence of MAO inhibition (24, 307). Moreover, radioactive tyrosine or dopamine can be converted to norepinephrine in the reserpine-treated brain (122). The same amount of radioactive dopa formed from ^{14}C-tyrosine in the brains of reserpine-treated and normal rats (122); hence reserpine fails to inhibit this rate-limiting step of norepinephrine synthesis. However, since reserpine removes the substrate, dopamine, from the site of norepinephrine synthesis, it can thus indirectly limit the formation of norepinephrine.

It seems well established that the mechanism of the catecholamine-depleting effect of reserpine involves interference with intraneuronal storage, which then allows catabolism to occur. Many studies of the effects of reserpine on intraneuronal binding have been done with granules prepared from the adrenal medulla (50, 174), or with vesicles prepared from peripheral sympathetic neurons (93, 292) or brain tissue (212). These studies demonstrated that reserpine, in very low concentrations *in vitro*, blocks the accumulation of norepinephrine in storage particles. Once norepinephrine was bound to peripheral sympathetic nerve granules (91, 255, 292) or brain vesicles (212), spontaneous release was augmented by high concentrations of reserpine, but actually inhibited by low concentrations (91, 292). However, these studies *in vitro* do not represent the state of the intact nerve ending. It is likely that neural activity is necessary to release stored norepinephrine and bring about the maximal effect of reserpine. Thus, denervation (152, 311), ganglionic blocking agents (152), or spinal cord section (35) can limit the norepinephrine-lowering effect of reserpine in peripheral tissues. Further work is necessary to elucidate the effect of reserpine on storage vesicles. Since the process of vesicular retention appears to be active and dependent on ATP and Mg^{++} (212), reserpine may inhibit active transport at the vesicular membrane as it does in platelets (160). Alternatively, it may alter the ability of a storage substance to retain and protect norepinephrine in vesicles, and perhaps in the cytoplasm as well.

The benzoquinolizine derivatives are similar to the Rauwolfia alkaloids in several ways, and these drugs have been extensively reviewed (244). The best studied member of this group is tetrabenazine (Ro 1-9569, 2-oxo-3-isobutyl-9,10-dimethoxy-hexahydro-benzoquinolizine). This agent produces a behavioral syndrome of central depression similar to that of reserpine, except for a shorter duration, which corresponds to the rapid catabolism of the drug. Tetrabenazine decreases the concentration of norepinephrine, dopamine, and serotonin in the brain, but seems to have little effect on their concentration in the periphery (244, 259). The decrease of brain amines is greatest for norepinephrine, which is

85 % depleted within 4 hours. Recovery of normal amine concentrations is nearly complete in 24 hours, while the behavioral effects last only 5 to 6 hours. Like reserpine, tetrabenazine produces a rapid disappearance of amine fluorescence from the cell bodies and terminals of central neurons (76). Fluorescence reappeared at about the same rate throughout the neurons after tetrabenazine (76), although it reappeared first at cell bodies after reserpine treatment. MAO inhibitors can reverse the depletion of brain amines produced by tetrabenazine (244). Tetrabenazine prevents the accumulation of amines in platelets (240) and medullary granules (50). Pretreatment with the drug appears to prevent the behavioral effects of reserpine, and this finding has been attributed to competition by the 2 agents for similar receptor sites (259). Thus in general, tetrabenazine appears to be similar to reserpine in many ways, and may also act primarily by interfering with the intraneuronal storage of amines.

Other types of compounds produce striking decreases of central amines. These include decaborane ($B_{10}H_{14}$) (216), which acts mainly on norepinephrine, triethyltin (227), which acts on catecholamines and serotonin, and several chlorinated aralkyl amines, which may either reduce all monoamine concentrations (247), or affect serotonin selectively (107, 190). The mechanisms of the amine-depleting action of these agents are not clear.

D. Alteration of release and reuptake

The evidence that drugs may influence release of central amines from nerve endings is largely indirect. It is sometimes assumed when the administration of a drug is followed by some manifestation of central excitation and by a diminution of brain monoamine concentrations, that release of a neurotransmitter-amine in a physiologically active form has occurred. Conversely, when manifestation of decreased central neural activity occur, and amine levels are not lowered, it is often assumed that either an inhibition of release, or a postsynaptic receptor blockade has occurred. These assumptions represent over-simplified views of the effect of drugs on the metabolism of brain norepinephrine and may not always be correct. The drugs included in the present discussion appear either to facilitate or inhibit amine release. However, most of these drugs have complex, sometimes subtle, and even apparently contradictory effects on the metabolism of brain monoamines. Furthermore, the actions of drugs on the release and postsynaptic effects of proposed central neurotransmitters generally have not been measured directly. For these reasons, it is difficult to draw conclusions about the mechanisms of action of the drugs within the framework of our present understanding of amine metabolism.

1. Chlorpromazine. Chlorpromazine [Thorazine, 2-chloro-10-(3-dimethylaminopropyl)-phenothiazine], produces a striking syndrome of tranquilization without altering the concentration of brain monoamines (110). This finding suggests that the release of a central neurotransmitter may be inhibited by this agent, or that interference with postsynaptic receptors may occur. The pharmacological and biochemical effects of chlorpromazine are very complex (112, 246). They include increases of catecholamine metabolites in the brain (8, 52, 112, 246) and the

inhibition of the amine depletion produced by reserpine (62, 110) and of the amine increase produced by MAO inhibitors (110, 229). Most of these effects appear to be related partly to the hypothermia induced by chlorpromazine (62, 112, 229, 246).

Several observations are consistent with the conclusion that chlorpromazine may affect membrane permeability. It appears that cortical neuronal membranes are more resistant to the effects of electrical depolarization in the presence of very low concentrations of chlorpromazine *in vitro* (215). There is evidence that this drug decreases membrane permeability to amines at various cellular and subcellular sites (112, 246). Chlorpromazine inhibits the accumulation *in vitro* of exogenous norepinephrine in brain slices (83), adrenal granules (50, 306), and platelets (191, 290). It inhibits the accumulation *in vivo* of exogenous norepinephrine in peripheral tissues (151) and of exogenous dopa or tryptamine in brain (246). Chlorpromazine inhibits the disappearance of radioactive norepinephrine, previously accumulated in rat brain (117), and can counteract the lowering of brain norepinephrine concentrations by stress (213). The effects observed *in vivo* could be due to nonspecific decreases in temperature (112, 246), blood-flow, or neural activity. However, it has been found recently that chlorpromazine inhibits the release by electrical stimulation of norepinephrine previously accumulated in brain slices (20).

2. Morphine. Morphine may also have effects on central amines. Although changes in brain catecholamine concentrations have been reported (136, 183, 211, 213, 302), they varied with dose, timing, and species and sometimes were correlated with hypoxia (302). It has generally been noted that with central stimulation brain norepinephrine concentrations were decreased, whereas with mild depressions of central neural activity, there were no changes in brain norepinephrine (183, 278). The reduction of brain norepinephrine concentrations by stress was not prevented by high doses of morphine (213).

3. Amphetamine. The amphetamines (Benzedrine, Dexedrine, *d* or *l* α-methylphenylethylamine) have striking excitatory effects on the central nervous system. Many hypotheses have been offered to explain these effects. Amphetamine may stimulate neuronal activity directly by acting on postsynaptic receptors of various central monoamine transmitters, or it could produce an indirect stimulalation by releasing catecholamines or preventing their destruction by MAO. These hypotheses have been reviewed recently (291).

In relatively high doses, amphetamine consistently decreased the endogenous content of brain norepinephrine (183, 268), but, in contrast, produced small and variable effects on dopamine and serotonin concentrations (183, 199, 225, 279). This effect on norepinephrine was greater with the dextro-isomer of amphetamine, which is the more potent stimulant (225). However, there does not appear to be a consistent correlation among the various structural analogs of amphetamine between stimulant potency and the degree of norepinephrine decrease produced (17). The neurological effects may precede observable alterations in norepinephrine concentrations (279). This may be due to a direct excitatory effect of amphetamines at postsynaptic sites. The toxicity of this drug is greater in grouped

animals, and these also appear to have lower concentrations of brain norepinephrine (226).

The alteration by amphetamine of the normal subcellular distribution of brain norepinephrine is the opposite of that produced by reserpine (124). That is, the norepinephrine decrease produced by amphetamine is reflected in a marked reduction of norepinephrine content in a particulate fraction, and almost no change in the soluble supernatant fraction. This finding suggests that norepinephrine may have been released selectively from synaptic structures. The conclusion is supported in histochemical studies by the disappearance of norepinephrine fluorescence from the terminals of central neurons after treatment with amphetamine (53).

When the endogenous stores of rat brain norepinephrine were labeled with ^{3}H-norepinephrine, and amphetamine was administered to the animals, the pattern of radioactive metabolites found (116) was also consistent with the conclusion that this compound may release brain norepinephrine in a physiologically active form. More normetanephrine and a smaller amount of deaminated catechol products were found; this suggests that norepinephrine had been released without exposure to intraneuronal MAO, and was metabolized primarily by extraneuronal COMT. The decrease of the deaminated catechols may be due partly to inhibition of MAO by amphetamine. The effects of amphetamine on MAO had been discussed many years ago (256, 291). Inhibition of the oxidative deamination of ^{3}H-norepinephrine with low concentrations of amphetamine *in vitro* has been reported (119). However, amphetamine does not decrease concentrations of O-methylated deaminated metabolites in the brain as occurs with one of the hydrazine MAO inhibitors (119). In general, the striking syndrome of rapid central excitation produced by amphetamine does not appear to be fully explained by its limited ability to inhibit MAO.

The data discussed are consistent with the hypothesis that amphetamine may increase the release of norepinephrine, either directly, or as an indirect consequence of increased neuronal activity. However, the decrease of brain norepinephrine produced by amphetamine could be partly due to the prevention of reuptake of physiologically released norepinephrine. In peripheral sympathetically innervated tissue, the accumulation of exogenous norepinephrine is impaired by amphetamine (38, 151, 205). In the central nervous system, a similar impairment of the accumulation of exogenous norepinephrine occurs in brain slices (53, 83, 265) or *in vivo* in whole brain (116), or to various degrees in different parts of the brain (53, 119). The uptake process could not be measured separately in these studies. Consequently, the decreased accumulation of norepinephrine could have been due to a block of uptake or to an active release of norepinephrine by amphetamine, or both.

The use of precursors has provided further indirect evidence that neuronal membrane uptake mechanisms may be inhibited by amphetamine. Catecholamine stores were replenished to a lesser extent by dopa administered after amphetamine to animals treated with reserpine and a MAO inhibitor as measured by histochemical or biochemical assays (53). Furthermore, when radioactive

dopamine was introduced into the intact brain after amphetamine treatment, less endogenously formed radioactive norepinephrine was found than in normal animals. In this experiment, the accumulation of exogenous dopamine throughout the brain was not impaired. The decreased accumulation of norepinephrine may be due to a specific inhibition of the norepinephrine reuptake process by amphetamine (119), but enhanced release of norepinephrine may have been a contributing factor. It is also possible that the decreased amount of labeled norepinephrine may also have been due to an inhibition of dopamine β-hydroxylase by amphetamine. The activity of β-hydroxylase assayed *in vitro* is inhibited by amphetamine, but only at very high concentrations (126). No other alterations in norepinephrine synthesizing enzymes have been reported, and it remains to be demonstrated whether an effect on norepinephrine synthesis is an important action of amphetamine.

The central stimulating action of amphetamine may result from its complex effects on norepinephrine metabolism, which include increased release, decreased reuptake, and decreased deamination. In addition, a direct nerve stimulating action of amphetamine may be important. The ability of amphetamine to produce central stimulation even after norepinephrine depletion by reserpine may be related to such a direct effect of amphetamine on postsynaptic sites (279, 291). However, since some norepinephrine can be synthesized even in the presence of reserpine, the stimulating effect of amphetamine may be due partly to an inhibition of norepinephrine reuptake and an inhibition of MAO (122).

4. Imipramine and related compounds. Although interest in possible relationships between antidepressant drugs and central amine metabolism was stimulated by discovery of the MAO inhibitors, imipramine (Tofranil, dimethylaminopropyl-dihydro-dibenzazepine) and desmethylimipramine (Norpramin, Pertofrane) produce significant effects on central norepinephrine metabolism without inhibiting MAO (245, 258). Desmethylimipramine can be formed *in vivo* by the demethylation of imipramine (114). The pharmacology and clinical importance of this class of antidepressant drugs has recently been reviewed extensively (94, 175), and the possible importance of brain catecholamines in affective states has been discussed (88, 271).

Unlike MAO inhibitors or the amphetamines, neither imipramine nor desmethylimipramine alters the concentration of norepinephrine or serotonin in the brain (293), nor does imipramine alter the catecholamine fluorescence in central nerve terminals (22). These compounds impair the ability of peripheral sympathetically innervated tissue to accumulate exogenous norepinephrine (151, 165, 205). This effect has been demonstrated in brain slices with imipramine, and also with chlorpromazine (83). In the intact rat brain the accumulation of ^{3}H-norepinephrine was inhibited by imipramine, desmethylimipramine, and amitriptyline (Elavil), but not by chlorpromazine (115). As with amphetamine, there was less labeled norepinephrine, but a normal accumulation of dopamine in various parts of the brain after the intraventricular administration of ^{3}H-dopamine in animals treated with desmethylimipramine. This result suggests that the drug may specifically decrease the reuptake of physiologically released

norepinephrine without affecting the accumulation of dopamine in catecholamine neurons (119). This finding does not appear to be due to enhanced release of norepinephrine, since previously accumulated radioactive norepinephrine disappears from brain tissue even more slowly after imipramine treatment than normally (116). This slowed disappearance is unexplained, but it may be one result of a generalized decrease of membrane permeability. The striking inhibition of uptake produced by imipramine and desmethylimipramine may be the dominant effect. A decreased reuptake of norepinephrine would help to explain the ability of desmethylimipramine pretreatment to induce central stimulation instead of decreased central neural activity, which usually follows the administration of amine-depleting agents like reserpine and tetrabenazine (293). This central excitatory effect can be produced only when norepinephrine stores are rapidly decreasing, and when an inhibitor of reuptake might prolong the effect of norepinephrine which initially spills outside the neuron. Since this excitatory effect of desmethylimipramine and reserpine was absent when the norepinephrine stores had been replaced with α-methyl analogs of norepinephrine, norepinephrine probably mediates the effects (293).

The possibility that these antidepressant drugs may affect enzymatic steps in the metabolism of norepinephrine should be considered. Although high concentrations of imipramine appear to inhibit the activity of dopamine β-hydroxylase *in vitro* (127), the significance of this observation for the intact organism is not clear. There is an accelerated turnover of norepinephrine after repeated doses of imipramine or amitriptyline, but not after chlorpromazine (236). The effects of chronic administration of imipramine may be more representative of the effects induced by the usual clinical application of such drugs.

5. Cocaine. Among the drugs known to inhibit uptake of norepinephrine in the peripheral sympathetic system, cocaine (151, 166, 189, 205, 315), which produces central excitatory effects, may act as an inhibitor of uptake in central norepinephrine-containing neurons. Cocaine produced small and variable effects on brain norepinephrine concentrations (17, 154). This drug prevented the accumulation of exogenous norepinephrine in brain slices (83, 143). It has been reported that cocaine inhibited the uptake of norepinephrine *in vivo* as studied histochemically (104), but, surprisingly, it did not prevent the accumulation of ^{3}H-norepinephrine in the intact rat brain (116).

In general, drugs which may increase the amount of norepinephrine available to a postsynaptic receptor by various mechanisms, tend to produce central stimulatory effects. The inhibition of the re-uptake of physiologically released norepinephrine is one such mechanism, which may play a major role in the action of the antidepressant and stimulant drugs discussed above.

E. Inhibition of catabolic enzymes

1. Monoamineoxidase inhibitors. The amount of norepinephrine available for function at synapses may also increase after inhibition of the enzymes that metabolize this biogenic amine in the brain. It is generally assumed that the inhibitors of MAO act in this way, but recent reviews of MAO inhibitors (176,

266) emphasize that although the drugs have a profound effect on MAO, they may affect norepinephrine metabolism at other sites, and that it is not firmly established that MAO inhibition itself fully explains the antidepressant effects of these drugs.

Since the discovery of iproniazid many drugs have been used as MAO inhibitors, and their pharmacology and chemistry have been studied intensively (36, 41, 239, 297, 316, 320, 321). They have been very useful in the study of the metabolism of brain amines. Those of major clinical importance as antidepressants include the hydrazines, isocarboxazid [Marplan, 1-benzyl-2(5-methyl-3-isoxazoylcarbonyl)-hydrazine], nialamide [Niamid, N-isonicotinyl-N′-β(N-benzylcarboxyamino)-ethylhydrazine], and phenelzine (Nardil, β-phenylethylhydrazine); the nonhydrazines include the amphetamine analog, tranylcypromine (Parnate, trans-2-phenylcyclopropylamine), and the acetylenic phenylalkylamine, pargyline (MO-911, Eutonyl, N-methylbenzyl-2-propylylamine), which is more useful as an antihypertensive agent than as an antidepressant. Other MAO inhibitors, such as the hydrazines, iproniazid (Marsalid, N-isopropyl-isonicotinylhydrazide) and pheniprazine (JB-516, Catron, β-phenylisopropylhydrazine) although too toxic for clinical use, have often been used in animal studies. Most of these inhibitors of MAO are noncompetitive and irreversible, though several of the harmala alkaloids (methoxymethylpyridindoles) appear to be reversible and competitive inhibitors of MAO, and produce only small transient changes in brain amine concentrations (239, 297).

Norepinephrine concentrations were increased by MAO inhibitors in the rabbit, rat, and mouse brain (24, 68, 132, 243, 287), but not in the cat or dog brain (288, 302, 303). Generally, dopamine and serotonin concentrations responded similarly, but occasionally dissimilar effects were observed. For example, serotonin concentration may increase in the cat brain, though norepinephrine levels are unchanged (36, 302). Increases of serotonin concentration were often greater and more rapid than those of norepinephrine (36). Several MAO inhibitors markedly increased the fluorescence of serotonin-containing neurons, while catecholamine neurons were less affected (22).

Generally, increases of brain norepinephrine are paralleled by increased concentrations of normetanephrine (11, 56). The accumulation of intraventricularly administered ^{3}H-norepinephrine in the brain was enhanced by pretreating rats with a MAO inhibitor (116, 119). Simultaneously, normetanephrine accumulated markedly, and there was a pronounced decrease of deaminated metabolites of norepinephrine in the whole brain (116) or in specific areas of the rat brain (119). In rats treated with pheniprazine, endogenously formed radioactive normetanephrine, normally detectable only in the soluble supernatant (124) was found partly in a synaptosomal subcellular fraction of brain (281). The distribution of norepinephrine between particulate and soluble subcellular fractions does not appear to be affected by MAO inhibition (134, 307). In the cat brain, there are no appreciable changes in the metabolism of labeled norepinephrine injected into the lateral ventricle after iproniazed treatment (203). The marked increase of normetanephrine which follows MAO inhibition in some species suggests that

the extraneuronal COMT pathway predominates. Brain concentrations of S-adenosylmethionine, the methyl donor utilized by COMT, were decreased by several MAO inhibitors. The administration of large doses of methionine prevented the decrease. These observations (18) support the conclusion that MAO inhibition increases the utilization of the methylation pathway. It is not known whether the accumulation of normetanephrine in brain, partly in synaptosomes, contributes to the central effects of MAO inhibitors.

MAO inhibitors enhance the central stimulation and elevations of catecholamine concentrations produced by precursors (57, 307). They reverse the central depression and reduce the depleting effects on brain amines produced by reserpine (243, 284). Furthermore, they counteract the effects of reserpine on the subcellular distribution of brain norepinephrine (307). MAO inhibitors enhance the central effects of stimulants like amphetamine, which affect brain norepinephrine metabolism (56). Most of these phenomena have been ascribed to the inhibition of MAO alone, but other effects of these drugs may also be important.

It has been suggested that MAO inhibitors may slow the disappearance of radioactive norepinephrine previously accumulated in peripheral tissue (14) and in the brain (116) by means other than inhibiting deamination. For example, in isolated perfused tissues MAO inhibitors diminish the increased norepinephrine outflow associated with nerve stimulation (80, 161). This is partly explained by the accumulation of octopamine, which competes with norepinephrine for release and thus indirectly inhibits release of norepinephrine (176, 178). Furthermore, it has been shown recently that treatment with an MAO inhibitor can reduce the rate of turnover of brain norepinephrine (64). This finding was ascribed to negative feedback control of the rate-limiting step of norepinephrine synthesis by product accumulation, since norepinephrine has been shown to inhibit tyrosine hydroxylase *in vitro* (235). It was suggested that differences in the efficiency of this feedback mechanism may help explain species differences in the accumulation of amines after MAO inhibition. The increased intraneuronal norepinephrine concentration might result in a net increase in the amount of norepinephrine appearing in the extraneuronal space. This net extraneuronal increase of norepinephrine would help to explain the stimulating effects and the increased production of normetanephrine that result from the administration of MAO inhibitors.

2. Catechol-O-methyltransferase inhibitors. Although there are many relatively nontoxic compounds that produce specific and lasting inhibitory effects on monoamineoxidase, there are few such compounds for the inhibition of COMT. Many compounds with various chemical structures do inhibit COMT *in vitro* (15, 23, 44, 263), but only a few of these are effective *in vivo*. Several produce short-lasting effects on brain O-methylation, but also produce many side effects, including interference with norepinephrine metabolism in several ways (264). The most effective known inhibitors of brain COMT activity *in vivo* are pyrogallol (1, 2, 3-benzenetriol) and several dopacetamide (dihydroxyphenylacetamide) derivatives, whereas tropolones (heptatrienolones) appear to be more effective in peripheral tissues (43, 264).

Pyrogallol failed to alter catecholamine concentrations in the brain (68, 307), except after direct administration into the lateral ventricle of the rabbit brain (209). Dopacetamide derivatives decreased the amount of normetanephrine and 3-methoxytyramine in mouse brain (44). A tropolone-acetamide did not affect the accumulation of exogenous norepinephrine in rat brain, but markedly reduced the formation of normetanephrine (116). The dopacetamides increased the accumulation of dopamine and norepinephrine in the brain after the administration of catecholamine precursors, and potentiated the central excitation they induce (43). After the administration of dopa, pyrogallol enhanced the accumulation of brain dopamine, but had no effect on the accumulation of norepinephrine (307). Pyrogallol administration led to striking decreases in brain concentrations of S-adenosylmethionine, the methyl donor utilized by COMT. Since pyrogallol is itself methylated, this effect may result from competitive utilization of the methyl donor (18). The development of more effective, less toxic, and longer lasting COMT inhibitors would contribute significantly to the study of catecholamine metabolism.

V. SUMMARY AND CONCLUSIONS

In the past few years a great deal of information has accumulated about the distribution and metabolism of catecholamines in the central nervous system, owing largely to new experimental approaches. These studies promise to enrich our conception of the functional anatomy of the brain.

Norepinephrine is contained in complex systems of specific neurons and is highly localized at nerve terminals, largely within synaptic vesicles. The metabolism of norepinephrine in the brain is generally similar to that in the peripheral sympathetic system. In both systems, tyrosine is converted to norepinephrine intraneuronally, following the same enzymatic pathway. Intraneuronal oxidative deamination and extraneuronal O-methylation are important catabolic processes in both systems. As in the periphery, the processes of active uptake and storage are remarkably efficient and greatly facilitate the study of the metabolism of this amine since radioactive tracers are readily taken up and retained by norepinephrine neurons. Reuptake may be a major means of inactivating physiologically released norepinephrine. The amine is stored in central neurons in a protected and inactive form, which appears to be a reservoir for future functional needs. Since the disappearance of endogenously formed radioactive norepinephrine is multiphasic, and since drugs have a differential depleting action on subcellular fractions of norepinephrine, it appears to occur in the brain in more than one form, as in the periphery. Even among relatively homogeneous populations of sympathetic nerve endings in peripheral organs, it is difficult to correlate the functional compartmentalization of norepinephrine with its intracellular distribution. In the central nervous system, the apparent compartmentalization of norepinephrine may be due not only to different forms of its storage within individual nerve terminals, but also to metabolic or storage differences between cell bodies and terminals, or between groups of neurons which are heterogenous in size and distribution.

Norepinephrine fulfills several criteria of neurotransmitter. The amine is highly localized at nerve terminals in vesicles. It can be synthesized and stored locally, and very efficient mechanisms exist for its inactivation at synapses. In the peripheral sympathetic system, other fundamental criteria for chemical neurotransmission have been fulfilled. They include the release of norepinephrine by nerve stimulation and the ability of exogenous norepinephrine to mimic postsynaptic effects of sympathetic stimulation. In the brain, it has not yet been possible to demonstrate direct release of norepinephrine by neural activity in the intact brain, although indirect evidence for release has been obtained. In the central nervous system, postsynaptic effects are more difficult to study than at peripheral neuroeffector junctions. Although it has been possible to study the sensitivity of neurons to norepinephrine applied by microelectrophoresis, specific electrical criteria for postsynaptic responses produced uniquely by noradrenergic neurons have not been established. Norepinephrine has both excitatory and inhibitory effects in various areas of the central nervous system. This work has recently been reviewed elsewhere (266, 267).

It is difficult to relate the neurological effects of centrally active drugs to their neurochemical effects, or to explain the mechanisms of such drugs by isolated effects on individual groups of neurons. Chemical effects have usually been studied in acute experiments with relatively large doses of drugs, and they may not correspond to the chemical changes resulting from chronic treatment.

Despite these limitations, it is possible to make certain conclusions about the actions of several important pharmacological agents on norepinephrine metabolism. Several drugs induce striking changes in brain catecholamine concentrations. Several mechanisms may lead to accumulation or depletion of norepinephrine, and significant metabolic changes may occur even without appreciable changes in norepinephrine concentration. Several metabolic alterations may occur simultaneously and may induce secondary metabolic responses, such as changes in the rate of turnover. The net effect produced may be the result of complementary or antagonistic actions of the drug. From the present survey of drug interactions with central norepinephrine metabolism, it appears that compounds that decrease synthesis, alter or compete for storage sites, or block release, may decrease central neural activity, most likely by limiting the availability of norepinephrine at the synapse. In contrast, the administration of precursors of norepinephrine, or of drugs which activate release, inhibit reuptake or prevent enzymatic inactivation, is followed by a syndrome of central excitation, probably due to increased availability of norepinephrine at the synapse. These interpretations are complicated by the uncertainty about the role of norepinephrine as an excitatory or inhibitory transmitter in the brain. Even though the present conclusions seem rather naive, more sophisticated concepts are rapidly emerging in the relatively new field of biochemical neuropharmacology.

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