REGULATION OF BIOSYNTHESIS OF CATECHOLAMINES AND SEROTONIN IN THE CNS

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INTRODUCTION

Brain contains several aralkylamines of considerable functional importance: norepinephrine (NE), dopamine (DA), and serotonin (5-hydroxytryptamine, 5HT). Their importance lies in an involvement in the exchange of information between two neurons where they function as neurotransmitters.

The overall process of neurotransmission and its changing frequency in different types of neurons can be studied either by recording the electrical activity of neurons or by examining the dynamics of the release of neurotransmitters. Because it is not possible to study the amount of neurotransmitter that reaches the receptors except in artificial conditions, the overall amount of neurotransmitter in the tissue is all that can be measured. The amount present extraneuronally is small in comparison with the amount of neurotransmitter present intraneuronally, so what is measured is largely intraneuronal. Additionally, some of the neurotransmitter molecules that are synthesized intraneuronally are destroyed before they can reach the receptor sites. The turnover rate in vivo of a neurotransmitter is a useful measure of the utilization rate of transmitter molecules as it may reflect the rate of synaptic transactions. The methods and the assumptions involved in measuring turnover and the limitations necessary in interpreting the results of turnover studies have been reviewed (1–3).

The rate of biosynthesis of neurotransmitters in the CNS is subject to alterations by many factors, but the nervous system is able to control this rate in response to varying demands. The object of this review is to bring into focus the molecular nature of the various processes involved in the regulation of the biosynthesis of catecholamines and serotonin. We review sites of physiological control that have emerged during the last few years and stress the information important in the study of the mode of action of drugs on the CNS.

The rate of aralkylamine biosynthesis in any part of the CNS reflects the local equilibrium among small molecules and macromolecules—the biosynthetic enzymes, their cofactors, substrates, products, inhibitors, and activators. Each of these molecules is itself subject to an equilibrium between renovation and disappearance. In different areas, the factors affecting these equilibria are functionally different due to the peculiar nature of the neuron. In the perikarya, enzyme molecules are renovated by de novo synthesis; in nerve terminals, however, the renovation of enzyme molecules depends on transport from the perikarya. Thus, in the former site, the regulation of one half of the equilibrium depends on the control of DNA transcription and RNA translation, while in the terminals, introduction of new enzyme molecules involves axoplasmic flow and transport which are as yet poorly understood. At steady state, the rate of enzyme degradation balances that of synthesis. Again, the processes involved in the two sites are different: in perikarya, the axoplasmic flow is probably the major process causing disappearance of enzyme molecules, whereas in the nerve terminals, destruction is caused by proteolysis or elimination by exocytosis. Because the nature of enzyme degradation in nerve terminals is still poorly understood, the role it may play in the regulation of transmitter biosynthesis is currently unknown.

Portions of our topic have been considered at recent symposia (4–6). Recent reviews have summarized other aspects of catecholamine and serotonin research including biochemistry of the catecholamines (7), regulation of catecholamine turnover in the periphery (8, 9), structure and function of synaptic vesicles (10), pteridine cofactors (11), biogenic amines and mental disorders (12–14), axonal transport (15), LSD and serotonin (16), drugs affecting biogenic amines (17), and amine uptake mechanisms (18).

HYDROXYLATION OF TRYPTOPHAN AND TYROSINE

Tryptophan and tyrosine are the amino acids that function as precursors in the biosynthesis of indolealkylamines and catecholamines respectively. The first step in the biosynthesis of these two amines involves ring hydroxylation by two mixed function oxygenases, tyrosine hydroxylase (EC 1.10.3.1) and tryptophan hydroxylase (EC 1.99.1.4). Although these two enzymes are themselves similar, the possibilities for regulation differ in the two types of neurons. For example, there is not enough tryptophan in brain to saturate tryptophan hydroxylase, while tyrosine hydroxylase is saturated with tyrosine. In addition, tryptophan hydroxylase is not inhibited by high concentrations of 5-hydroxytryptophan or 5HT, whereas tyrosine hydroxylase is inhibited by NE ($10^{-4}M$) (19). To understand the phenomena involved in the regulation of these two enzymes better we discuss the hydroxylation step from the standpoint of cofactor requirements, end-product inhibition, and the possibility of allosteric changes in conformation.

Pterin Cofactors

Pterins are necessary cofactors for hydroxylation (11, 19–21), but the exact chemical structure of the natural cofactor in brain is not certain. Most of the studies on the

kinetic constants of tryptophan hydroxylase have been made with the synthetic cofactor, 6,7-dimethyltetrahydropterin ($DMPH_4$) (22). When these constants are compared with those obtained with tetrahydrobiopterin (BH_4), the probable natural cofactor, it is clear that many of the inferences made concerning the regulation of tryptophan hydroxylase in vivo do not hold. For instance, the K_m of this enzyme using $DMPH_4$ as a cofactor is 290 μM (22), a finding which supports the view that enzyme in vivo is not saturated by tryptophan since its concentration in brain is about 50 μM. However, the K_m for tryptophan measured using BH_4 is 50 μM (22). Analogous differences have been reported measuring the K_m for O_2 with $DMPH_4$ (20%) and with BH_4 (2.5%) (22). The K_m values obtained with the probable natural cofactor for tryptophan and O_2 are therefore similar to the concentrations of these substrates in vivo. These data are consistent with the magnitude of increase in hydroxylation which is obtained by loading animals with tryptophan or subjecting them to hyperbaric oxygen. It has been suggested that most enzymes operate in vivo with substrates at a concentration approximately similar to the K_m (23).

Assuming that BH_4 or a pterin with similar biological properties is the natural cofactor, the pterin can function in hydroxylation only in the reduced form. Dihydropteridine reductase which has been isolated and purified from liver homogenates (24) can maintain the cofactor in the reduced state. This enzyme is also present in high concentrations in liver, kidney, brain, and adrenal medulla. This reductase might be regulatory for the hydroxylation of tyrosine and tryptophan (25, 26) but such a possibility is not substantiated by the available data (24). It would seem that the amount of reduced BH_4 rather than the enzyme could perform a regulatory function. The concentration of BH_4 in adrenal is 10 μM (20) and in brain is estimated to be 0.75 $\mu g/g$ (3 μM if evenly distributed) (27). Inferences as to regulatory potential for the biosynthesis of NE, DA, and 5HT in brain are premature until we know more about the regional and cellular distribution of BH_4. Nonetheless, we can speculate that the BH_4 concentrations may be limiting in some circumstances because the K_m of adrenal tyrosine hydroxylase is 20 μM (19) and that of brain tryptophan hydroxylase is 31 μM (22). The value of such speculation for catecholamine biosynthesis is further supported if one considers that the catechol products of tyrosine hydroxylase inhibit their own formation by competing with the pterin cofactor. If the pterin were to be in concentrations below the K_m, its potential as a regulatory mechanism would be enhanced. In addition, conformational changes of tyrosine hydroxylase (28–30) have been described and these are associated with changes of K_i values for the catecholamines, increasing the possibility of a physiological regulation by this mechanism.

The cofactors for dopamine-β-hydroxylase are ascorbic acid and copper. Ascorbic acid is probably not limiting in norepinephrine synthesis because the apparent K_m for ascorbate with the adrenal enzyme (0.9 mM) (31) is less than or similar to the reported content of ascorbate in adrenal and brain (32). Before the discovery of the noradrenergic pathways in the brain, histochemical evidence (33) suggested that there might be high ascorbic acid concentrations in the cells of the locus coeruleus and in the hypothalamus, areas rich in NE. Although it would be tempting to speculate that cells containing dopamine-β-hydroxylase are rich in ascorbic acid,

it might be that the silver stain used to detect ascorbic acid was actually being reduced by NE. Peripherally injected ^{14}C-ascorbic acid does not concentrate in brain in areas with high concentrations of NE (34).

pO_2

Molecular oxygen is required for hydroxylation of tyrosine, tryptophan, and DA. The K_m of tryptophan hydroxylase from rabbit brain for oxygen is approximately 2.5%, which is a concentration in the order of magnitude of those reported in brain (22), although pO_2 varies in different brain parts. When rats inhale 100% O_2, the pO_2 in various areas approximately doubles (35, 36). Under these conditions, the turnover rate of brain 5HT and cathecholamines increases (37, 38). Anoxia produces a decrease in the hydroxylation of tryptophan in vivo; this parallels the fall in arterial pO_2 (39). Tyrosine hydroxylase is also not saturated with oxygen (40), and breathing a concentration of oxygen lower than normal decreases the hydroxylation of tyrosine in vivo (39). Dopamine-β-hydroxylase is apparently also not saturated at normal pO_2 (41). Although changes in pO_2 may alter the rate of amine formation, oxygen is not likely to play a physiological role in regulating the catecholamine turnover rate. However, it would be desirable to know whether conformational changes in these hydroxylases alter the K_m for O_2 before ruling out changes in pO_2 as a physiological control mechanism for hydroxylation.

Allosteric and Other Conformational Changes of Hydroxylases

It is well established that nerve stimulation can increase the synthesis of catecholamines by a mechanism which is too rapid to be caused by de novo synthesis of tyrosine hydroxylase (8, 9). Part of the increased catecholamine synthesis might be related to a decrease in end-product inhibition, but Weiner and colleagues have concluded that additional factors may be operating (42). The conformation of tyrosine hydroxylase (and its activity) might vary with nerve firing rate, due to changes in the ionic environment or binding to membranes.

Potassium and sulfate ions can stimulate tyrosine hydroxylase activity in vitro. Sulfate ions in high concentrations (30) appear to interact with the enzyme molecules, but the high concentrations involved indicate that the effect is probably devoid of physiological importance. The activation by potassium (43) is probably not via direct interaction with the enzyme molecules, because it occurs only in slices, and thus requires the integrity of nerve endings. Potassium might affect tyrosine hydroxylase by interacting with nerve ending constitutents such as membranes, but the action is probably related to the release of catecholamines and the resulting decrease in end-product inhibition.

Tyrosine hydroxylase appears to occur partially free and partially bound to membranes (28, 44). The enzyme molecules are presumed to have the same amino acid sequence because the fraction that is bound depends on the homogenization conditions (28, 45). However, the conformations of the two forms are probably different because their catalytic properties are not identical. According to Kuczenski & Mandell (28), the membrane bound form of tyrosine hydroxylase, compared with the soluble form exhibits a smaller apparent K_m for $DMPH_4$ and a lower K_i for

dopamine. They suggested that the membrane binding of the enzyme has an important regulatory value in synaptic function. The kinetic parameters of the soluble enzyme become like those of the particulate form if heparin is added: it lowers the K_m for $DMPH_4$, decreases the K_i for dopamine and increases the apparent V_{max} of the enzyme (28, 30). Heparin is not an important constituent of the brain, but other sulfated mucopolysaccharides are components of cellular and synaptic membranes. This activation occurs only in the presence of tris buffer at certain pHs and has not yet been achieved with preparations of naturally occurring brain membranes.

The ratio of the particulate form to the soluble form is smaller in areas rich in aminergic cell bodies. This finding supports the possibility that the apparent two forms of subcellular localization are real. In adrenal chromaffin cells, tyrosine hydroxylase was thought to occur primarily in a bound form (46, 47). Recent work suggests that the adrenal enzyme is mainly free and that the apparent particulate nature was due to an aggregation of enzyme molecules (48, 49). This question is still controversial (50).

Whether or not the adrenal enzyme is particulate, it may be that the activity of the brain enzyme can be modified by a shift in location from a free to a bound form. It is quite possible that the control mechanisms operating in the brain differ from the adrenal where end-product inhibition and enzyme induction are more likely to be important.

The ratio in brain of free to bound tyrosine hydroxylase can be changed by pharmacological manipulations. A single dose of methamphetamine (2.5 mg/kg) produced a slight but significant shift in localization of tyrosine hydroxylase from the soluble to the particulate form (51). A similar effect was seen after reserpine and α-methyltyrosine. However, imipramine, foot shock, and/or electroconvulsive shock (all of which increase the turnover rate of brain NE) failed to increase the activity of tyrosine hydroxylase (51).

In our laboratory, Drs. Zivkovic and Guidotti have found that free, but not bound striatal tyrosine hydroxylase activity is doubled within 30 min after injection of small doses of tranquilizers of the phenothiazine or butyrophenone type. These compounds are dopamine receptor blockers, and increase the firing rate of dopaminergic neurons, probably via a neuronal feedback loop (see below). The effect on tyrosine hydroxylase can be reversed by apomorphine, which stimulates dopamine receptors and slows the firing rate of the dopaminergic neurons. These changes in tyrosine hydroxylase activity were observed in the dopaminergic neurons of the striatum, but did not occur in the noradrenergic neurons of the hypothalamus.

The occurrence of two forms of tryptophan hydroxylase in brain and their varying regional distribution have been discussed by Knapp & Mandell (52–55). Homogenizing tissues with isotonic sucrose, they found that the enzyme of brain stem (cell bodies) is soluble, whereas that of other parts of brain is particle bound because it is associated with synaptosomes (the particle bound tyrosine hydroxylase discussed above occurs even after lysis of the synaptosomes). This tryptophan hydroxylase is not activated by the synthetic cofactor ($DMPH_4$), because the cofactor cannot penetrate the synaptosomal membrane (56). After chronic morphine administra-

tion, Knapp & Mandell have described an increase in tryptophan hydroxylase activity compensatory to an initial inhibitory action. The question of whether two forms of tryptophan hydroxylase exist in vivo and their regulatory significance deserves further investigation.

The best direct evidence that allosteric effects alter the conformation of one of the mixed function oxygenases acting upon an amino acid comes from studies of liver phenylalanine hydroxylase, which shares with tyrosine hydroxylase many kinetic properties, cofactor requirements, and antigenic nature (57). Lysolecithin, a detergent-like lipid, reversibly stimulates phenylalanine hydroxylase activity and causes a conformational change of the enzyme which results in exposing a SH group (58). A similar stimulation of brain tyrosine hydroxylase (59) occurs with the synthetic detergent Triton X-100, although the enzyme was not purified enough to study directly a conformational change. When solubilized hydroxylases from brain or adrenal medulla are treated with proteolytic enzymes, the enzyme's molecular weight is reduced (29, 44, 50, 58, 60) but the catalytic activity tends to increase (29, 58). The increased activity is associated with a decreased K_m for substrate and cofactor. These findings support the possibility that if depolymerization occurs in vivo it may produce significant increases in enzyme activity.

End-product Inhibition

Tyrosine hydroxylase can be inhibited in vitro by its end-product 3,4-dihydroxyphenylalanine (Dopa) or by its metabolites DA and NE (46). DA is more active than NE in competing with the pteridine cofactor for tyrosine hydroxylase (28, 46). The first demonstration that feedback inhibition may be important in the control of tyrosine hydroxylation in vivo came from studies with monoamine oxidase inhibitors (MAO) (61–64). Pargyline causes a decrease in the rate of NE and DA biosynthesis in the CNS and peripheral tissues. In the CNS, the DA and NE concentrations increase rapidly and the turnover rate is decreased within 1 or 2 hr after the injection of paragyline. This increase in concentration of catecholamines could inhibit tyrosine hydroxylase by competition with cofactors. However, the possibility cannot be ruled out that pargyline slows down the rate of catecholamine synthesis by slowing the firing rate of catecholamine neurons, or by other actions associated with MAO inhibitors.

The importance of endproduct inhibition in the regulation of peripheral catecholamine biosynthesis can be documented in heart. Here, due to the slow synthesis rate of catecholamines, they accumulate more slowly after inhibition of MAO (65). It is therefore possible to show that the time course of reduction of the rate of catecholamine biosynthesis parallels the accumulation rate of catecholamines and not the concentration of the MAO inhibitor in tissues.

If tyrosine hydroxylase is located outside the granules, then only the extragranular pool of Dopa and catecholamines can function in the physiological control of catecholamine biosynthesis by end-product inhibition. While the extragranular pool may be large after MAO inhibition, approximate estimates of pool sizes can be made. In the dopaminergic terminals of the striatum, the total dopamine concentra-

tion has been calculated to be at least 10^{-2} *M* (66). Since the K_i of striatal tyrosine hydroxylase for inhibition by dopamine is less than 10^{-4} *M* (28), significant inhibition would occur if only 1% of the dopamine were extragranular. The estimate of the K_i for dopamine is made with saturating concentrations of cofactor. If the cofactor is limiting, the inhibition would occur at even lower concentrations.

In noradrenergic terminals of spinal cord, the NE concentration is also calculated to be greater than 10^{-2} *M*. Because the DA concentration in various tissues may be 12–18% (67) that of NE, the total concentration of DA in the terminals might be 10^{-3} *M*. The distribution of DA in NE terminals is not established, but significant amounts are likely to occur free. Since DA is a more effective feedback inhibitor than NE, the possibility that DA operates in the feedback control of NE biosynthesis cannot be disregarded (68). The availability of specific MAO inhibitors which cause DA but not NE to increase, makes it possible to test this hypothesis (69).

Despite the difficulty of providing conclusive evidence on the physiological role of feedback control by product inhibition, the possibility that such control contributes to noradrenergic regulation should be investigated further.

Tryptophan hydroxylase is not inhibited in vitro by high concentrations of 5HTP, 5-hydroxyindole acetic acid (5HIAA) or 5HT. The enzyme is inhibited by catecholamines, but such inhibition is probably only important under artificial conditions such as after treatment of animals with large doses of Dopa. The turnover rate of brain 5HT is the same whether measured when the concentration of brain 5HT is normal or when it is increased threefold by MAO inhibition (70). In experiments with labeled tryptophan, the rate of synthesis of 5HT was measured in vivo without considering the change with time of the specific activity of the precursor and product (71). It was concluded that the 5HT synthesis could be controlled by end-product inhibition. Because the validity of these experiments has been questioned on technical grounds (72) the issues involved are still unsettled.

LONG-TERM INCREASES IN ENZYME ACTIVITIES IN MONOAMINERGIC CELLS

Persistent increases of impulse traffic in noradrenergic or dopaminergic cells or in the afferents to chromaffin cells bring about an increase of tyrosine hydroxylase (73) and dopamine-β-hydroxylase activities of tissue homogenates (74). Perhaps, this increase is due to enzyme induction (an increased synthesis of enzyme molecules) because it can be blocked by inhibition of protein synthesis (75, 76). The increase in the adrenal enzyme is described as trans-synaptically induced, a term seldom applied to the CNS. However, in both cases the delayed long-term increases in enzyme activity probably arise from long-term increases of firing rate (CNS) or rate of stimulation (chromaffin cells) and require the presence of an intact afferent system. These long-term increases have been best studied in the adrenal; less information is available for sympathetic ganglia and brain. The peripheral systems will be considered first to provide a framework for discussion of the data available for the brain.

Adrenal Medulla

Studies from this laboratory (77–82) have suggested that the increase of tyrosine hydroxylase activity in adrenal medulla involves the following sequence of events: 1. stimulation of nicotinic receptors, 2. increase of the concentration ratio of 3',5'-adenosine monophosphate (cAMP) to 3',5'-guanosine monophosphate (cGMP), 3. elaboration of the stimulus and 4. increase of enzyme activity.

1. Stimulation of nicotinic receptors: In the adrenal medulla, catecholamine secretion from chromaffin cells is elicited by cholinergic stimuli acting via nicotinic receptors (83). Exposure to 4°C for 1 hr or injection of a cholinomimetic (carbamylcholine or mecholine) can increase the activity of tyrosine hydroxylase after a latency of about 12 to 16 hr. Denervation of adrenals prevents the increase of tyrosine hydroxylase activity elicited by cold exposure but not that following the injection of the two cholinomimetics. Pretreatment with mecamylamine or hexamethonium (nicotinic blockers) prevents the increase of tyrosine hydroxylase activity elicited by either type of stimulus.

2. Increase of cAMP/cGMP concentration ratio: Exposure of rats to 4°C for 60 min or the injection of nicotinic stimulants causes an increase of the cAMP/cGMP concentration ratio which persists for about 2 hr even when the rats are exposed to cold for several hours. Denervation of the adrenal gland fails to change their phosphodiesterase activity or the concentration of cAMP but reduces the activity of cyclic nucleotide synthesizing enzymes and the concentrations of cGMP. If slices of adrenal medulla are incubated with carbamylcholine ($5X10^{-5}M$), cAMP tends to accumulate in these slices at a rate faster than that of slices incubated without the nicotinic receptor stimulant. Chromaffin cells contain high concentrations of cAMP (about 40 pmol/mg protein) (78, 84, 85) and possess a very high adenylcyclase activity (39 pmol/mg protein/min). Cold exposure or the injection of nicotinic receptor stimulants (86) increases the catecholamine output from medulla and other peripheral stores. However, the concentration of cAMP in the adrenal medulla is not affected by drugs that increase circulating catecholamines but do not stimulate adrenal nicotinic receptors. The increase of medullary cAMP can therefore be dissociated from the release of catecholamine from adrenal medulla, but not from conditions that cause long-term increase of tyrosine hydroxylase activity. The report by Kvetnansky and colleagues (87) that the injection of dibutyryl cAMP restores the activity of tyrosine hydroxylase which had been reduced by hypophysectomy supports this relationship. Recent studies have shown that acetylcholine can cause accumulation of cGMP in heart, brain, vas deferens, and intestine; this effect is inhibited by atropine (88–91). Actually, Lee et al (90) recently suggested that excitation of muscarinic receptors increases the cGMP/cAMP concentration ratio in target cells whereas adrenergic receptor stimulation should increase the cAMP/cGMP concentration ratio in these cells. Because carbamylcholine injections increase the cAMP/cGMP concentration ratio in the adrenal medulla, this change is not exclusively elicited by adrenergic stimulation. Perhaps, we should refrain from explaining excitation of specific receptors in terms of changes of cyclic

nucleotide concentration ratios until we understand the location where cyclic nucleotides accumulate in response to receptor stimulation.

The view that a sustained increase of cAMP is an important signal in eliciting an increase of tyrosine hydroxylase activity in the adrenal medulla has been challenged (92). Thoenen et al (92) made 100 g rats swim in a water bath at 15°C for 7 to 10 min, and repeated this stress six times in 2 hr. This procedure caused a delayed and prolonged increase of medullary tyrosine hydroxylase although cAMP concentrations in this tissue were not increased at the end of each swimming stress. These authors (92) concluded that an increase of cAMP concentration did not necessarily precede trans-synaptic induction of tyrosine hydroxylase activity. However, we (80) found that swimming stress at 15°C in 100 g rats caused a decrease in body temperature of about 17°C which lasted more than 40 min. In adrenal medulla, the increase of cAMP/cGMP concentration ratio occurs only after the body temperature has returned to normal. Thus, swimming stress experiments do not negate the concept that in adrenal medulla an increase of cAMP/cGMP concentration ratio precedes the increase of tyrosine hydroxylase activity elicited trans-synaptically.

3. Elaboration of the stimulus: As mentioned previously, the increase of cAMP/cGMP concentration ratios occurs several hours before the long-term increase of tyrosine hydroxylase activity. There is no information yet on the phenomena involved in the elaboration of the message brought about in the chromaffin cells by the increase of cAMP/cGMP concentration ratios. Although we know from the work of Axelrod and colleagues (76) that the control of protein concentration is involved, we have no data to suggest whether it is at the transcription or at the translation level, or whether there is an increase in the synthesis rate of tyrosine hydroxylase or a reduction of its catabolism. The only information available is that a blockade of the nicotinic receptor during the elaboration of the stimulus fails to impair the increase of tyrosine hydroxylase activity (80).

4. Increase of enzyme activity: The term enzyme induction implies either an increased synthesis rate of enzyme molecules or an increase in the number of molecules from whatever cause. The latter use is more common, because unless the number of molecules can be estimated immunologically (93), it is difficult to determine the exact cause of long-term increases in enzyme activity. The concentration of an enzyme in a neuron depends on the balance of two processes: synthesis which occurs in the cell body and breakdown which can occur anywhere in the neuron. In the chromaffin cell, these sites are much closer together. In discussing activity of tyrosine hydroxylase in adrenal medulla one should keep in mind that there is a certain degree of functional alternation among cells of this tissue and that a masking or an unmasking of enzyme activity could be associated to this functional alternation. If this were the case, it would seem quite unusual that the mechanisms involved in this chemistry have a time constant of several hours. Another consideration concerns the possible hyperplasia or hypertrophy of the tissue. In chromaffin tissue, hyperplasia is probably a process of limited importance in the genesis of increases of tyrosine hydroxylase enzyme molecules. An increase in the number of cells is also not a cause for increased amounts of enzyme in brain, because adrenergic

neurons in brain stop dividing early in life. Noradrenergic terminals in brain can sprout in response to injury (94), but there is no evidence that sprouting is a mechanism involved in the long-term increase of enzyme activity elicited transsynaptically.

Sympathetic Ganglia

Thoenen (95) first reported that exposure to 4°C for 48 hr increases tyrosine hydroxylase activity in sympathetic ganglia. Similar studies were extended to dopamine-β-hydroxylase. Hanbauer et al (96) have analyzed whether a sequence of events, similar to that reported for adrenal medulla is operative in the increase of tyrosine hydroxylase activity of rat cervical sympathetic ganglia elicited by cold exposure. Normally, rats require long-lasting exposure to cold before one can detect an increase of tyrosine hydroxylase activity in their ganglia. But, if the rats are demedulated at least 3 weeks prior to the experiment, then an exposure to 4°C for only 4 hr is sufficient to elicit an increase of tyrosine hydroxylase activity 48 hr later. Decentralization or pretreatment with mecamylamine or hexamethonium abolishes the delayed increase of tyrosine hydroxylase. However, if hexamethonium or mecamylamine are injected, after the stimulus, they fail to inhibit the increase of tyrosine hydroxylase activity (96). In ganglia of demedullated rats there is an increase of cAMP concentration and a decrease of cGMP concentration during the stimulus application (Hanbauer & Guidotti, unpublished). The increase of the cAMP/cGMP concentration ratios persists for the duration of the stimulus application (exposure to 4°C). Treatment with hexamethonium before exposure to cold prevents the increase of cAMP/cGMP concentration ratios as well as the delayed increase of tyrosine hydroxylase activity.

Pretreatment with atropine before or after the stimulus application fails to change the increase of tyrosine hydroxylase activity elicited by exposure to 4°C. In contrast, decentralization performed before or at various times after the cold exposure prevents the delayed increase of tyrosine hydroxylase activity in the ganglia. In addition, the injection of carbamylcholine to rats with unilateral decentralization of the superior cervical ganglion fails to elicit an increase of cAMP/cGMP concentration ratios or a delayed long-term increase of tyrosine hydroxylase activity in either ganglion. It is therefore apparent that in rats exposed to 4°C the correlation between the early increase of cAMP/cGMP concentration ratios and the delayed long-term increase of tyrosine hydroxylase activity which appears to be valid in the adrenal medulla might not be extrapolated to the sympathetic ganglia. This is not surprising because the cell population of adrenal medulla is rather uniform functionally and morphologically whereas the cells of sympathetic ganglia differ in both respects. The cyclic nucleotides in ganglia could be localized in presynaptic cholinergic endings, noradrenergic neurons, small intensely fluorescent cells and in a variety of supporting cells.

Central Nervous System

The delayed increase in long-term activity of brain tyrosine hydroxylase (95) and tryptophan hydroxylase differ significantly from those seen in the adrenal: the

magnitude of the change is much less, and the delay is longer. Moreover, the interpretation of brain tyrosine hydroxylase increases is complicated, because in several brain areas both dopaminergic and noradrenergic neurons are present. In the brain, another factor which must be considered is that the enzyme is synthesized in cell bodies and must be moved a considerable distance to the nerve terminals by axonal transport. Thus, in brain there is a considerable lag between onset of increased protein synthesis and any increase in enzyme in the nerve terminals (97–99) (especially in large species); whereas in the adrenal, the only lag is the time interval needed for protein synthesis.

The tyrosine hydroxylase activity of several brain areas is increased 2 to 8 days after reserpine or cold exposure, but the extent of the increase is rather small (73, 95, 97, 100). In caudate, the extent of this increase is greater, but occurs within a few hours after the injection of reserpine or within 30 min after phenothaizines Zivkovic and Guidotti have shown that protein synthesis inhibition does not affect this enzyme increase, suggesting that a mechanism different from the long-term increase of enzyme activity is involved. Repeated convulsive shocks increase brain tyrosine hydroxylase activity after 7 days (101) while phenothiazine-neuroleptics decrease it (97). Attempts at correlating the long term-delayed increase of tyrosine hydroxylase and the increase of cAMP/cGMP concentration ratios is impractical because the two nucleotides are present in a variety of other neurons present in the same brain areas and in numerous supporting cells.

There are conflicting reports as to whether morphine increases tryptophan hydroxylase activity (102–104). When the studies were conducted distinguishing between tryptophan hydroxylase in cell bodies and nerve endings, chronic morphine administration produced an immediate decrease (53) and a long-term increase of tryptophan hydroxylase in areas rich in serotonergic nerve endings, but it did not change enzyme activity in an area containing cell bodies. Short-term treatment with lithium chloride stimulates the uptake of tryptophan and its conversion to serotonin by synaptosomes (54). Cell body tryptophan hydroxylase activity is reduced at 5 days of treatment even if the uptake of tryptophan is increased. After 10 days of lithium treatment, the tryptophan hydroxylase activity in the nerve endings is decreased while the tryptophan uptake remains increased. The delay in the transfer of the alteration from cell bodies to nerve terminals corresponds in time to the axoplasmic transport for tryptophan hydroxylase (105). Lysergic acid diethylamide (LSD) is reported to decrease tryptophan hydroxylase activity (99). The mechanism is unknown but LSD, which is possibly a 5HT agonist at postsynaptic receptors, decreases the firing rate of serotonergic neurons.

Bilateral adrenalectomy reduces the tryptophan hydroxylase activity and the turnover rate of 5HT in the midbrain (106). The adrenocortical function plays a role in regulating tryptophan hydroxylase activity, and many drug effects appear to be mediated by adrenocortical secretion. Reserpine in high doses (5 mg/kg ip)(99) causes an increase of midbrain tryptophan hydroxylase activity beginning 10–14 hr after the injection. This increase reaches a plateau between 36 and 48 hr after the injection of the drug. The increase extends to the serotonergic endings after a latency time compatible with the axoplasmic transport of the enzyme. Inhibition of protein

synthesis by injection of cycloheximide into the cerebral vertricles abolishes the reserpine-induced long-term delayed increase of tryptophan hydroxylase activity. The increase of enzyme activity elicted by reserpine appears to involve the increased secretion of corticosteroids elicited by this drug. Pargyline prevents this action of reserpine, possibly by inhibiting the increase of plasma corticosteroids (99). Forebrain tryptophan hydroxylase is increased 4 hr after an injection of reserpine, presumably via a different mechanism (107).

In conclusion, various drugs can elicit a delayed long-term increase in tyrosine or tryptophan hydroxylase activity in the CNS, possibly via mechanisms that are similar to those seen in the periphery. In general, the long-term responses require prolonged and drastic treatment. Perhaps, because the brain has greater plasticity and the neurons have complex patterns of innervation, enzyme induction may be less important as a control mechanism. The functional significance of the long-term increase of hydroxylase activity remains to be established.

FEEDBACK CONTROL OF MONOAMINE SYNTHESIS VIA A NEURONAL LOOP

In brain, the short-term control of monoamine synthesis involves feedback control of the firing rate of monoaminergic neurons, which is linked in some unknown way to the regulation of monoamine synthesis rate. The neuronal feedback can be illustrated by the increase in turnover rate of dopamine in the striatum, which is elicited by many blockers of dopaminergic receptors, including chlorpromazine (108–110). Carlsson & Lindqvist (111) proposed the following explanation: chlorpromazine blocks the dopamine receptors in the striatum, thus altering the firing rate of the postsynaptic neurons. These neurons via collaterals or a series of interneurons increase the firing rate of the dopaminergic neurons, thus causing an increased release of dopamine in attempt to overcome the receptor inhibition caused by the phenothiazine. The resulting changes in striatal tyrosine hydroxylase activity might be explained by a decreased end-product inhibition, or by a change of the K_m of the enzyme for the pteridine cofactor.

There is now a considerable body of evidence consistent with this model not only for dopaminergic neurons, but also for serotonergic and noradrenergic neurons (112–115). Direct evidence for drug-induced changes in firing rate of aminergic neurons has been made possible by extracellular recordings of the cell bodies of serotonergic neurons in the raphe, of the dopaminergic neurons in the substantia nigra, and of the noradrenergic neurons in the locus coereleus (113–115). These electrophysiological experiments confirm that phenothiazines increase the firing rate of dopaminergic neurons. Monoamine oxidase inhibitors, which raise the transmitter amine's concentrations by blocking their destruction, decrease the firing rate of serotonergic and dopaminergic neurons, and decrease the synthesis of norepinephrine. LSD, which is claimed to be a serotonergic agonist (116), slows the firing rate of serotonergic neurons and decreases the turnover of 5HT. Apomorphine, which stimulates dopamine receptors, slows the firing rate of dopaminergic neurons and slows dopamine synthesis (114). Carlsson and co-workers have attempted to alter

directly the firing rate of aminergic neurons by axotomy and have then studied the "in vivo activity" of tyrosine and tryptophan hydroxylase (117). They measured the accumulation of Dopa and 5HTP induced by a decarboxylase inhibitor. The results with the different amines and in the different areas of the CNS do not form a consistent picture: spinal transection markedly slows the accumulation of 5HTP in the caudal half of the spinal cord suggesting that axotomy decreased amine turnover. In contrast, brain transection rostral to the cell bodies in mesencephalon does not alter the rate of Dopa accumulation in noradrenergic neurons, or that of 5HTP in serotonergic axons and endings in the forebrain. A still different effect was seen in the dopaminergic system: cerebral hemisection increases the Dopa accumulation in striatum and causes a rapid rise in DA (118).

The negligible effect of axotomy on 5HTP and Dopa accumulation in the cerebral hemispheres cannot be readily explained because of a number of uncontrolled factors involved in these experiments. When measuring Dopa, in the forebrain one includes Dopa measurements in dopaminergic and noradrenergic neurons, and this discrepancy is reflected in diminishing the specificity of this estimation.

A model can be proposed to explain these effects, based on a hypothetical system of interneurons and presynaptic receptors (114, 119). It suggests that the dopaminergic neurons have presynaptic inhibitory dopamine receptors on their terminals (Figure 1a). Activation of these receptors inhibits the release and synthesis of dopamine. When the axons are cut, impulse flow stops, and due to a decrease in stimulation of the inhibitory receptor, synthesis of dopamine increases. Serotonergic neurons in the cord might not have serotonin presynaptic receptors, but they might be controlled presynaptically through either a collateral of the target cell or an interneuron. These can be activated by other synaptic inputs but inhibit presynaptically the serotonergic neuron (Figure 1b). When the serotonergic neuron is cut by spinal section at the midthoracic level, the collateral is not cut, the postsynaptic cell continues to fire by virtue of its innervation by other neurons, and synthesis and release of 5HT is inhibited. A similar situation may exist in noradrenergic and serotonergic endings in the forebrain, but transection is much closer to the terminals and also involves the collaterals mediating the feedback. Alternately, the feedback loop in the brain extends to the aminergic cell body and is cut by the hemisection. In either case, whether or not the postsynaptic cell fires, there would be no inhibition of release or synthesis, because there is no input to the terminals.

The following evidence is consistent with this model:

1. Presynaptic receptors exist in the CNS and have been shown in the peripheral adrenergic system. Presynaptic inhibition in the CNS has been reviewed (120, 121). The best studied case involves the primary afferent terminals (which innervate motor neurons) and are innervated by terminals of another system. This second system makes axo-axonal synapses on the primary afferent terminals. Stimulation of the second system depolarizes the primary afferent terminals, blocking action potentials, and inhibiting release of transmitter. In the peripheral nervous system there is evidence for prejunctional α-adrenergic (122, 123) and muscarinic receptors (124), both of which can affect NE release.

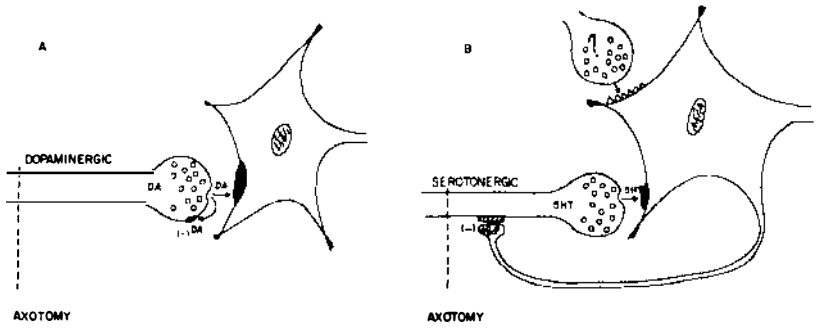

Figure 1 Model illustrating possible feedback loops in (*A*) dopaminergic terminals of the striatum, and (*B*) serotonergic terminals in the spinal cord. Dopamine via a presynaptic receptor inhibits its own synthesis and release. The inhibition is removed by axotomy. Serotonin inhibits its own synthesis via a collateral from the post-synaptic neuron. The inhibition continues or is increased after axotomy.

2. Dopamine receptor stimulators can decrease DA synthesis in striatal slices. This effect is seen both when the compounds are given in vivo, or added in vitro at 10^{-6} *M* concentrations (125). This effect is not seen in homogenates, and the compounds do not affect tyrosine hydroxylase directly at this concentration. The slices are probably too small to allow an extensive feedback system, and the post-synaptic cell might not be able to respond to apomorphine stimulation even if feedback were possible. The situation in slices is not completely clear because the apomorphine effect is blocked by haloperidol when it is given in vivo but not in vitro. Tyrosine hydroxylase in slices is increased by dibutyryl cyclic AMP (125) while release of norepinephrine is enhanced (126), but it is not clear whether receptors are involved.

3. Apomorphine injections reduce the amine accumulation seen in the striatum after cerebral hemisection (118), and apomorphine antagonizes the increase in enzyme activity elicited by injection of haloperidol.

PRECURSOR CONCENTRATIONS

Serotonin is the only one of the three putative neurotransmitters considered in this review whose synthesis can be accelerated by injections of its precursor, tryptophan. It is believed that tryptophan hydroxylation may function as a rate limiting step in 5HT formation, because its maximum velocity is lower than that of the aromatic decarboxylase which controls the successive step. Because the amount of tryptophan present in brain does not saturate the enzyme, the tryptophan concentration in brain determines the rate of 5HT formation. If an enzyme is saturated, that is the substrate concentrations present at the catalytic sites are at least several times the K_m, then small modifications in the substrate concentration will not affect the rate of reaction. It is well known that administration of tryptophan, or withholding it from the diet

will increase, and decrease, respectively the concentration of 5HT in brain (127–131). In contrast, loading rats with tyrosine increases brain tyrosine, but does not affect the concentrations of the catecholamines in brain (132). In vitro, very high tyrosine concentrations can inhibit catechol formation (50) but this effect has not been observed in vivo (132).

Early work with tryptophan hydroxylase in vitro (133) showed that the K_m for tryptophan using a synthetic cofactor ($DMPH_4$) was 300 μM. This K_m is high compared with the concentration of tryptophan in brain (approximately 30 μM). Jequier et al (133) therefore suggested that "the enzyme may not be fully saturated with substrate normally, and that the overall rate of serotonin synthesis may be partially dependendent upon availability of tryptophan." It has since appeared that the K_m of tryptophan hydroxylase is much lower (50 μM) when BH_4 is used as a cofactor (22). This K_m value appears to be consistent (22) with data from experiments with tryptophan loading. A dose of 50 mg/kg ip, tryptophan causes the brain tryptophan concentration to be about 100 μM (threefold normal), and the brain serotonin concentration doubles (134). Larger doses of tryptophan increase the brain tryptophan content proportionately, but produce no further appreciable increase in brain 5HT. Grahame-Smith (135) noted a similar effect, but found that the maximum rise in 5HT occurred with a dose of 120 mg/kg tryptophan which produced a brain tryptophan concentration of approximately 350 μM. Thus, serotonin formation in vivo is maximal when the brain tryptophan concentration rises by 3–6 times (reaching 3–6 times the K_m of tryptophan hydroxylase measured in vitro with BH_4 as the cofactor). A similar conclusion was derived from estimates of the "in vivo activity" of tryptophan hydroxylase (117). Therefore, it appears that tryptophan hydroxylase is not saturated in vivo and that by changing the brain tryptophan concentrations it is possible to control the rates of 5HT synthesis and accumulation.

A major current controversy concerns whether drugs and physiological variables that affect 5HT synthesis do so by means of an effect on brain tryptophan concentrations. Tagliamonte et al (136–140) have shown that there are many drugs that produce parallel changes in brain tryptophan and brain 5HT turnover rate. (In these studies and most of the others to be discussed, changes in brain 5HT turnover rate are inferred from changes in steady-state 5HIAA concentrations). They showed that amphetamine, lithium salts, dibutyryl cyclic AMP, reserpine, 40°C environment, and electroconvulsive shock increase brain 5HT turnover rate and also increase the concentration of tryptophan in brain. In contrast, *p*-chlorophenylalanine, which slowly inhibits tryptophan hydroxylase and promptly lowers brain tryptophan, decreases 5HT turnover (136, 137) when only brain tryptophan is lowered. They therefore suggested that changes in the serotonin turnover produced by these drugs might be secondary to their effects on brain tryptophan concentrations. Although in many cases plasma tryptophan was increased when brain tryptophan rose, there was not a complete correlation. Brain tryptophan is, of course, derived from plasma, and after a tryptophan load the concentrations in brain and plasma are directly proportional to each other (135). However, tryptophan requires a transport mechanism to pass the blood brain barrier, and so factors that affect this uptake can alter the distribution between plasma and brain which is normally 4–6:1.

Fernstrom & Wurtman have examined in detail factors that affect brain and plasma tryptophan concentrations. Plasma tryptophan is subject to diurnal variations (21 μg/ml at noon; 32 μg/ml at midnight), due to variations in diet corticosteroid levels and the activity of hepatic catabolizing enzymes (134, 141). Intraperitoneal doses of tryptophan which change plasma tryptophan concentrations by an extent smaller than the circadian variation also produce a significant rise in brain 5HT content (134). However, a rise in plasma tryptophan does not increase brain tryptophan if high concentrations of other amino acids are present to inhibit tryptophan uptake completely. Oldendorf (142) has studied penetration into brain of tryptophan that was injected into the carotid artery. The amino acids that inhibited this process were tyrosine, phenylalanine, histidine, leucine, isoleucine, valine, and methionine. Excess phenylalanine is known to decrease brain 5HT, either when present in the diet (130) or given acutely (143, 144), or in phenylketonuria (145, 146) due to an enzymatic defect. A diet low in tryptophan leads to a decrease in brain tryptophan and 5HT (130, 147). A diet rich in carbohydrates increases brain tryptophan and 5HT, apparently because insulin is released causing plasma trytophan to rise (148–150).

Tryptophan is one of the few amino acids that is bound to plasma albumin. The ratio of bound to free (20:1) (151) or 2:1 (152) can be altered by agents that compete for binding sites including nonesterified fatty acids and salicylates (153). Curzon and co-workers suggest that the tryptophan that actually influences 5HT turnover is that which is free in the plasma (151). They showed that food deprivation and immobilization stress increase brain tryptophan, 5HT turnover, and free tryptophan in plasma, while total plasma tryptophan remained constant. Administration of nonesterified fatty acids or of heparin (which increases plasma nonesterified fatty acids) can also release tryptophan bound to plasma (154), but it hasn't yet been shown that these treatments increase brain 5HT turnover rate.

TRYPTOPHAN CONCENTRATIONS AND SEROTONERGIC FUNCTION

All of these studies show that brain 5HT and 5HIAA concentrations correlate fairly well with brain tryptophan and with free and total tryptophan in plasma. However, the correlations are not perfect. There are conditions when brain or plasma tryptophan levels (free or total) are high, while 5HT and/or 5HIAA concentrations are below normal, and vice versa (150, 155–157). This incomplete correlation means that there are other parameters that can control brain 5HT synthesis, but they do not necessarily negate the possibility that control of 5HT synthesis via brain tryptophan concentrations is operative. A major unanswered question is the functional consequences of the increase of brain 5HT synthesis brought about by increases in tryptophan brain concentrations. It seems unlikely that a neurotransmitter such as 5HT, which is possibly involved in sleep, sexual activity, motor behavior, and emotional states, would be subject to functional control by dietary composition and so on, although the firing rate of raphe neurons is affected by tryptophan loading (115). Changes in blood tryptophan may be read out by the brain in a yet unknown

way (148), but it also seems likely that the diverse brain functions mediated by serotonergic neurons are not controlled by gross changes in plasma tryptophan concentration. Several authors have suggested that the amount of 5HT formed is in excess of the amount needed, and that this excess is not stored in vesicles, but is immediately degraded by monoamine oxidase (135, 158, 159). As evidence, Grahame-Smith (135) points out that animals receiving tryptophan display an increase of 5HT concentrations, but that motor behavior does not change. On the other hand, when the rats are treated with tryptophan and a monoamine oxidase inhibitor even when smaller amounts of 5HT are formed, motor behavior is strikingly increased, probably due to a spillover of "active" 5HT onto receptors. Welch has also suggested (159) that an excess of 5HT is formed and hypothesized that the activity of monoamine oxidase is modulated to control the amount of 5HT available for use. It is also possible that MAO activity is constant, but that access of the amines to the mitochondrial MAO is variable, depending, for example, upon the availability of vesicles for storage. Unfortunately, no method exists for measuring what fraction of the 5HT synthesized is eventually released into the synaptic cleft.

CONCLUSIONS

The synthesis of neurotransmitter amines in the CNS normally reflects the rate of impulse flow in aminergic neurons, but when drugs are administered such a relationship does not necessarily hold. A number of other factors can change amine synthesis rate. Neither the effects of psychotropic drugs on these rates, nor the normal control mechanisms can be understood until we have a deeper insight into the function of the various regulatory processes involved. It is now clear that control mechanisms involved are the concentration of pterin cofactors, end-product inhibition by the catecholamines, tryptophan concentration, activation of enzymes by allosteric changes, and long-term increases in enzyme activity. However, large gaps in our knowledge make it difficult to assess the importance of these variables.

For a clear understanding of these processes, we need to know the following: 1. The exact chemical structure of the cofactor present in brain with each of the hydroxylases, and the amount present in the reduced form in vivo; 2. whether or not the hydroxylases are saturated with cofactor in vivo, and whether a decrease in the K_m for the cofactor by allosteric changes increases enzyme activity; 3. whether the particulate and soluble forms of the hydroxylases represent functionally different but interconvertible forms.

Other possibilities can, in turn, be examined only when we begin to find answers to the questions formulated above. In this review we have discussed general mechanisms that control monoamine synthesis that might be possible sites for drug action. Many pharmacologically interesting compounds affect aminergic neurons by mechanisms of action which are completely unknown. For example, the benzodiazepines (minor tranquilizers) block the increase in NE turnover produced by stress, in doses devoid of any effect on amine turnover in normal animals (160, 161). One of the mechanisms discussed above might be directly affected by the benzodiazepines, but they might directly affect another system of neurons that in stress influence the

noradrenergic neurons. It is apparent that much remains to be learned about the anatomical inputs to the monoaminergic neurons as well as their biochemistry.

At a recent symposium, "Frontiers in Catecholamine Research," many of the topics considered dealt with the questions discussed above. It appears that within the next few years, great strides will be made in our understanding of the functional complexity of the aminergic systems. In addition to our current knowledge of the anatomy of the aminergic pathways, we can hope to understand better their biochemical and neurophysiological nature and their significance in terms of behavior and psychiatry.

Literature Cited

1. Costa, E., Neff, N. H. 1970. *Handb. Neurochem.* 4:45–90
2. Costa, E. 1970. *Advan. Biochem. Psychopharmacol.* 2:169–204
3. Costa, E. 1972. *Proc. Int. Congr. Pharmacol., 5th, Basel, Switzerland* 4: 215–26
4. Cotten, M., Ed. 1972. *Pharmacol. Rev.,* Vol. 24 (2) (Proc. NY Heart Assoc. Symp. Regul. Catecholamine Metab. Sympathetic Nervous System)
5. Usdin, E., Snyder, S., Eds. *Frontiers in Catecholamine Research.* (Proc. Int. Catecholamine Symp., 3rd.) London: Pergamon. In press
6. Costa, E., Gessa, G. L., Sandler, M., Eds. 1974. *Advan. Biochem. Psychopharmacol.* Vols. 10, 11. In press (Proc. Int. Symp. 5-Hydroxytryptamine and other Indolealkylamines in Brain)
7. Molinoff, P. B., Axelrod, J. 1971. *Ann. Rev. Biochem.* 40:465–500
8. Weiner, N. 1970. *Ann. Rev. Pharmacol.* 10:273–90
9. Udenfriend, S., Dairman, W. 1970. *Advan. Enzyme Regul.* 9:1445–65
10. Geffen, L. B., Livett, B. G. 1971. *Physiol. Rev.* 51:98–157
11. Kaufman, S. 1967. *Ann. Rev. Biochem.* 36:171–81
12. Boulton, A. 1971. *Nature* 231:22–28
13. Chase, T. N., Murphy, D. L. 1973. *Ann. Rev. Pharmacol.* 13:181–97
14. Schildkraut, J. J. 1973. *Ann. Rev. Pharmacol.* 13:427–54
15. Dahlstrom, A. 1971. *Phil. Trans. Roy. Soc. London* 261B:325–58
16. Aghajanian, G. K. 1972. *Ann. Rev. Pharmacol.* 12:157–68
17. Creveling, C. R., Daly, J. W. 1971. *Med. Res. Ser. Monogr.* 5:355–411
18. Iversen, L. L. 1971. *Brit. J. Pharmacol.* 41:571–91
19. Nagatsu, T., Mizutani, K., Nagatsu, I., Matsura, S., Sugimoto, T. 1972. *Biochem. Pharmacol.* 21:1945–53
20. Lloyd, T., Weiner, N. 1971. *Mol. Pharmacol.* 7:569–80
21. Lovenberg, W., Jéquier, E., Sjoerdsma, A. 1967. *Science* 155:217–19
22. Friedman, P. A., Kappelman, A. H., Kaufman, S. 1972. *J. Biol. Chem.* 247: 4165–73
23. Cleland, W. W. 1967. *Ann. Rev. Biochem.* 36:77–112
24. Craine, J. E., Hall, E. S., Kaufman, S. 1972. *J. Biol. Chem.* 247:6082–91
25. Musacchio, J. M. 1969. *Biochem. Biophys. Acta* 191:485–87
26. Musacchio, J. M., D'Angelo, G. L., McQueen, C. A. 1971. *Proc. Nat. Acad. Sci. USA* 68:2087–91
27. Guroff, G., Rhoads, C. A., Abramowitz, A. 1967. *Anal. Biochem.* 21: 273–78
28. Kuczenski, R. T., Mandell, A. J. 1972. *J. Biol. Chem.* 247:3114–22
29. Kuczenski, R. T. 1973. *J. Biol. Chem.* 248:2261–65
30. Kuczenski, R. T., Mandell, A. J. 1972. *J. Neurochem.* 19:131–37
31. Foldes, A., Jeffrey, P. L., Preston, B. N., Austin, L. 1973. *J. Neurochem.* 20: 1431–32
32. Kirk, J. E. 1962. *Vitam. Horm. New York* 20:67–139
33. Shimizu, N., Matsunami, T., Onishi, S. 1960. *Nature* 186:479–80
34. Hammarström, L. 1966. *Acta Physiol. Scand.* 70:Suppl. 289,1–84
35. Cater, D. B., Garattini, S., Marina, F., Silver, I. A. 1961. *Proc. Roy. Soc. London* B155:136–57
36. Jamieson, D., Van Den Brenk, H. A. S. 1963. *J. Appl. Physiol.* 18:869–76
37. Diaz, P. M., Ngai, S. H., Costa, E. 1968. *Advan. Pharmacol.* 6B:75–92
38. Neff, N. H., Costa, E. 1967. *Fed. Proc.* 26:463
39. Davis, J. N., Carlsson, A. 1973. *J. Neurochem.* 20:913–15

40. Fisher, D. B., Kaufman, S. 1972. *J. Neurochem.* 19:1359–65
41. Goldstein, M., Joh, T. H., Garvey, T. 1968. *Biochemistry* 7:2724–30
42. Weiner, N., Cloutier, G., Bjur, R., Pfeffer, R. I. 1972. *Pharmacol. Rev.* 24:203–21
43. Harris, J. E., Roth, R. H. 1971. *Mol. Pharmacol.* 7:593–604
44. Nagatsu, T., Sudo, Y., Nagatsu, I. 1971. *J. Neurochem.* 18:2179–89
45. Coyle, J. T. 1972. *Biochem. Pharmacol.* 21:1935–44
46. Nagatsu, T., Levitt, M., Udenfriend, S. 1964. *J. Biol. Chem.* 239:2910–17
47. Petrack, B., Sheppy, F., Fetzer, V. 1968. *J. Biol. Chem.* 243:743–48
48. Laduron, P., Belpaire, F. 1968. *Biochem. Pharmacol.* 17:1127–40
49. Wurzburger, R. J., Musacchio, J. M. 1971. *J. Pharmacol. Exp. Ther.* 177: 155–68
50. Shiman, R., Akino, M., Kaufman, S. 1971. *J. Biol. Chem.* 246:1330–40
51. Mandell, A. J., Knapp, S., Kuczenski, R. T., Segal, D. S. 1972. *Biochem. Pharmacol.* 21:2737–50
52. Knapp, S., Mandell, A. J., 1972. *Life Sci.* 11:761–71
53. Knapp, S., Mandell, A. J. 1972. *Science* 177:1209–11
54. Knapp, S., Mandell, A. J. 1973. *Science* 180:645–47
55. Knapp, S., Mandell, A. J. *Serotonin and Behavior*, ed. J. Barchas. New York: Academic. In press
56. Grahame-Smith, D. G. 1967. *Biochem. J.* 105:351–60
57. Friedman, P. A., Lloyd, T., Kaufman, S. 1972. *Mol. Pharmacol.* 8:501–10
58. Fisher, D. B., Kaufman, S., 1973. *J. Biol. Chem.* 248:4345–53
59. Kuczenski, R. 1973. *J. Biol. Chem.* 248:5074–80
60. Musacchio, J. M., Wurzburger, R. J., D'Angelo, G. L. 1971. *Mol. Pharmacol.* 7:136–46
61. Carlsson, A., Lindqvist, M., Magnusson, T. 1960. *Adrenergic Mechanisms.* ed. J. R. Vane, G. E. W. Wolstenhome, M. O'Connor, 432–439. London: Churchill
62. Spector, S., Gordon, R., Sjoerdsma, A., Udenfriend, S. 1967. *Mol. Pharmacol.* 3:549–55
63. Neff, N. H., Costa, E. 1966. *Life Sci.* 5:951–59
64. Neff, N. H., Costa, E. 1968. *J. Pharmacol. Exp. Ther.* 160:40–47
65. Ngai, S. H., Neff, N. H., Costa, E. 1968. *Life Sci.* 7: Part II, 847–55
66. Andén, N. E., Fuxe, K., Hamberger, B., Hökfelt, T. 1966. *Acta. Physiol. Scand.* 67:306–12
67. Costa, E., et al 1972. *Pharmacol. Rev.* 24:167–90
68. Musacchio, J. M., McQueen, C. A., Craviso, G. L. 1973. *New Concepts in Neurotransmitter Regulation*, ed. A. J. Mandell, 69–88. New York: Plenum
69. Neff, N. H., Yang, H-T., Goridis, C. See Ref. 5
70. Lin, R. C., Neff, N. H., Ngai, S. H., Costa, E. 1969. *Life Sci.* 8:Pt.1, 1077–84
71. Macon, J. B., Sokoloff, L., Glowinski, J. 1971. *J. Neurochem.* 18:323–31
72. Millard, S. A., Costa, E., Gál, E. M. 1972. *Brain Res.* 40:545–51
73. Mueller, R. A., Thoenen, H., Axelrod, J. 1969. *J. Pharmacol. Exp. Ther.* 169: 74–79
74. Molinoff, P. B., Brimijoin, S., Axelrod, J. 1972. *J. Pharmacol. Exp. Ther.* 182: 116–29
75. Patrick. R. L., Kirshner, N. 1971. *Mol. Pharmacol.* 7:87–96
76. Axelrod, J. 1972. *Science* 173:598–606
77. Guidotti, A., Costa, E. 1973. *Science* 179:902–4
78. Guidotti, A., Zivkovic, B., Pfeiffer, R., Costa, E. 1973. *Naunyn Schmiedebergs Arch. Pharmakol. Exp. Pathol.* 278: 195–206
79. Costa, E., Guidotti, A., See Ref. 68, pp. 135–52
80. Guidotti, A., Mao, C. C., Costa, E. 1974. *Advan. Cytopharmacol.* 2: In press
81. Guidotti, A., Mao, C. C., Costa, E. See Ref. 5
82. Hanbauer, I., Kopin, I. J., Costa, E. *Naunyn Schmiedebergs Arch. Pharmakol. Exp. Pathol.* In press
83. Douglas, W. W., Rubin, R. P. 1961. *Nature* 192:1087–89
84. Paul, M. I., Kvetnansky, R., Cramer, H., Silbergeld, S., Kopin, I. J. 1971. *Endocrinology* 88:338–44
85. Serck-Hanssen, G., Christoffersen, T., Mørland, J., Osnes, J. B. 1972. *Eur. J. Pharmacol.* 19:297–300
86. Douglas, W. W. 1966. *Pharmacol. Rev.* 18:471–80
87. Kvetnansky, R., Gewirtz, G. P., Weise, V. K., Kopin, I. J. 1971. *Endocrinology* 89:50–55
88. George, W. J., Polson, J. B., O'Toole, A. G., Goldberg, N. D. 1970. *Proc. Nat. Acad. Sci. USA* 66:398–403
89. Ferrendelli, J. A., Steiner, A. L., McDougal, D. B., Kipnis, D. M. 1970.

Biochem. Biophys. Res. Commun. 41: 1061–67
90. Lee, T. P., Kuo, J. F., Greengard, P. 1972. *Proc. Nat. Acad. Sci. USA* 69: 3287–91
91. Schultz, G., Hardman, J. G., Davis, J. W., Schultz, K., Sutherland, E. W. 1972. *Fed. Proc.* 31:440
92. Thoenen, H., Otten, U., Oesch, F. See Ref. 5
93. Hartman, B. K., Udenfriend, S. 1972. *Pharmacol. Rev.* 24:311–30
94. Björklund, A., Katzman, R., Stenevi, U., West, K. 1971. *Brain Res.* 31:21–33
95. Thoenen, H. 1970. *Nature* 228:861–62
96. Hanbauer, I. See Ref. 5
97. Besson, M. J., Cheramy, A., Gauchy, C., Musacchio, J. 1973. *Eur. J. Pharmacol.* 22:181–86
98. Zivkovic, B., Guidotti, A., Costa, E. 1973. *Brain Res.* 57:522–26
99. Zivkovic, B., Guidotti, A., Costa, E. 1974. *Advan. Biochem. Psychopharmacol.* 10: In press
100. Segal, D. S., Sullivan, J. L., Kuczenski, R. T., Mandell, A. J. 1971. *Science* 173:847–49
101. Musacchio, J. M., Julou, L., Kety, S. S., Glowinski, J. 1969. *Proc. Nat. Acad. Sci. USA* 63:1117–19
102. Azmitia, E. C. 1970. *Life Sci.* 9: Pt. 1, 633–37
103. Fukui, K., Shiomi, H., Takagi, H. 1972. *Eur. J. Pharmacol.* 19:123–25
104. Schecter, P. J., Lovenberg, W., Sjoerdsma, A. 1972. *Biochem. Pharmacol.* 21:751–53
105. Meek, J. L., Neff, N. H. 1972. *J. Neurochem.* 19:1519–25
106. Azmitia, E. C., McEwen, B. S. 1969. *Science* 166:1274–76
107. Gál, E. M., Heater, R. D., Millard, S. A. 1968. *Proc. Soc. Exp. Biol. Med.* 128: 412–15
108. Andén, N. E., Corrodi, H., Fuxe, K., Ungerstedt, U. 1971. *Eur. J. Pharmacol.* 15:193–99
109. Neff, N. H., Costa, E. 1967. *Proc. Int. Symp. Antidepressant Drugs,* ed. S. Garattini, M. N. C. Dukes, 28–33. New York: Excerpta Med. Found. Ser. 122
110. Nybäck, H., Sedvall, G. 1971. *J. Pharm. Pharmacol.* 23:322–26
111. Carlsson, A., Lindqvist, M. 1963. *Acta. Pharmacol. Toxicol.* 20:140–44
112. Andén, N. E., Carlsson, A., Häggendal, A. J. 1969. *Ann. Rev. Pharmacol.* 9: 119–34
113. Aghajanian, G. K., Bunney, B. S., Kuhar, M. J. 1973. See Ref. 68, pp. 115–19
114. Aghajanian, G. K., Bunney, B. S. See Ref. 5
115. Aghajanian, G. K. 1972. *Fed. Proc.* 31:91–96
116. Andén, N. E., Corrodi, H., Fuxe, K., Hökfelt, T. 1968. *Brit. J. Pharmacol.* 34:1–7
117. Carlsson, A., Kehr, W., Lindqvist, M., Magnusson, T., Atack, C. V. 1972. *Pharmacol. Rev.* 24:371–84
118. Andén, N. E., Magnusson, T., Stock. G. 1973. *Naunyn. Schmiedebergs Arch. Pharmacol. Exp. Pathol.* 278:363–72
119. Andén, N. E. See Ref. 5
120. Eccles, J. C., Schmidt, R., Willis, W. D. 1963. *J. Physiol.* 168:500–30
121. Schmidt, R. F. 1971. *Ergeb. Physiol.* 63:21–101
122. Starke, K., Altmann, K. P. 1973. *Neuropharmacol.* 12:339–47
123. Enero, M. A., Langer, S. Z., Rothlin, R. P., Stefano, F. J. E. 1972. *Brit. J. Pharmacol.* 44:672–88
124. Loffelholz, K., Muscholl, E. 1969. *Naunyn Schmiedebergs Arch. Pharmacol. Exp. Pathol.* 265:1–15
125. Goldstein, M., Anagnoste, B., Shirron, C. 1973. *J. Pharm. Pharmacol.* 25: 348–51
126. Wooten, G. F., Thoa, N. B., Kopin, I. J., Axelrod, J. 1973. *Mol. Pharmacol.* 9:178–83
127. Moir, A. T. B., Eccleston, D. 1968. *J. Neurochem.* 15:1093–1108
128. Weber, L. J., Horita, A. 1965. *Biochem. Pharmacol.* 14:1141–49
129. Ashcroft, G. W., Eccleston, D., Crawford, T. B. B. 1965. *J. Neurochem.* 12: 483–92
130. Green, H., Greenberg, S. M., Erickson, R. W., Sawyer, J. L., Ellison, T. 1962. *J. Pharmacol. Exp. Ther.* 136:174–78
131. Hess, S. M., Doepfner, W. 1961. *Arch. Int. Pharmacodyn.* 134:89–99
132. Dairman, W. 1973. See Ref. 68, pp. 1–20
133. Jéquier, E., Lovenberg, W., Sjoerdsma, A. 1967. *Mol. Pharmacol.* 3:274–78
134. Fernstrom, J. D., Wurtman, R. J. 1971. *Science* 173:149–52
135. Grahame-Smith, D. G. 1971. *J. Neurochem.* 18:1053–66
136. Tagliamonte, A., Tagliamonte, P., Perez-Cruet, J., Gessa, G. L. 1971. *Nature New Biol.* 229:125–26
137. Tagliamonte, A., Tagliamonte, P., Perez-Cruet, J., Stern, S., Gessa, G. L. 1971. *J. Pharmacol. Exp. Ther.* 177: 475–80

138. Perez-Cruet, J., Tagliamonte, A., Tagliamonte, P., Gessa, G. L. 1971. *J. Pharmacol. Exp. Ther.* 178:325–30
139. Tagliamonte, A. et al 1971. *J. Neurochem.* 18:1191–96
140. Tagliamonte, A., Tagliamonte, P., DiChiara, G., Gessa, R., Gessa, G. L. 1972. *J. Neurochem.* 19:1509–12
141. Fernstrom, J. D., Larin, F., Wurtman, R. J. 1971. *Life Sci.* 10: Part 1, 813–19
142. Oldendorf, W. H. 1971. *Am. J. Physiol.* 221:1629–39
143. Yuwiler, A., Loutit, R. T. 1961. *Science* 134:831–32
144. McKean, C. M., Boggs, D. E., Peterson, N. A. 1968. *J. Neurochem.* 15:235–41
145. McKean, C. M. 1972. *Brain Res.* 47: 469–76
146. Oldendorf, W. H. 1973. *Arch. Neurol.* 28:45–48
147. Fernstrom, J. D., Wurtman, R. J. 1971. *Nature New Biol.* 234:62–64
148. Fernstrom, J. D., Wurtman, R. J. 1971. *Science* 174: 1023–25
149. Fernstrom, J. D., Wurtman, R. J. 1972. *Metabolism* 21:337–42
150. Madras, B. K., Cohen, E. L., Munro, H. N., Wurtman, R. J. 1974. *Advan. Biochem. Psychopharmacol.* 10: In press
151. Knott, P. J., Curzon, G. 1972. *Nature* 239:452–53
152. Lipsett, D., Madras, B. K., Wurtman, R. J., Munro, H. N. 1973. *Life Sci.* 12:Pt. 2, 57–64
153. McArthur, J. N., Dawkins, P. D. 1969. *J. Pharm. Pharmacol.* 21:744–50
154. Curzon, G., Friedel, J., Knott, P. J. 1973. *Nature* 242:198–200
155. Korf, J., Van Praag, H. M., Sebens, J. B. 1972. *Brain Res.* 42:239–42
156. Bruinvels, J. 1972. *Eur. J. Pharmacol.* 20:231–37
157. Leonard, B. E., Shallice, S. A. 1972. *Neuropharmacology* 11:373–84
158. Moir, A. T. B. 1971. *Brit. J. Pharmacol.* 43:715–23
159. Welch, B. L., Welch, A. S. 1970. *Amphetamines and Related Compounds,* ed. E. Costa, S. Garattini, 415–445. New York: Raven
160. Corrodi, H., Fuxe, K., Lidbrink, P., Olson, L. 1971. *Brain Res.* 29:1–16
161. Doteuchi, M., Costa, E. 1973. *Neuropharmacology,* 1059–73