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PHARMACOLOGY OF BRAIN EPINEPHRINE NEURONS

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INTRODUCTION

The metabolic pathway by which catecholamines are synthesized is now well known (see Figure 1). The amino acid L-tyrosine, derived from hydroxylation of L-phenylalanine or the hydrolysis of dietary or tissue proteins, is hydroxylated in the *meta* postion of the phenyl ring to form L-dihydroxyphenylalanine or L-Dopa. L-Dopa is decarboxylated to form dopamine, which is then β-hydroxylated to norepinephrine. The final step in epinephrine biosynthesis is the transfer of a methyl group from *S*-adenosyl-L-methionine to the amino group of norepinephrine. The enzyme catalyzing this methylation step is the only one unique to epinephrine synthesis, the earlier enzymes being involved also in dopamine or norepinephrine synthesis, or both. The recommended name for this methylating enzyme (EC 2.1.1.28) is norepinephrine *N*-methyltransferase (1), but it has commonly been called phenylethanolamine *N*-methyltransferase and abbreviated PNMT. That abbreviation will be used in this review because it is known by those working in the field.

The major site of epinephrine formation in the body is the adrenal medulla; epinephrine is formed there by neuroendocrine transducer cells which release it into the blood as a circulating hormone. The focus of this paper is on a quantitatively minor site of epinephrine biosynthesis, namely discrete neurons in the brain which apparently make and use epinephrine as a neurotransmitter.

Although the presence of epinephrine in mammalian brain has long been known (2–4) and the ability of brain tissue to form epinephrine in vitro was also demonstrated (5–7), only within the past decade has there been much

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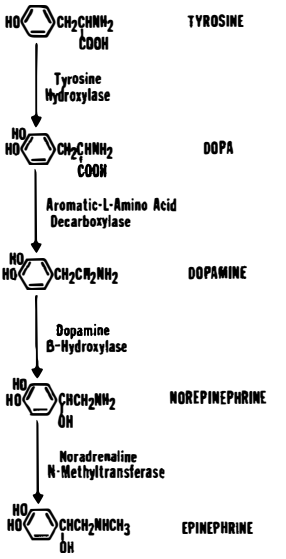

Figure 1 Pathway of epinephrine biosynthesis.

study of this brain catecholamine. Dopamine and norepinephrine neurons in brain, in contrast, had previously been mapped in detail and studied physiologically and pharmacologically with widely varied techniques and in regard to numerous physiologic functions. Epinephrine in brain had been relatively ignored primarily because its concentration was so low in comparison to concentrations of the other two catecholamines. Histofluorescence methods could not discriminate epinephrine and norepinephrine well enough to permit tracking of the relatively sparse epinephrine-containing neurons. Quantitative fluorometric methods distinguished epinephrine from norepinephrine, but measurement of low concentrations of epinephrine in the presence of much higher concentrations of norepinephrine was difficult and unreliable. Thus there had been few studies of drug effects on epinephrine concentration or turnover in brain.

The recent focus of attention on epinephrine-forming neurons in brain began when Hokfelt et al (8, 9) used antibodies to adrenal PNMT to map epinephrine-forming neurons in rat brain. PNMT in brain is similar to that in adrenal gland in substrate specificity, susceptibility to inhibitors, and also immunologically. Antibodies prepared to PNMT purified from bovine adrenal medulla cross-reacted with PNMT from rat brain, permitting the antibodies to be used in immunohistofluorescence studies to locate PNMT-containing cell bodies and track PNMT-containing nerve fibers.

The recognition that separate and distinct epinephrine-forming neurons occur in brain created interest in these neurons and in their possible physi-

ologic functions. During the past several years, a body of literature on the pharmacologic modification of these epinephrine neurons has arisen, and this paper surveys that literature. Although the detailed mapping and most of the experimental study of brain epinephrine neurons has been in rats, PNMT and epinephrine are also present in the brains of primates, including man, and the distribution of these neurons is at least as widespread in these species as in the rat (4, 10–13). In contrast, PNMT activity and epinephrine concentration in brain are very low in guinea pigs and mice compared to rats (R. W. Fuller and S. K. Hemrick-Luecke, unpublished data).

Although the low concentration of epinephrine in brain has until recently posed technological problems in studying it, the fact that epinephrine-forming neurons are less abundant than neurons using other catecholamines as neurotransmitters should not cause one to infer that epinephrine neurons are of little importance. Arvid Carlsson, a leader in the field of central catecholamines, has made this point:

> In the brain adrenaline occurs in small amounts, only a few percent of the total catecholamines; therefore we tend to disregard adrenaline, which is very bad because even if the adrenaline neurons form a really quite small minority among the catecholamine neurons in the brain, we have to take into account the fact that the brain is not a democracy, and that is perhaps the reason why it works so well most of the time (14).

METHODS FOR MEASURING EPINEPHRINE CONCENTRATION IN BRAIN

Fluorometric methods for measuring catecholamines have been widely used and have played a major role in the study of drug effects on brain neurons containing dopamine and norepinephrine during the past twenty years. These fluorometric methods can distinguish epinephrine from norepinephrine when both are present in large amounts and work well for measuring epinephrine and norepinephrine separately in the adrenal gland. The fluorometric measurement of epinephrine in brain, however, has been much more difficult. Although a few workers have used fluorometric methods to quantitate epinephrine concentration in brain (15), the low ratio of epinephrine to norepinephrine in brains of commonly studied species such as rats makes it very difficult to measure epinephrine concentrations reliably with most current fluorometric procedures.

Brain epinephrine has been measured by gas chromatography with electron capture detection (16). The combination of gas chromatography with mass fragmentography (17) provides the necessary sensitivity and specificity, but the measurement of brain epinephrine by this procedure has found very limited use. The introduction of radioenzymatic methods for analysis of catecholamines offered a highly sensitive method that has been applied

to epinephrine in brain (18–20). Several groups of workers have reported the effects of drugs on epinephrine concentration measured radioenzymatically (21–23), and this method apparently yields satisfactory results with regard to sensitivity and specificity. Nonetheless, these methods are tedious and not devoid of problems when the catecholamine of interest, epinephrine, is present with large excesses of dopamine and norepinephrine, as is true for all brain regions in most common laboratory animals.

The most recently introduced method for measuring epinephrine in brain couples high performance liquid chromatography with electrochemical detection (LCEC). Electrochemical detection in the analysis of catecholamines has been pioneered by Dr. Ralph Adams and his students. Several specific LCEC methods for measuring epinephrine in brain have been reported, differing in details of column packing, eluants, and electrochemical detector (24–27). LCEC methods for measuring brain epinephrine have proven satisfactory in many laboratories, and most data on epinephrine alterations by drugs have been generated with this analytical method. In my opinion, LCEC is the preferred method for measuring epinephrine in brain.

DRUG EFFECTS ON EPINEPHRINE CONCENTRATION AND TURNOVER

Drugs That Lower Epinephrine Concentration

SYNTHESIS INHIBITORS Inhibition of tyrosine hydroxylation, dopa decarboxylation, or dopamine β-hydroxylation could lower epinephrine concentration, but only PNMT inhibition would decrease epinephrine formation without also decreasing dopamine or norepinephrine formation. Thus PNMT inhibition has been the focus of attention in drug-induced inhibition of epinephrine synthesis.

PNMT was first isolated and characterized from adrenal medulla (28), where it is much more abundant than in brain. The enzyme was shown to accept various phenylethanolamines in addition to norepinephrine as substrates, and recently non-phenyl ethanolamines have also been shown to be substrates for the enzyme (29, 30). The β-hydroxyl group of phenylethanolamines can be replaced with a β-amino group with retention of some substrate activity (31), but removal of the β-hydroxyl group essentially abolishes substrate activity. Phenylethylamines can combine with PNMT but are not methylated; they are thus competitive inhibitors rather than substrates (32, 33). Certain substituted phenylethylamines are reasonably potent inhibitors of PNMT (34, 35).

Much of the earlier work in the discovery and development of PNMT inhibitors was done with the adrenal medulla enzyme (32–38). When atten-

tion was turned to PNMT-containing neurons in brain, the brain enzyme was found to be similar to the adrenal enzyme in susceptibility to inhibitors (39–44). By now several potent inhibitors of brain PNMT that inhibit the enzyme both in vitro and in vivo have been described (see Figure 2).

One widely studied inhibitor is DCMB or 2,3-dichloro-α-methyl-benzylamine (24, 39, 41, 42, 45–50). This compound was one of the most potent from a series of benzylamines that were compared in vitro (51). The α-methyl substituent on DCMB provides biological stability, the parent benzylamines being rapidly deaminated by amine oxidases when they are administered to animals (52). DCMB produces a rapid decline in epinephrine concentration in rat brain and has been used in studying the turnover of brain epinephrine, as discussed in a later section of this paper.

Three other compounds (*b–d*) in Figure 2 can be viewed as conformationally rigid analogs of 2,3-dichlorobenzylamine, of which DCMB is the α-methyl analog. These other three compounds are also potent inhibitors of brain PNMT both in vitro and in vivo (37, 39, 43, 44, 53–59). All decrease epinephrine concentration in rat brain. One of these compounds, SK&F 64139, currently is being studied clinically (60).

LY87130, compound (*e*) in Figure 2, has also been found to produce nearly complete depletion of hypothalamic epinephrine in rats (61). This compound is structurally related to SK&F 7698, a compound earlier described as a potent inhibitor of adrenal PNMT (36). No data have yet been published on SK&F 7698 effects on brain epinephrine concentration.

Compound (*f*) in Figure 2, 2-cyclooctyl-2-hydroxyethylamine, is one of a series of non-phenyl ethanolamine substrates for the enzyme (29); it has recently been reported to lower epinephrine concentration in brain (62), presumably inhibiting PNMT action on norepinephrine as a competitive substrate.

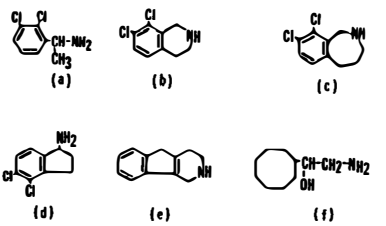

Figure 2 Structures of some PNMT inhibitors that lower brain epinephrine concentration: (*a*) 2,3-dichloro-α-methylbenzylamine (DCMB or LY78335); (*b*) 7,8-dichloro-1,2,3,4-tetrahydroisoquinoline (SK&F 64139); (*c*) 8,9-dichloro-2,3,4,5-tetrahydro-1H-2-benzazepine (LY134046); (*d*) 4,5-dichloro-1-aminoindan; (*e*) 2,3,4,5-tetrahydro-1H-indeno[1,2c]pyridine (LY87130); (*f*) 2-cyclooctyl-2-hydroxyethylamine.

One finding that has become apparent in the study of PNMT inhibitors that deplete brain epinephrine is that some of the inhibitors have high affinity for α_2 adrenergic receptors (63–65). A possible interpretation is that the α_2 receptor and the active site of PNMT have some structural similarities, not surprising since both are macromolecules for which norepinephrine has high affinity. However, recent studies comparing LY134046 and SK&F 64139 (compounds *b* and *c* in Figure 2) have indicated that the relative affinity of these two compounds for α_2 receptors and for PNMT differs (56). Both compounds appear to inhibit PNMT because they are conformationally rigid analogs of 2,3-dichlorobenzylamine. SK&F 64139 appears to have high affinity for the β_2 receptor because it is also a conformationally rigid analog of 3,4-dichlorophenylethylamine. LY134046 has lower affinity for the α_2 receptor because it is not a phenylethylamine. If PNMT inhibitors are to be useful tools for elucidating functional roles of epinephrine-forming neurons in brain, they need to be as specific as possible in their effects. Future development of PNMT inhibitors may have the separation of α_2 receptor activity as a goal. Recently we have found a lack of correlation between potency of inhibition of brain PNMT and α_2 receptor affinity (competition with tritiated clonidine for binding to brain membranes) among several PNMT inhibitors representing different chemical classes (R. E. Toomey, J. S. Horng, and R. W. Fuller, data to be published), suggesting that these two activities can be dissociated.

RELEASERS Epinephrine concentration in rat brain is also depleted by agents previously known to deplete other catecholamines, including Ro 4-1284 (2-ethyl-2,3,4,6,7,11b-hexahydro-3-isobutyl-9,10-dimethoxy-2H-benzo[a]quinolizin-2-ol hydrochloride) (66), reserpine (23, 67, 68), α-methyldopa (69, 70) and α-methyl-*m*-tyrosine (42, 67). The depletion of catecholamines by the latter two compounds is thought to be due to the formation of decarboxylated metabolites that are transported into the nerve terminals causing release of endogenous stores of catecholamines. The ability of these compounds to deplete epinephrine as well as dopamine and norepinephrine implies that epinephrine nerve terminals have an uptake carrier on the neuronal membranes as dopamine and norepinephrine nerve terminals were known to have.

6-HYDROXYDOPAMINE 6-Hydroxydopamine has been widely used as a chemical neurotoxin to destroy dopamine and norepinephrine nerve fibers. Epinephrine-forming neurons were at first thought to be resistant to 6-hydroxydopamine, because PNMT activity was not decreased after the intracisternal or intraventricular injection of 6-hydroxydopamine into adult

rats or the systemic injection of 6-hydroxydopamine into newborn rats (71–73). Subsequently epinephrine concentration was found to be decreased by 6-hydroxydopamine (26, 42, 67, 68). Fuxe et al (71) reported that PNMT activity in hypothalamus was reduced after intrategmental injection of large amounts of 6-hydroxydopamine and suggested the route of administration accounted for the failure of PNMT to be lowered after intracisternal or intraventricular injection of 6-hydroxydopamine. However, a decrease in epinephrine concentration but not PNMT activity measured in hypothalamus of the same rats has been found after intraventricular injection of 6-hydroxydopamine (26, 42). Since the previous studies with 6-hydroxydopamine had used phenylethanolamine to assay PNMT activity, we thought nonspecific enzyme activity might have been measured. However, several experiments in our laboratory and in collaboration with Dr. Arthur D. Loewy at Washington University have confirmed that 6-hydroxydopamine treatment lowers epinephrine concentration but not PNMT activity in rat hypothalamus and brain stem, PNMT activity in our studies being measured with L-norepinephrine as the methyl-accepting substrate so that epinephrine is the product (24). Why epinephrine but not PNMT activity is lowered by 6-hydroxydopamine is not yet understood. The possibility that these are present in different neurons directly contradicts the assumption that both are present in the same neuronal structures, an assumption that formed the basis for the histologic studies mapping epinephrine-forming neurons with PNMT antibodies coupled to a fluorescent tag [see (74)]. Possibly the best explanation for the apparent discrepancy between epinephrine depletion and lack of PNMT reduction after 6-hydroxydopamine is suggested by the findings of Burgess et al (75). They reported that PNMT activity in rat hypothalamus was lowered one day after 6-hydroxydopamine injection. By 10 days, a compensatory increase in enzyme activity had occurred, whereas epinephrine concentration remained depleted. This situation resembles that reported for norepinephrine neurons in hippocampus, in that tyrosine hydroxylase activity and norepinephrine concentration were reduced to similar extents soon after 6-hydroxydopamine injection, yet enzyme activity recovered much more quickly (76).

OTHER AGENTS Epinephrine concentration in rat brain is also decreased by various α-blocking drugs, including piperoxan (58, 59) and other benzodioxanes (67), prazosin (59, 67), and yohimbine (67).

Bromocriptine has also been reported to decrease epinephrine concentration in the hypothalamus and medulla oblongata in rats (58). We have observed a dose-dependent lowering of epinephrine in rat hypothalamus by a more potent and selective dopamine agonist, pergolide, and the lowering

by pergolide was prevented by small doses of spiperone (R. W. Fuller and K. W. Perry, unpublished data). These findings suggest a dopaminergic influence on hypothalamic epinephrine neurons.

Drugs That Increase Epinephrine Concentration

The only drugs so far known to increase epinephrine concentration in brain are inhibitors of monoamine oxidase (MAO) (EC 1.4.3.4). Several workers observed an increase in hypothalamic epinephrine concentration (to approximately double within a few hours) after treating rats with pargyline, an MAO inhibitor (23, 42, 67, 68). MAO is known to exist in at least two forms, type A and type B, which differ in their substrate specificity and susceptibility to inhibitors [see (77)]. Pargyline is a relatively nonselective inhibitor of MAO; it is somewhat more effective in blocking type B MAO than type A MAO, but at high doses it substantially inhibits both (77, 78). We therefore did experiments to ascertain if type A or type B MAO was involved in the metabolism of epinephrine in rat hypothalamus.

Harmaline, a short-acting reversible inhibitor that is highly selective for type A MAO in vitro, caused an increase in hypothalamic epinephrine concentration and marked inhibition of type A but not type B MAO in vivo in rats. The inhibition of type A MAO and the increase in hypothalamic epinephrine concentration were essentially over by 24 hours (79).

Several irreversible inhibitors of MAO in addition to pargyline also increased hypothalamic epinephrine concentration in rats, and this increase persisted for much longer times than with harmaline (several days). These inhibitors include LY51641, LY54832, LY54761, and deprenyl (80). Two of these, LY51641 and LY54832, are highly selective inhibitors of type A MAO. They increased epinephrine concentration to a degree that was directly proportional to the percentage inhibition of type A MAO and caused no inhibition of type B MAO at the doses used. LY54761 inhibited type B as well as type A MAO, but the percentage increase in epinephrine concentration was not greater at a given percentage inhibition of type A MAO than with LY51641 or LY54832, suggesting that inhibition of type B MAO did not contribute to the elevation of epinephrine. Deprenyl was the most selective inhibitor of type B MAO studied. At low doses which inhibited only type B MAO, epinephrine concentration was not increased. Epinephrine was elevated only at higher doses of deprenyl that began to inhibit type A MAO as well.

Further evidence that inhibition of type A MAO was the causative factor in the elevation of hypothalamic epinephrine concentration by pargyline and LY51641 was obtained by co-administration of harmaline. Pargyline and LY51641 are "suicide substrates" for MAO, being acted upon by the

enzyme to form reactive intermediates that then react with the enzyme to inactivate it. Harmaline, by inhibiting type A MAO selectively, antagonizes the inactivation of type A MAO by these irreversible inhibitors but does not interfere with inactivation of type B MAO. Thus 24–48 hrs after the injection of pargyline, both type A and type B MAO activities are ordinarily reduced, but when harmaline was co-administered with pargyline only type B MAO activity was reduced (78, 81). The elevation of epinephrine concentration in rat hypothalamus that ordinarily accompanies pargyline administration was completely prevented at this time, providing strong evidence that the increase in epinephrine was a consequence of type A MAO inhibition by pargyline (81). The inhibition of type A MAO by LY51641 and the elevation of hypothalamic epinephrine concentration were also antagonized by harmaline (80).

In addition, the rapid decrease in epinephrine concentration that accompanies the administration of an amine-releasing agent, Ro 4-1284, was antagonized by pargyline pretreatment (66). This antagonism by pargyline was prevented when harmaline was co-administered, suggesting that inhibition of type A MAO by pargyline was responsible for protection of epinephrine released from intraneuronal storage granules from MAO (66). Thus epinephrine neurons in rat hypothalamus may contain mainly type A MAO.

All of these data are consistent with the idea that in the rat hypothalamus, oxidative deamination is an important metabolic pathway by which epinephrine is degraded, and type A MAO is involved predominantly or entirely in this degradation.

Drugs That Alter Epinephrine Turnover

The turnover of brain monoamines has been measured by various techniques. One is the measurement of steady state concentrations of metabolites of the amine, such as 3,4-dihydroxyphenylacetic acid and homovanillic acid in the case of dopamine or of 3-methoxy-4-hydroxyphenylethyleneglycolsulfate in the case of norepinephrine. Deaminated metabolites of epinephrine would be common to norepinephrine as well, as these could not be used to evaluate epinephrine turnover. Metanephrine is a metabolite formed from epinephrine but not directly from norepinephrine; metanephrine levels in brain might reflect epinephrine turnover, but this possibility has not yet been evaluated. Another technique of measuring brain monoamine turnover is the rate of disappearance of radioactivity after injection of radiolabeled amine or after injection of a radiolabeled precursor. In the case of epinephrine, the former method has limitations because of uncertainty about specific labeling (radioactive epinephrine may be taken up by the heavily predominant norepinephrine neurons) and the latter method has

limitations because such a small percentage of a labeled precursor would be incorporated into epinephrine.

A technique that has been used to evaluate epinephrine turnover in brain is the rate of decline in epinephrine concentration after inhibition of epinephrine synthesis. Inhibitors of dopamine β-hydroxylase and of PNMT have been used for this purpose.

Drugs reported to decrease epinephrine turnover in hypothalamus or other brain regions include clonidine (67, 68, 82, 83), guanabenz (42, 84), and desipramine (68). These drugs attenuate the decline in epinephrine concentration following inhibition of dopamine β-hydroxylase or PNMT. Clonidine and guanabenz probably do so by direct stimulation of α_2 receptors modulating epinephrine synthesis and release. Desipramine may slow turnover by blocking epinephrine reuptake, enhancing the neurotransmitter's action on pre- or post-synaptic receptors that modulate synthesis and release.

Drugs reported to increase epinephrine turnover (to enhance the decline in epinephrine concentration following inhibition of dopamine β-hydroxylase or PNMT) in hypothalamus or other brain regions include piperoxan (59, 67, 68, 85), yohimbine (68), prazosin (68), phenoxybenzamine (68), mianserin (68), and high doses of chlorpromazine and haloperidol (68). These effects may be secondary to blockade of α-receptors that modulate epinephrine formulation and release. In addition, oxotremorine has been reported to increase epinephrine turnover in rat hypothalamus (68); cholinergic modulation of epinephrine turnover is suggested, a possibility that needs further study.

Fuxe et al (86) have compared drug effects on epinephrine turnover in two brain regions, the hypothalamus and the dorsal midline area of the caudal medulla oblongata (DCMO). They reported that clonidine at low doses preferentially reduced epinephrine turnover in the DCMO without altering epinephrine turnover in hypothalamus or norepinephrine turnover in the DCMO. They also reported that prazosin, an α_1 receptor antagonist, increased epinephrine turnover in hypothalamus but not in DCMO. These findings suggest heterogeneity among α-receptors modulating turnover of epinephrine and norepinephrine in brain regions.

EPINEPHRINE RECEPTORS IN BRAIN

Characterization of adrenergic receptors in various tissues led early to the definition of α- and β-receptors and subsequently to subclassification of those receptor types [see (87)]. Although the brain is known to contain both α- and β-receptors, there is presently no understanding of the nature of receptors specifically innervated by epinephrine-containing nerve terminals. Tritiated epinephrine has been used as a radioligand and shown to bind to

brain membranes (88, 89), but there is no assurance that this binding is primarily to receptors that actually see epinephrine (as opposed to norepinephrine) as a neurotransmitter physiologically.

U'Prichard & Snyder (88) have designated the sites labeled with tritiated clonidine, norepinephrine, and epinephrine as α_2 receptors. Because the concentration of norepinephrine in rat brain is 50–100 times the concentration of epinephrine, these receptors may be predominantly receptors for norepinephrine nerve terminals. If receptors for epinephrine nerve terminals are included in the sites labeled by these radioligands, one cannot be sure if their properties resemble or are identical to those of norepinephrine receptors. In fact, the receptors for epinephrine terminals and norepinephrine terminals may have to differ considerably if they are to be studied separately by ligand binding techniques.

Presynaptic autoreceptors for epinephrine in brain may with present methodology be more amenable to study than postsynaptic receptors. In general, the action of presynaptic receptor agonists and antagonists can be demonstrated by studying their effect on basal or stimulated release of neurotransmitter (90). For instance, dopamine agonists suppress and dopamine antagonists enhance the potassium or electrical stimulation-induced dopamine release from synaptosomes or brain slices in vitro. So far there has been little study of epinephrine receptor agonists and antagonists in this way.

The release of tritiated epinephrine from brain tissue in vitro has been studied after incubation of the tissue with tritiated epinephrine. Potassium-evoked release was suppressed by clonidine and enhanced by yohimbine (82, 91, 92). However, tritiated epinephrine may not have been taken up exclusively by epinephrine-forming nerve terminals; the possibility seems great that the tritiated epinephrine was taken up by the more numerous norepinephrine terminals as well and therefore that the epinephrine terminals were not specifically labeled in these experiments.

A means of avoiding this uncertainty would be to measure the release of endogenous epinephrine. Burgess & Tessel (93) have used LCEC to measure the release of epinephrine from minced hypothalamic tissue in vitro. High potassium evoked a calcium-dependent release of epinephrine, and this system may be suitable for the study of agonists and antagonists acting on presynaptic epinephrine receptors.

The in vivo release of endogenous epinephrine from cat brain has been studied by means of a push-pull cannula in the posterior hypothalamus (94). Yohimbine enhanced epinephrine release, and the effect was prevented by caudal transection, indicating the effect of yohimbine was dependent on nerve impulses coming from cell bodies located caudal to the hypothalamus. Both β_1 and β_2 agonists increased epinephrine release. In these experiments, parallel changes in dopamine and norepinephrine release were observed.

Whether epinephrine release can be modulated independently of the other two catecholamines remains to be shown. A preliminary observation by Tessel & Myers (95) suggests that desipramine increases norepinephrine and dopamine efflux from hypothalamic tissue in vitro at low concentrations but increases epinephrine efflux only at higher concentrations.

Makman et al (96) have shown that epinephrine stimulates cyclic AMP formation by the brain stem nuclei richest in epinephrine-forming neurons. These brain regions also responded to norepinephrine, but some evidence that epinephrine and norepinephrine may have acted on different subpopulations of receptors was presented. For instance, epinephrine and norepinephrine were equipotent in stimulating adenylate cyclase activity in tissue punches from the C-1 area, but norepinephrine was more potent than epinephrine in C-2 area punches in normotensive rats. In spontaneously hypertensive rats, however, epinephrine was approximately twice as potent as norepinephrine in stimulating cyclic AMP formation by C-2 punches. A possible interpretation is that supersensitivity of a specific epinephrine receptor existed in the spontaneously hypertensive rats. These interesting observations may lead to clearer differentiation of epinephrine and norepinephrine receptors.

Possible pharmacologic differentiation of epinephrine and norepinephrine receptors has also been suggested from in vivo experiments. Bolme et al (97) suggested that the autonomic effects of clonidine are due to epinephrine receptor stimulation, whereas the effects of clonidine on spinal reflexes are due to stimulation of norepinephrine receptors. They observed that piperoxan and yohimbine blocked the hypotensive and respiratory actions of clonidine at doses that did not antagonize clonidine-induced increases in flexor reflex activity, the latter effect known to involve norepinephrine receptors. They also reported that low doses of piperoxan and yohimbine selectively increased norepinephrine turnover in the cortex cerebri and not in the rest of the brain, possibly by blocking receptors for inhibitory epinephrine nerve terminals innervating the norepinephrine cell bodies of the locus coeruleus.

From these findings, they hypothesized that adrenaline neurons may be involved in vasomotor and respiratory control and that clonidine may influence blood pressure and respiration by acting on epinephrine receptors (as distinct from norepinephrine receptors). Piperoxan at a low dose (5 mg/kg i.p.) increased the percentage of awake time in rats, leading Fuxe et al (98) to postulate that clonidine caused sedation by activation of receptors at inhibitory epinephrine synapses controlling the noradrenergic arousal system and that piperoxan caused arousal by blocking these epinephrine synaptic receptors. Electrophysiological studies showing that iontophoresed epinephrine inhibited the firing of locus coeruleus cells, whereas

intravenously injected piperoxan increased the firing of these cells and prevented or reversed the inhibition of the cells by epinephrine or clonidine (99), are compatible with the hypothesis that receptors for epinephrine nerve terminals have an inhibitory effect on the norepinephrine-containing cell bodies in the locus coeruleus. Drugs that more selectively influenced epinephrine or norepinephrine receptors would be valuable to test these hypotheses.

INHIBITION OF UPTAKE INTO EPINEPHRINE NEURONS

The in vitro uptake and accumulation of radiolabeled dopamine and norepinephrine by synaptosomes or brain slices have provided straightforward and useful means of studying agents that block the uptake pump on these neurons. Caution has to be used, however, even with these catecholamines, inasmuch as radiolabeled dopamine added to synaptosomes from some brain regions can be taken up by norepinephrine neurons [see (100)]. This problem can be avoided by using synaptosomes prepared from striatum, where dopamine terminals predominate by a large extent over norepinephrine terminals; in this way, the radiolabeled dopamine can be assured of being taken up into dopaminergic terminals. Thus similar studies of uptake of radiolabeled epinephrine are fraught with dangers. Any brain region studied would have norepinephrine terminals far out-numbering epinephrine terminals, and the likelihood seems great that any uptake of radiolabeled epinephrine might be largely into norepinephrine terminals. Presently there is no proven means of avoiding this difficulty, which accounts for the lack of studies on the epinephrine uptake pump in vitro.

One way of avoiding this potential problem is to study uptake inhibitors in vivo, based on their ability to antagonize the depletion of catecholamines by drugs whose action requires active uptake into the catecholamine-containing neurons. To this end, we have used α-methyl-*m*-tyrosine, which leads to depletion of dopamine, norepinephrine, and epinephrine in rat brain. The mechanism is thought to involve metabolic conversion of α-methyl-*m*-tyrosine to decarboxylated products, α-methyl-*m*-tyramine and metaraminol, which are accumulated by catecholamine neurons and stored as false transmitters within the nerve terminals at the expense of endogenous catecholamines (101). Not only can the depletion of norepinephrine by α-methyl-*m*-tyrosine be blocked, as had been shown previously (102), but we have found also that the depletion of epinephrine is blocked by certain uptake inhibitors (42). This is illustrated for imipramine in Table 1. We have studied various tricyclic and other antidepressant drugs in this way and found that they antagonize the depletion of epinephrine as well as

Table 1 Antagonism of α-methyl-*m*-tyrosine induced depletion of hypothalamic epinephrine by imipramine

Treatment	Hypothalamic epinephrine (pmoles/g)
None	180 ± 13
α-Methyl-*m*-tyrosine	84 ± 7[a]
α-Methyl-*m*-tyrosine + imipramine	160 ± 18

[a] $P < 0.01$

α-Methyl-*m*-tyrosine (100 mg/kg sc) was injected 6 hrs before rats were killed and 10 min after imipramine hydrochloride (30 mg/kg ip). Values are mean ± S.E. for five rats per group.

norepinephrine by α-methyl-*m*-tyrosine. Drugs that are active in this respect include several marketed tricyclic antidepressant drugs—amitriptyline, protriptyline, nortriptyline, and desipramine in addition to imipramine. Nisoxetine, an experimental compound shown to be effective in the treatment of mental depression in limited efficacy trials after it was recognized to be a potent inhibitor of norepinephrine uptake in animal studies, also antagonized epinephrine as well as norepinephrine depletion by α-methyl-*m*-tyrosine. Fluoxetine, an analog that inhibits serotonin uptake selectively, did not antagonize epinephrine depletion by α-methyl-*m*-tyrosine. Nisoxetine and the tricyclic drugs do not block the depletion of dopamine by α-methyl-*m*-tyrosine, suggesting the uptake systems on epinephrine and norepinephrine neurons may be similar but different from those on dopamine neurons. This apparent similarity further implies that radiolabeled epinephrine might be taken up by norepinephrine terminals if slices or synaptosomal preparations are used in in vitro studies. Mazindol, a drug used as an appetite suppressant but which was previously known to be a potent inhibitor of uptake into norepinephrine and dopamine neurons (103), was also found to block epinephrine depletion by α-methyl-*m*-tyrosine. Mazindol then is an agent that blocks uptake into all three catecholamine neurons.

Tessel et al (26) and Scatton & Bartholini (68) have also shown that an uptake inhibitor, desipramine, blocks the depletion of hypothalamic epinephrine by intraventricular 6-hydroxydopamine.

Iprindole, a drug reported to have antidepressant activity (104), did not block depletion of epinephrine (or norepinephrine or dopamine) by α-methyl-*m*-tyrosine. Iprindole had previously been shown not to block dopa-

mine or norepinephrine uptake in vitro or in vivo. VonVoigtlander & Losey (105), however, had reported that iprindole did antagonize the depletion of epinephrine in mouse brain by 6-hydroxydopa given in combination with an MAO inhibitor and suggested that iprindole might be a specific blocker of uptake into epinephrine neurons. Our data with α-methyl-*m*-tyrosine do not support that suggestion. Scatton & Bartholini (68) also have reported that iprindole failed to antagonize the 6-hydroxydopamine-induced depletion of epinephrine in the rat hypothalamus and brain stem.

We are continuing to search for uptake inhibitors that discriminate between the uptake pumps on epinephrine and norepinephrine neurons. An agent that selectively blocked epinephrine uptake presumably would enhance epinephrine neuronal function (if neuronal reuptake is an important means of inactivating epinephrine released into the synaptic cleft as is true for dopamine and norepinephrine). Presently there is no other known means of enhancing epinephrine function selectively, since agents like MAO inhibitors increase the concentration of other catecholamines as well as epinephrine, and the prospects of finding an MAO inhibitor that influenced only epinephrine seem small. An agent that selectively blocked norepinephrine uptake and not epinephrine uptake would also be of interest. Such a compound could be co-administered with 6-hydroxydopamine, keeping it out of norepinephrine neurons but letting it be taken into epinephrine neurons. That could provide a means of selective chemical lesioning of epinephrine nerve terminals, something which cannot at present be accomplished.

FUNCTIONS OF EPINEPHRINE NEURONS IN BRAIN

Based on the neuroanatomic sites of highest density of PNMT-containing terminals in rat brain, Hokfelt et al (9) listed some physiological functions these epinephrine-forming neurons might serve. The dense innervation of the nucleus paraventricularis suggested oxytocin secretion might be regulated by these neurons. Regulation of food and water intake was suggested as a possible function based on the dense innervation of the perifornical area and nucleus dorsomedialis hypothalami. The innervation of various parts of the hypothalamus known to participate in thermoregulation suggested body temperature as a physiologic variable possibly controlled by these neurons. Innervation of the arcuate nucleus, along with the observation that epinephrine was more effective than dopamine and norepinephrine in triggering ovulation in proestrus pentobarbital blocked rats (106), implied that gonadotrophin secretion might be influenced by epinephrine neurons. The

presence of PNMT-containing fibers in the nucleus dorsalis motorius nervi vagi, nucleus tractus solitarii, and the sympathetic lateral column of the spinal cord suggested that blood pressure and respiration might be regulated by epinephrine neurons.

Epinephrine neurons may also have a role in behavior. As discussed earlier, behavioral effects of PNMT inhibitors have been noted in animals. The fact that both MAO-inhibiting and uptake-inhibiting antidepressant drugs affect epinephrine neurons as well as other monoaminergic neurons in brain raises the possibility that epinephrine neurons might contribute to the mood-elevating properties of these drugs.

Regulation of Cardiovascular Function

The greatest focus of attention on possible physiologic roles of epinephrine-forming neurons in brain has been on the central regulation of blood pressure. Bolme & Fuxe (107) postulated that low doses of clonidine and high doses of morphine lowered blood pressure by stimulating central epinephrine receptors, probably in the nucleus tractus solitarii (108). Epinephrine turnover in the dorsal midline area of the caudal medulla oblongata was reduced by the low doses of clonidine (109). Consistent with the idea that the epinephrine neurons in the medulla oblongata represent a vasodepressor system is the finding that a PNMT inhibitor injected intracisternally or intraventricularly increased blood pressure (110–112), except that the effects were so rapid they were thought not to be due to inhibition of epinephrine synthesis.

The systemic injection of several different PNMT inhibitors has been reported to lower blood pressure. Saavedra et al (113) reported that SK&F 7698, a PNMT inhibitor, lowered blood pressure in DOCA-salt hypertensive rats. This compound was known to be as potent an α-blocker as it was a PNMT inhibitor, so no conclusions can be drawn from studies with it about a possible role of PNMT-containing neurons in blood pressure regulation. Other PNMT inhibitors have also been reported to lower blood pressure after system injection. These include 2,3-dichloro-α-methylbenzylamine (22, 49), SK&F 64139 (112), 2-cyclooctyl-2-hydroxyethylamine (62), LY87130 (T. T. Yen, unpublished data), and LY134046 (R. A. Hahn, unpublished data). In no case has there been compelling evidence that the lowering of blood pressure is a *consequence of* PNMT inhibition. The concept that inhibition of epinephrine synthesis in brain would lower blood pressure seems contrary to the suggestion that clonidine lowers blood pressure by stimulating central epinephrine receptors. Additional study of pharmacologic agents with the highest specificity attainable will be necessary to clarify the role of epinephrine neuronal systems in blood pressure modification by drugs.

Cardiovascular responses to hemorrhage, namely bradycardia and hypotension, are prolonged after treatment with a PNMT inhibitor that decreases brain epinephrine synthesis and may be influenced by central epinephrine neurons (114).

Apart from the pharmacologic studies, evidence for involvement of brain epinephrine neurons in blood pressure regulation has also come from studies of epinephrine concentration and PNMT activity in brain regions of hypertensive rats. Many workers have found increases in PNMT activity or in epinephrine concentration in brain stem and hypothalamic regions of genetically hypertensive rats of different ages (112, 113, 115–121). These increases are apparently related to a genetically determined increase in the number of epinephrine-forming neurons in various medullary regions of spontaneously hypertensive rats (122). Increased PNMT activity has also been observed in the brain stem of Goldblatt rats (renal-impaired) during development of hypertension (123) and in salt-sensitive Dahl rats after institution of a high salt diet to increase blood pressure (124). A few reports on changes in epinephrine turnover in brain regions of hypertensive rats have also appeared (86, 125–127). There is also evidence of changes in epinephrine receptor function in hypertensive rats (95, 128).

Additional studies are necessary to elucidate more fully the role of epinephrine neurons in the development and maintenance of increased blood pressure. Drugs capable of acting more specifically on brain epinephrine neurons are needed to establish causal relationships between changes in epinephrine neuron function and blood pressure. There is the obvious possibility that some such agents might have therapeutic potential as antihypertensive drugs based on their actions on central epinephrine neurons.

Behavior

Several behavioral effects of PNMT inhibitors in rats or mice have been reported, including decreased responding for intracranial reward (45, 47), increased ambulation, rearing and defecation during open field behavioral testing (47), and enhancement of behavioral stimulation by amphetamine (48), morphine (48), and L-dopa (129). Proof that the behavioral effects were consequences of inhibition of epinephrine synthesis in brain and not due to other effects possibly caused by the particular PNMT inhibitors used was not offered.

Neuroendocrine Regulation

The possibility that epinephrine neurons in brain might have a role in regulation of pituitary function has been explored in a preliminary way. Gold et al (130) found that clonidine increased growth hormone in monkeys and that piperoxan prevented or reversed the increase. Based on earlier

suggestions that these drugs affect epinephrine receptors in brain, they postulated that epinephrine synapses in the hypothalamus might augment arcuate nucleus activity and stimulate growth hormone secretion. The finding that dopamine turnover in the lateral palisade zone was reduced following PNMT inhibition led Fuxe et al (131) to suggest that epinephrine neurons might have a facilitatory influence on the tuberinfundibular dopamine systems and in this way modulate neuroendocrine function. A PNMT inhibitor, SK&F 64139, has been reported to decrease plasma levels of luteinizing hormone in ovariectomized rats, to suppress the surge of luteinizing hormone induced by estrogen plus progesterone, and to suppress episodic growth hormone release (132–134). Two other PNMT inhibitors, 2,3-dichloro-α-methylbenzylamine and 2-chloro-3-trifluoromethyl-α-methylbenzylamine, were found to increase serum corticosterone concentration in rats (135). Additional evidence is needed that these effects relate specifically to the inhibition of epinephrine synthesis by these agents and not, for example, to blockade of α receptors by SK&F 64139 or to nonspecific "stress" effects of high doses of the other compounds. These provocative findings suggest the need for more specific pharmacologic tools with which brain epinephrine neuronal function can be manipulated to prove causal relationships to changes in neuroendocrine function.

SUMMARY

Neurons that contain PNMT, the epinephrine-forming enzyme, have their cell bodies in brain stem regions in rat brain and send projections mainly into other brain stem areas, hypothalamus, and spinal cord. These neurons can be affected pharmacologically by various kinds of drugs. Epinephrine neuronal systems might play a part in some pharmacologic actions of MAO inhibitors and uptake inhibitors as well as α and β agonists and antagonists. PNMT inhibitors currently represent the only means of modifying epinephrine neurons pharmacologically without also altering norepinephrine or dopamine neurons in brain. The continued study of drugs affecting epinephrine neurons should be useful in elucidating functions of these neurons. Drugs that affect epinephrine neurons may be of use in the treatment of hypertension, psychiatric disorders, neuroendocrine dysfunction, and possibly other diseases.

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