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Catecholamine Metabolism: A Contemporary View with Implications for Physiology and Medicine

GRAEME EISENHOFER, IRWIN J. KOPIN, AND DAVID S. GOLDSTEIN

Clinical Neurocardiology Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

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***Abstract*——This article provides an update about catecholamine metabolism, with emphasis on correcting common misconceptions relevant to catecholamine systems in health and disease. Importantly, most metabolism of catecholamines takes place within the same cells where the amines are synthesized. This mainly occurs secondary to leakage of catecholamines from vesicular stores into the cytoplasm. These stores exist in a highly dynamic equilibrium, with passive outward leakage counterbalanced by inward active transport controlled by vesicular monoamine transporters. In catecholaminergic neurons, the presence of monoamine oxidase leads to formation of reactive catecholaldehydes. Production of these toxic aldehydes depends on the dynamics of vesicular-axoplasmic monoamine exchange and enzyme-catalyzed conversion to nontoxic acids or alcohols. In sympathetic nerves, the aldehyde produced from norepinephrine is converted to 3,4-dihydroxyphenylglycol, not 3,4-dihydroxymandelic acid. Subsequent extraneuronal *O*-methylation consequently leads to production of 3-methoxy-4-hydroxyphenylglycol, not vanillylmandelic acid. Vanillylmandelic acid is instead formed in the liver by oxidation of 3-methoxy-4-hydroxyphenylglycol catalyzed by alcohol and aldehyde dehydrogenases. Compared to intraneuronal deamination, extraneuronal *O*-methylation of norepinephrine and epinephrine to metanephrines represent minor pathways of metabolism. The single largest source of metanephrines is the adrenal medulla. Similarly, pheochromocytoma tumor cells produce large amounts of metanephrines from catecholamines leaking from stores. Thus, these metabolites are particularly useful for detecting pheochromocytomas. The large contribution of intraneuronal deamination to catecholamine turnover, and dependence of this on the vesicular-axoplasmic monoamine exchange process, helps explain how synthesis, release, metabolism, turnover, and stores of catecholamines are regulated in a coordinated fashion during stress and in disease states.**

I. Introduction

The catecholamines, dopamine, norepinephrine, and epinephrine, constitute a class of chemical neurotransmitters and hormones that occupy key positions in the regulation of physiological processes and the development of neurological, psychiatric, endocrine, and cardiovascular diseases. As such, these chemicals and the catecholamine neuronal and endocrine systems in which they are produced continue to receive considerable research attention.

Because of the long-standing and extensive nature of research in the area of catecholamines and catechol-

Address correspondence to: Graeme Eisenhofer, Building 10, Room 6N252, National Institutes of Health, 10 Center Dr., MSC-1620, Bethesda, MD 20892-1620. E-mail: ge@box-g.nih.gov

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amine systems, it should be expected that the pathways of catecholamine metabolism would by now be well understood and clearly described. In fact, almost all textbooks and most reviews on the subject contain inaccurate and misleading descriptions. As a result, misunderstandings in the area are common and persist.

Early work on catecholamines centered on their disposition and metabolism. The endogenous catecholamines were not easily quantified so that their routes of metabolism were largely deduced from isotopic tracer studies of the fate of exogenously administered radiolabeled catecholamines. Pathways of catecholamine metabolism depicted in textbooks and reviews remain based largely on results of these early studies. In several important respects, those depictions simply do not apply and should not be generalized to the sources, fate, and significance of endogenous catecholamines and their metabolites.

Subsequent work showed much more complex dynamics, involving multiple processes for catecholamine synthesis, storage, release, inactivation, and metabolism that differ markedly among tissues and cell types, before and after release of the catecholamines into the extracellular fluid and circulation. Over the last 20 years, a more correct understanding of the disposition and metabolism of catecholamines has emerged.

This article reviews and updates current understanding about catecholamine metabolism with emphasis on correcting common misconceptions, clarifying how these arose, and outlining the importance of a correct understanding for advancing progress about catecholamine systems in health and disease.

II. Facts and Fallacies

Probably the most common area of misunderstanding concerns the deamination of norepinephrine and epinephrine by monoamine oxidase (MAO[1]) and the subsequent formation of vanillylmandelic acid (VMA), the principal end-product of norepinephrine and epinephrine metabolism (Table 1). Contrary to usual depictions of catecholamine metabolism, VMA is produced mainly by oxidation of 3-methoxy-4-hydroxyphenylglycol (MHPG), catalyzed by the sequential actions of alcohol and aldehyde dehydrogenases (Kopin, 1985). This pathway is rarely considered. Instead, formation of VMA is usually and erroneously ascribed to sequential oxidative deamination of norepinephrine to form 3,4-dihydroxymandelic acid (DHMA) followed by *O*-methylation of DHMA to form VMA. This fallacious depiction implies independent sources of MHPG and VMA. Indeed, for several years, levels of MHPG in plasma or urine were thought, incorrectly, to provide an index of norepinephrine metabolism in the brain.

A second area of misunderstanding is that catecholamines are usually considered to be metabolized at sites distant from their sites of synthesis and release, after their entry into the extracellular fluid or even the bloodstream. In fact, most metabolism of catecholamines takes place in the same cells where the amines are produced. Importantly, most of this metabolism occurs independently of exocytotic release, and only a small fraction of catecholamine metabolites is formed from circulating catecholamines. Failure to recognize these facts probably reflects another misconception that vesicular stores of catecholamines exist in a static state until a stimulus evokes release into the extracellular space. In fact, vesicular stores of catecholamines exist in a highly

[1]Abbreviations: MAO, monoamine oxidase; COMT, catechol-*O*-methyltransferase; DHMA, 3,4-dihydroxymandelic acid; DHPG, 3,4-dihydroxyphenylglycol; DOPEGAL, 3,4-dihydroxyphenylglycolaldehyde; DOPAC, 3,4-dihydroxyphenylacetic acid; DOPET, 3,4-dihydroxyphenylethanol; DOPAL, 3,4-dihydroxyphenylacetaldehyde; MHPG, 3-methoxy-4-hydroxyphenylglycol; VMA, vanillylmandelic acid; HVA, homovanillic acid; CSF, cerebrospinal fluid.

TABLE 1
Fallacies and facts about catecholamine metabolism

Fallacy	Fact
Deamination of norepinephrine and epinephrine produces an inactive acid metabolite, DHMA	Deamination of norepinephrine and epinephrine produces a reactive aldehyde that is reduced to form DHPG
The major pathway of VMA formation is via *O*-methylation of DHMA formed from deamination of norepinephrine	The major pathway of VMA formation is via oxidation of MHPG formed by *O*-methylation of DHPG
Catecholamine stores are static	Catecholamine stores are dynamic
Most neuronal catecholamines are metabolized after release	Most neuronal catecholamines are metabolized intraneuronally after leakage from stores
Neuronal catecholamine turnover depends mainly on nerve activity	Neuronal catecholamine turnover is much more dependent upon vesicular leakage than neuronal activity
Metanephrines are formed mainly in the liver and kidneys after release of catecholamines into the circulation	Catecholamine *O*-methylation in the adrenal medulla is the predominant source of metanephrine and a major source of normetanephrine.
MHPG is derived mainly from the brain	Most MHPG is derived from DHPG formed in peripheral sympathetic nerves
MHPG sulfate reflects brain norepinephrine turnover	MHPG sulfate reflects norepinephrine turnover in the gastrointestinal tract
Dopamine metabolites (mainly HVA) are mainly derived from the central nervous system.	Dopamine metabolites are derived mainly from peripheral non-neuronal sources, particularly the gastrointestinal tract
Urinary dopamine is derived mainly from renal dopaminergic neurons or from plasma	Urinary dopamine is derived mainly from plasma DOPA decarboxylated in the kidney parenchyma

dynamic equilibrium with catecholamines in the surrounding cytoplasm. Avid and rapid active transport from cytoplasm into vesicles, mediated by vesicular monoamine transporters, counterbalances passive outward leakage from vesicles. Although only a small fraction of the catecholamines in the cytoplasm escapes vesicular sequestration, that fraction represents a major source of catecholamine metabolites.

A. Catecholamine Deamination

MAO catalyzes only the first step of a two-step reaction (Fig. 1). As shown when oxidative deamination of catecholamines was originally described in the mid 1930s, the first step involves formation of deaminated aldehydes (Richter, 1937). Dopamine is deaminated to 3,4-dihydroxyphenylacetaldehyde (DOPAL), whereas norepinephrine and epinephrine are both deaminated to 3,4-dihydroxyphenylglycolaldehyde (DOPEGAL).

The deaminated aldehyde metabolites of catecholamines are short-lived intermediates that undergo further metabolism in a second step catalyzed by another group of enzymes, forming more stable alcohol or acid deaminated metabolites (Erwin and Deitrich, 1966; Tabakoff and Erwin, 1970; Wermuth and Munch, 1979; Tank et al., 1981). Aldehyde dehydrogenase metabolizes DOPAL to 3,4-dihydroxyphenylacetic acid (DOPAC) and DOPEGAL to DHMA, whereas aldehyde reductase metabolizes DOPAL to 3,4-dihydroxyphenylethanol (DOPET) and DOPEGAL to 3,4-dihydroxyphenylglycol (DHPG).

In addition to aldehyde reductase, the related enzyme, aldose reductase, is also capable of reducing catecholadehydes to the corresponding alcohols. The latter enzyme is present in sympathetic neurons and adrenal chromaffin cells and is in fact more effective than aldehyde reductase in converting DOPEGAL to DHPG (Kawamura et al., 1999, 2002).

Theoretically, the redundancy of enzymes could lead to production of multiple deaminated catecholamine metabolites. In fact, only one is normally formed in significant quantities from each catecholamine precursor, this depending on the presence or absence of a β-hydroxyl group (Breese et al., 1969; Tabakoff et al., 1973; Duncan and Sourkes, 1974; Kawamura et al., 2002). The absence of the β-hydroxyl group on dopamine, and on its deaminated metabolite, DOPAL, favors oxidation by aldehyde dehydrogenase, whereas the presence of the β-hydroxyl group on norepinephrine, epinephrine, and their common deaminated metabolite, DOPEGAL, favors reduction by aldehyde or aldose reductase (Fig. 1). This explains why dopamine is preferentially metabolized to the acid metabolite, DOPAC, whereas the β-hydroxylated catecholamines, norepinephrine and epinephrine, are preferentially converted to the alcohol metabolite, DHPG.

Why then are norepinephrine and epinephrine usually shown to be deaminated to DHMA rather than to DHPG? Presumably this originates from early studies of amine oxidases, which were shown to act on a variety of amines, ultimately forming acid products (Blaschko, 1952). The actions of these same enzymes on catecholamines were therefore also considered to lead to formation of the acid metabolites, a view that was supported by findings that the acid metabolite, VMA, was the main end-product of norepinephrine metabolism excreted in urine (Armstrong et al., 1957). The above considerations led to emphasis on aldehydye dehydrogenase as the crucial enzyme acting in concert with MAO to metabolize catecholamines. Two sets of early findings further supported the plausibility of this pathway. First, incubation of partially purified preparations of MAO and aldehyde dehydrogenase with norepinephrine or epinephrine was shown to result in production of DHMA (Leeper et al., 1958); second, oral and intravenous administration of DHMA led to production of VMA (Armstrong and McMillan, 1959; Goodall and Alton, 1969).

Grossly overestimated plasma concentrations of DHMA, as measured by radioenzymatic assays (Sato

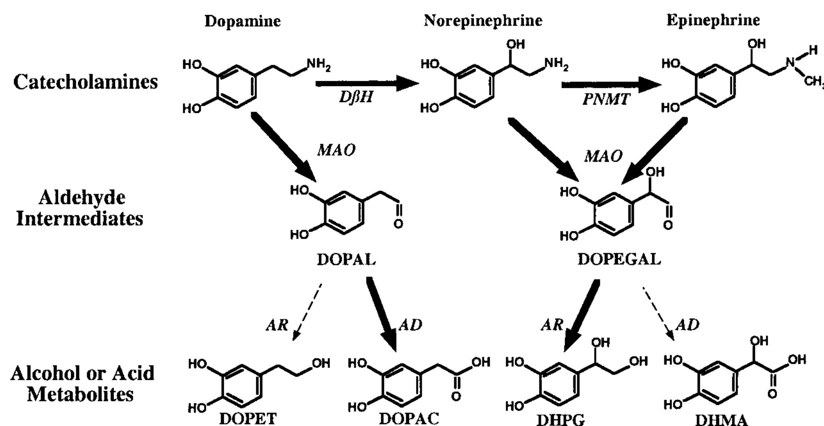

FIG. 1. Pathways of oxidative deamination of catecholamines to their corresponding biogenic aldehyde intermediates and acid derivative or alcohol metabolites. The β-hydroxylated catecholamines, norepinephrine and epinephrine, are preferentially metabolized to the alcohol, whereas dopamine, which lacks the β-hydroxy group, is preferentially metabolized to the acid derivative. DβH, dopamine β-hydroxylase; PNMT, phenolethanolamine-*N*-methyltransferase; AR, aldose or aldehyde reductase; AD, aldehyde dehydrogenase.

and DeQuattro, 1969; Vlachakis et al., 1979; Izzo et al., 1985), probably contribute to persistence of the incorrect pathway of norepinephrine deamination in today's textbooks. Using these same radioenzymatic assays, Dong and Ni (2002) more recently showed that MAO inhibition causes greater than 98% reductions in plasma concentrations of DHPG but has little effect on concentrations of DHMA. This supports the view that estimates of DHMA by radioenzymatic assays do not reflect deamination and are therefore erroneous. Use of more specific liquid chromatographic assays established that DHMA is normally present in plasma at barely detectable levels, less than 2% those of DHPG (Eisenhofer et al., 1987, 1988a; Eriksson and Persson, 1987; Kawamura et al., 1997). Only when aldehyde and aldose reductase are both inhibited do plasma levels of DHMA exceed those of DHPG (Kawamura et al., 1997). Thus, under normal circumstances DHMA should be considered an insignificant metabolite of norepinephrine and epinephrine. DHPG, not DHMA, is the main metabolite produced by deamination of these catecholamines.

B. Formation of Vanillylmandelic Acid

In humans, VMA is the major end-product of norepinephrine and epinephrine metabolism (Armstrong et al., 1957; Armstrong and McMillan, 1959). Because VMA is an acid metabolite, and due to the above misconceptions about products of catecholamine deamination, VMA came to be considered to be formed by *O*-methylation of DHMA (Armstrong and McMillan, 1959; Goodall, 1959; Goodall and Alton, 1969). Despite subsequent evidence to the contrary, this view persists more than 40 years later.

The other route usually described as a major pathway leading to formation of VMA involves *O*-methylation of norepinephrine to normetanephrine and of epinephrine to metanephrine, followed by oxidative deamination of both these metabolites to VMA. As further detailed below, this is only a minor pathway for formation of VMA.

The above pathways ignore early observations by Axelrod and colleagues (1959) indicating that MHPG is an important metabolite of norepinephrine and epinephrine. As reviewed comprehensively elsewhere (Kopin, 1982, 1985; Kopin et al., 1984a), a considerable body of evidence over the following two decades established that MHPG is a major metabolite of norepinephrine formed predominantly by *O*-methylation of DHPG (Fig. 2). MHPG can also be formed by deamination of normetanephrine and metanephrine. As originally noted by Axelrod et al. (1959), the *O*-methylated amines are first deaminated by MAO to 3-methoxy-4-hydroxyphenylglycolaldehyde. Because these compounds are β-hydroxylated, the preferred subsequent step in most extraneuronal tissues, excluding the liver, is reduction by aldose or aldehyde reductase to MHPG (Schanberg et al., 1968; Kawamura et al., 2002).

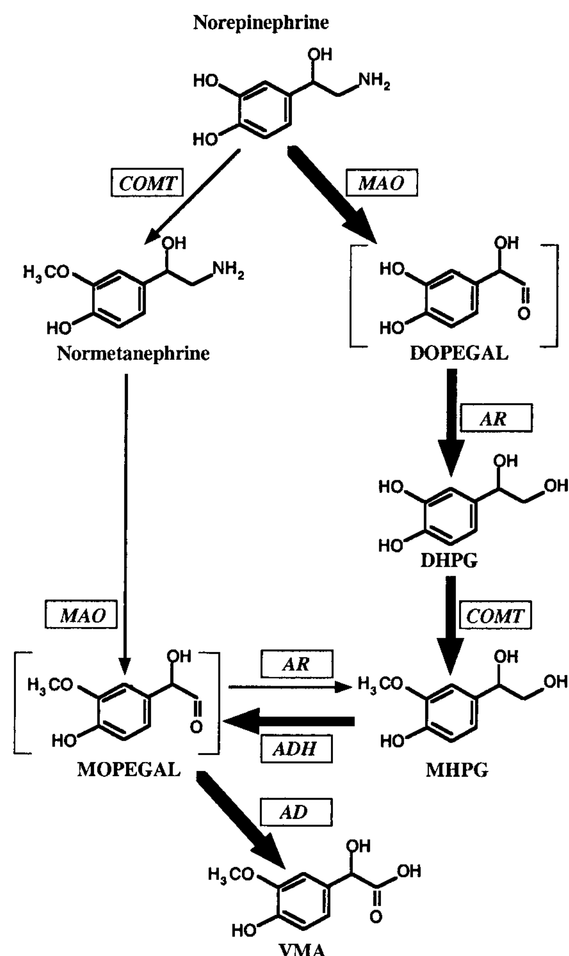

FIG. 2. Pathways of metabolism of norepinephrine to VMA. Deamination of norepinephrine to DOPEGAL by MAO and subsequent reduction to DHPG by aldehyde reductase (AR) occurs mainly in sympathetic nerves and represents the major pathway of norepinephrine metabolism. COMT catalyzed conversion of norepinephrine to normetanephrine and of DHPG to MHPG occurs in extraneuronal tissues. The sequential actions of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (AD) lead to subsequent conversion of MHPG to 3-methoxy-4-hydroxyphenylglycolaldehyde (MOPEGAL) and formation of VMA in the liver. DOPEGAL and MOPEGAL are short-lived aldehyde intermediates.

The above considerations raise an apparent paradox. If deamination and reduction to the alcohol metabolites is the favored pathway for metabolism of β-hydroxylated catecholamines, then why is the acid metabolite, VMA, the major urinary excretion product of norepinephrine and epinephrine? An explanation was first suggested by LaBrosse (1970) from studies in children with neuroblastoma who received intravenous infusions of tritium-labeled MHPG. These infusions resulted in production of tritium-labeled VMA. Subsequent studies, involving intravenous infusions of deuterated MHPG, established that most VMA is produced by oxidation of circulating MHPG (Blombery et al., 1980; Mårdh et al., 1983; Mårdh and Änggård, 1984). Human class I alcohol dehydrogenase, located mainly in the liver, was then identified as the enzyme responsible for this conversion (Mårdh et al., 1985).

In addition to alcohol dehydrogenase, conversion of MHPG to VMA also requires the actions of aldehyde dehydrogenase (Fig. 2). The roles of hepatic alcohol and aldehyde dehydrogenases in this step explain the classical observation that ethanol consumption decreases excretion of VMA while increasing that of MHPG (Smith and Gitlow, 1966; Davis et al., 1967b). These alterations are partly due to ethanol-induced decreases in the NAD^+/NADH ratio and inhibitory influences of this and acetaldehyde, the metabolite of ethanol, on NAD^+-dependent aldehyde dehydrogenase-catalyzed formation of acid metabolites (Walsh et al., 1970). This results in a shunting in production of acid end-products to alcohol end-products of monoamine metabolism. The reciprocal ethanol-induced increases in alcohol deaminated metabolites and decreases in acid metabolites of dopamine and serotonin reflect this effect on metabolism of the aldehyde intermediates produced from these monoamines (Davis et al., 1967a; Yoshimoto et al., 1992) but don't fully explain the changes in norepinephrine metabolism. In particular, since deamination of norepinephrine leads to negligible production of DHMA, ethanol consumption does not increase plasma DHPG levels (Howes and Reid, 1985). Thus, increased MHPG levels after ethanol do not reflect a shunting in production of DHMA to DHPG. Rather, it is now clear that decreased levels of VMA and increased levels of MHPG after ethanol reflect inhibitory influences on hepatic alcohol dehydrogenase and aldehyde dehydrogenase-catalyzed conversion of MHPG to VMA (Mårdh et al., 1985).

More recent studies of hepatic catecholamine metabolism, involving sampling of blood from inflowing arterial and portal venous sites and from the outflowing hepatic vein, have confirmed the importance of the liver for production of VMA (Eisenhofer et al., 1995a, 1996a). More than 94% of VMA production in the body occurs in the liver. In effect, the liver acts as a metabolic "vacuum cleaner", converting almost all inflowing catecholamines and catecholamine metabolites into VMA and leaving very little else to escape into the hepatic venous effluent. Of the VMA produced by the human liver, 87% is derived from hepatic extraction and metabolism of circulating MHPG and DHPG, 11% from norepinephrine and epinephrine, and less than 2% from circulating normetanephrine and metanephrine (Eisenhofer et al., 1996a).

Remarkably, despite the above experimental findings, few of today's textbooks note that MHPG is a major catecholamine metabolite or that VMA is produced mainly by oxidation of MHPG. Instead VMA is invariably depicted as being formed by *O*-methylation of DHMA and deamination of normetanephrine and metanephrine.

C. Contribution of Vesicular Leakage to Catecholamine Metabolism

An important but poorly recognized fact about catecholamine metabolism is that this mainly occurs in the cytoplasm of the same cells in which the catecholamines are synthesized, and most of this is due to passive leakage of catecholamines from vesicular storage granules into the cytoplasm (Fig. 3). The vesicular monoamine transporter avidly sequesters about 90% of the catecholamines leaking into the cytoplasm back into storage vesicles, but about 10% escapes sequestration and is metabolized (Eisenhofer et al., 1992a). Similarly, most norepinephrine released by sympathetic nerves is recaptured and then recycled into vesicles, so that only about 30% is lost to metabolism (Eisenhofer, 2001). Although only a small proportion of the norepinephrine that is recaptured or leaks from vesicles is metabolized, rates of leakage considerably exceed those of baseline exocytotic release and reuptake. Thus, under resting conditions much more catecholamine is metabolized secondary to leakage from vesicles than is metabolized after exocytotic release.

The high rate at which catecholamines leak from storage vesicles is revealed by the actions of reserpine, a drug that blocks sequestration of catecholamines into vesicles. Unchecked by the balancing sequestration process, loss of catecholamines due to metabolism after leakage then occurs at much higher rates, so that catecholamine stores are rapidly depleted. More than half of the contents of catecholamine stores are lost within the first hour of reserpine administration (Kopin and Gordon, 1962). This compares with the normal half-life of catecholamine stores of 8 to 12 h, as determined from the tissue disappearance of exogenous tritiated norepinephrine (Montanari et al., 1963) or the decline in endogenous norepinephrine after inhibition of catecholamine synthesis (Brodie et al., 1966). Catecholamine turnover, reflecting the continuous loss by metabolism and replenishment by synthesis, is therefore driven primarily by leakage of catecholamines from vesicular stores (Fig. 3).

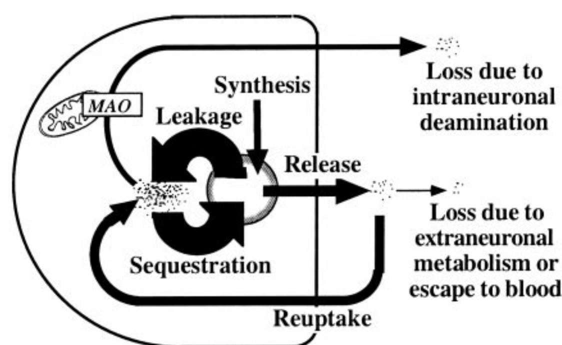

FIG. 3. Diagram illustrating the contribution of the vesicular-axoplasmic monoamine exchange process (leakage counter-balanced by sequestration) to catecholamine metabolism and turnover. To maintain vesicular stores of catecholamines constant, the rate of catecholamine turnover (i.e., loss of catecholamine due to metabolism or escape to the bloodstream) must be balanced by an equal rate of synthesis. The contribution of exocytotic release to catecholamine turnover is minimized by the high efficiencies of neuronal reuptake and vesicular sequestration. The highly efficient sequestration also minimizes the contribution of vesicular leakage to turnover. Because rates of leakage are larger than baseline rates of release, the contribution of leakage to catecholamine metabolism and turnover exceeds that of release.

The important contribution of intraneuronal metabolism of norepinephrine to catecholamine turnover, and the significant dependence of this on leakage of norepinephrine from vesicular storage granules, was originally hypothesized by Kopin (1964). Later, Maas et al. (1970) used i.v. infusions of tracer doses of tritium-labeled norepinephrine to examine the kinetics and metabolism of circulating and neuronally produced norepinephrine in humans. These investigators concluded that at least 75% of norepinephrine turnover in man reflects intraneuronal metabolism without prior release from the nerve endings.

Numerous studies in isolated tissue preparations, and in vivo in animals and humans, have now amply confirmed that leakage of catecholamines from storage granules represents a key determinant of catecholamine metabolism and turnover (Eisenhofer et al., 1988a,b; Goldstein et al., 1988; Halbrügge et al., 1989; Tyce et al., 1995). Use of norepinephrine radiotracer techniques and regional blood sampling has most recently provided estimates of the contributions of the various pathways to norepinephrine turnover in the normal resting human heart in vivo (Eisenhofer et al., 1996b, 1998b). These studies indicated that 18% of norepinephrine turnover results from extraneuronal uptake and metabolism or loss of the transmitter to the circulation, 12% from intraneuronal metabolism after reuptake, and 70% from intraneuronal metabolism of norepinephrine leaking from storage vesicles. The latter figure agrees closely with the 75% value calculated by Maas et al. (1970) more than 25 years earlier.

Using purified synaptic vesicles from vas deferens, the half-life of vesicular-axoplasmic exchange of norepinephrine was estimated to be 43 min (Fried, 1981), which is similar to that in the human heart in vivo (Eisenhofer et al., 1996b). Studies utilizing rat brain synaptic vesicles have indicated that the exchange is more rapid for dopamine than for norepinephrine (Floor et al., 1995). Thus, leakage of transmitter from storage vesicles into the neuronal cytoplasm may make a larger contribution to turnover of dopamine in central dopaminergic neurons than the contribution to turnover of norepinephrine in sympathetic neurons.

In summary and in contrast to usual descriptions, vesicular stores of catecholamines do not exist in a static state simply awaiting a signal for exocytotic release. Instead, catecholamine stores exist in a highly dynamic state, with passive outward leakage of catecholamines counterbalanced by inward active transport under the control of vesicular monoamine transporters. Because of this dynamic equilibrium, a substantial amount of catecholamine metabolism takes place within the cytoplasm of the same cells in which the amines are synthesized. Thus, under resting conditions, catecholamine metabolism reflects more the passive process of vesicular leakage than the active processes of exocytosis followed by cellular uptake.

D. Neuronal and Extraneuronal Catecholamine Metabolism

Sympathetic nerves contain MAO, but not catechol-*O*-methyltransferase (COMT). Therefore, intraneuronal metabolism of norepinephrine leads to production of the deaminated metabolite, DHPG, but not the *O*-methylated metabolite, normetanephrine (Graefe and Henseling, 1983). Consequently, almost all of the DHPG in plasma has a neuronal source, whereas normetanephrine and metanephrine are derived exclusively from non-neuronal sources (Fig. 4). As discussed below, these non-neuronal sources include chromaffin cells in the adrenal medulla.

At rest, about 80% of circulating DHPG is produced by deamination of norepinephrine leaking from vesicles in sympathetic nerves (Eisenhofer et al., 1991b). In contrast to this large and relatively constant source of pro-

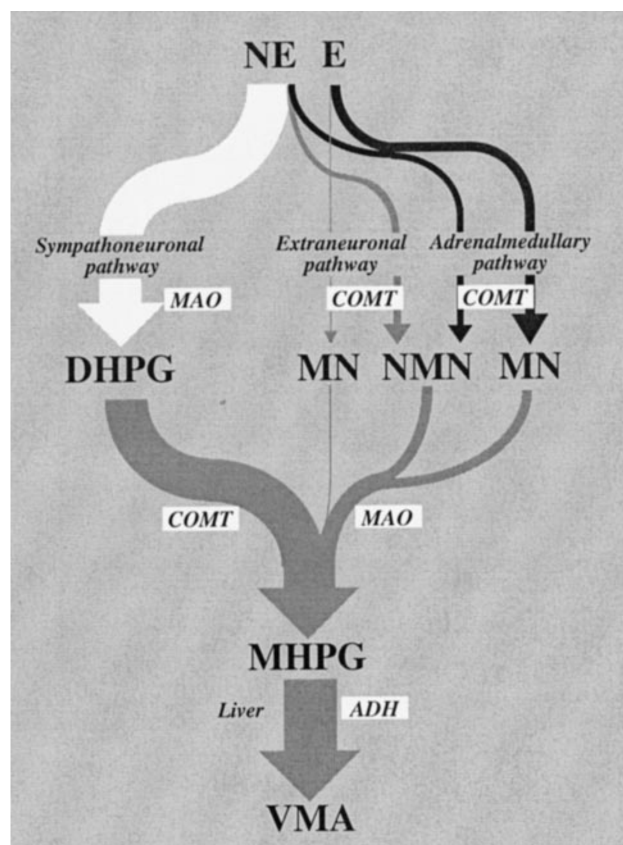

FIG. 4. Diagram showing the three main pathways for metabolism of the norepinephrine (NE) and epinephrine (E) derived from sympathoneuronal or adrenalmedullary sources. The sympathoneuronal pathway (white) is the major pathway of catecholamine metabolism and involves intraneuronal deamination of norepinephrine leaking from storage granules or of norepinephrine recaptured after release by sympathetic nerves. The extraneuronal pathway (gray) is a relatively minor pathway of metabolism of catecholamines released from sympathetic nerves or the adrenal medulla, but is important for further processing of metabolites produced by neuronal and adrenalmedullary pathways. The adrenalmedullary pathway (black) involves *O*-methylation of catecholamines leaking from storage granules into the cytoplasm of adrenalmedullary cells. Metabolism by sulfate conjugation which operates as part of the extraneuronal pathway is not shown. MN, metanephrines; NMN, normetanephrine; ADH, alcohol dehydrogenase.

duction of DHPG, the DHPG derived from deamination of recaptured norepinephrine varies depending on sympathetic outflow (Eisenhofer et al., 1990, 1991a,d). At high rates of norepinephrine release, DHPG production from reuptake can exceed that from leakage.

Because of the importance of intraneuronal metabolism, production of DHPG considerably exceeds that of normetanephrine (Eisenhofer, 1994; Eisenhofer et al., 1996b). Consequently, most MHPG comes from extraneuronal *O*-methylation of DHPG produced in, and diffusing readily from sympathetic nerves into the extracellular fluid (Eisenhofer et al., 1994); relatively little MHPG comes from deamination of normetanephrine formed in extraneuronal tissues.

In contrast to sympathetic nerves, which contain only MAO, adrenal chromaffin cells contain both MAO and COMT (Fig. 5). Importantly, the COMT in chromaffin cells is mainly present as the membrane-bound form of the enzyme (Eisenhofer et al., 1998a; Ellingson et al., 1999). This isoform has a much higher affinity for catecholamines than does the soluble form found in most other tissues, such as in the liver and kidneys (Roth, 1992). As a result, in adrenal chromaffin cells, leakage of norepinephrine and epinephrine from storage granules leads to substantial intracellular production of the *O*-methylated metabolites, normetanephrine and metanephrine. In fact, the adrenals constitute the single largest source out of any organ system—including the liver—for both circulating metanephrine and normetanephrine. In humans, about 93% of circulating metanephrine and between 25% to 40% of circulating normetanephrine are derived from catecholamines metabolized within adrenal chromaffin cells (Eisenhofer et al., 1995b,c).

The importance of intraneuronal and adrenomedullary intracellular metabolism to the overall turnover of catecholamines is rarely appreciated. More usually it is erroneously believed that most metabolism of catecholamines takes place in extraneuronal tissues after release from sympathetic nerves into the interstital fluid and from the adrenals into the bloodstream. The sequence of cellular uptake and metabolism of catecholamines in extraneuronal tissues, such as the liver and kidneys, is important for clearance of circulating and exogenously administered catecholamines; however, this pathway contributes at most 25% to the total metabolism of endogenous catecholamines produced in sympathetic nerves and adrenal chromaffin cells (Maas et al., 1970; Eisenhofer, 1994).

E. Central and Peripheral Contributions to Norepinephrine Metabolism

Although norepinephrine and epinephrine are mainly first metabolized in the same cells in which they are synthesized, the extent and nature of this and subsequent steps in catecholamine metabolism vary considerably among the various body organs. These variations,

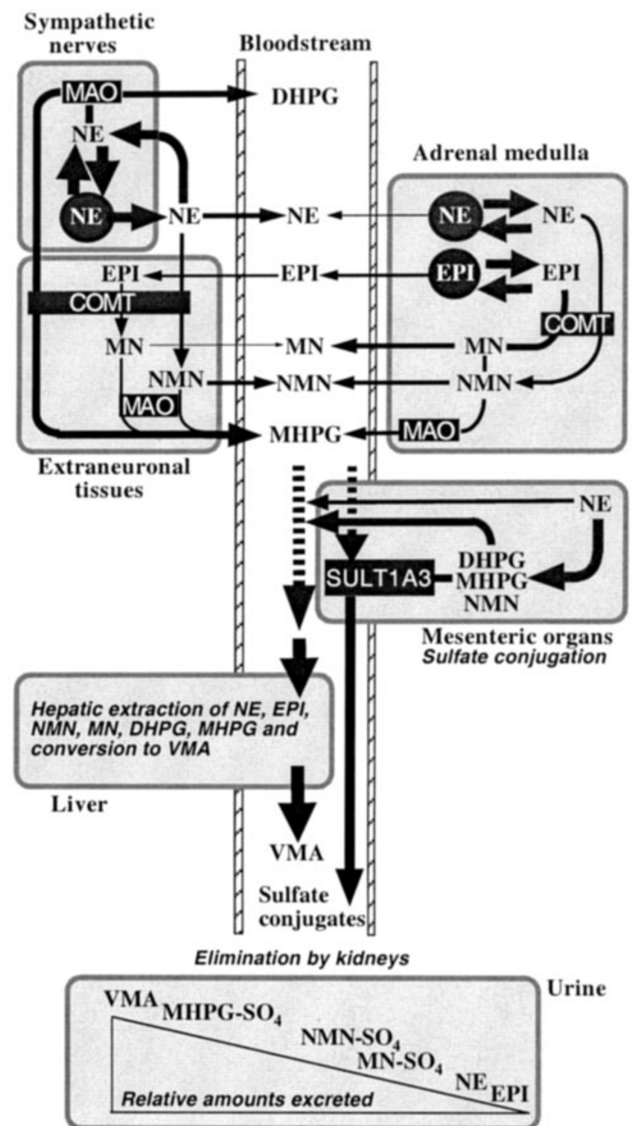

FIG. 5. Model illustrating the regional pathways of norepinephrine and epinephrine metabolism. Most norepinephrine is released and metabolized within sympathetic nerves, including up to a half produced in sympathetic nerves of mesenteric organs. Sulfate conjugation of catecholamines and catecholamine metabolites, particularly MHPG, occurs mainly in mesenteric organs, whereas production of VMA occurs mainly in the liver. These represent the main metabolites excreted in urine. SULT1A3, sulfotransferase type 1A3; NE, norepinephrine; EPI, epinephrine; NMN, normetanephrine; MN, metanephrine; NMN-SO_4, normetanephrine sulfate; MN-SO_4, metanephrine sulfate; MHPG-SO_4, 3-methoxy-4-hydroxyphenylglycol sulfate.

and the contributions of different tissues and organs to catecholamine release and metabolism, have been clarified only relatively recently (Fig. 5). Erroneous assumptions about the sources and significance of catecholamines and catecholamine metabolites in plasma and urine therefore remain relatively common.

One such assumption concerns the brain as a major site of norepinephrine turnover and a source of catecholamine metabolites, particularly MHPG. Findings of relatively high levels of free MHPG in brain tissue, close correlations of plasma and cerebrospinal fluid (CSF) MHPG levels, and increases in plasma concentrations of

MHPG from arterial to internal jugular venous sampling sites led to suggestions that measurements of this metabolite in urine, plasma, and CSF would provide a means for assessing the activity of central nervous noradrenergic systems (DeMet and Halaris, 1979; Maas et al., 1979; Elsworth et al., 1982).

The above notion was supplemented by the belief, subsequently proven incorrect, that VMA and MHPG represent distinct end-products of norepinephrine metabolism, the former involving an oxidative deamination pathway in the periphery and the latter a reductive pathway in the brain. At the time, it was not appreciated that substantial amounts of MHPG were oxidized to VMA in the liver. Consequently, findings that amounts of free MHPG produced by the brain were 80% those excreted in urine led to the mistaken belief that the brain was the main source of urinary free MHPG (Maas et al., 1979). Later findings that most free MHPG is not excreted in urine, but is converted to VMA, led to a reevaluation of the contribution of the brain to urinary and plasma levels of MHPG (Kopin et al., 1983, 1984c). These analyses showed that the brain makes at most a 20% contribution to these levels. Also, because MHPG diffuses freely from plasma into CSF, the close correlation between plasma and CSF levels of MHPG was shown to be expected (Kopin et al., 1983, 1984b). Thus, CSF MHPG levels cannot be interpreted as reflecting central norepinephrine metabolism unless corrected for MHPG levels in plasma.

The relatively minor contribution of the brain to circulating MHPG can be accurately appreciated from studies involving sampling of blood from arterial and internal jugular venous sites, where rates of release of free MHPG from the brain into plasma were estimated at 0.9–3.2 nmol per minute (Maas et al., 1979; Lambert et al., 1995). Comparison of these rates with the summed release of free MHPG into plasma from all tissues in the body, calculated at 33.5 nmol per minute (Mårdh et al., 1983), indicates a 3–10% contribution of the brain to levels of circulating free MHPG.

Apart from the substantial hepatic conversion of MHPG to VMA, large amounts of MHPG are also converted to sulfate and glucuronide conjugates, by specific sulfotransferases and glucuronidases. All three forms of MHPG—MHPG sulfate, MHPG glucuronide, and unconjugated or free MHPG—are present in human plasma at similar levels (Karoum et al., 1977). In urine, however, only about 3% of the MHPG is in the free form; most is excreted as the sulfate and glucuronide conjugates (Boobis et al., 1980). After VMA, these two conjugates of MHPG represent the second most abundant end-products of norepinephrine and epinephrine metabolism in human urine. Along with VMA, they account for over 90% of all the metabolites of norepinephrine and epinephrine excreted in urine.

After measurements of urinary free and total MHPG were discounted as markers of brain norepinephrine turnover, it was suggested that measurements of MHPG-sulfate might provide an alternative marker of central nervous system noradrenergic function (Filser et al., 1988; Peyrin, 1990). This was based on the premise that MHPG-glucuronide and MHPG-sulfate have different peripheral and central sources. More recent findings comparing the renal elimination of MHPG-sulfate with release of the metabolite into the portal venous drainage of mesenteric organs indicated that most if not all MHPG-sulfate is formed in the gastrointestinal tract (Eisenhofer et al., 1996a). The exact peripheral source of MHPG glucuronide remains unclear, but the liver is a likely possibility.

The considerable production of MHPG-sulfate and other norepinephrine metabolites in mesenteric organs indicates that about half of all norepinephrine in the body is produced in the gastrointestinal tract, pancreas, and spleen (Åneman et al., 1995, 1996; Eisenhofer et al., 1995a, 1996a). However, with the exception of the sulfate conjugates, very little of the norepinephrine and its metabolites that are produced in mesenteric organs and released into the portal venous outflow make it to the systemic circulation. Most are removed by the liver and converted there to VMA (Fig. 5). Thus, plasma levels and urinary excretion of MHPG-sulfate, while not providing a marker of norepinephrine turnover in the brain, may provide a unique and useful marker of norepinephrine turnover in sympathetic nerves innervating the gastrointestinal tract.

F. Central and Peripheral Contributions to Dopamine Metabolism

The mesenteric organs also constitute a major site of dopamine synthesis, accounting for about 45% of all the dopamine produced in the body (Eisenhofer et al., 1995a, 1997). The major end-product of dopamine metabolism is homovanillic acid (HVA), which is excreted in urine at up to twice the rate of VMA (Änggård et al., 1974; Elchisak et al., 1982). Unlike VMA, most HVA is formed outside the liver, by *O*-methylation of DOPAC or oxidative deamination of methoxytyramine (Eisenhofer et al., 1995a, 1997). Based on measurements of HVA concentrations in arterial and internal jugular venous plasma, the brain makes at most a 12% contribution to circulating HVA, indicating that most of the metabolite is produced in peripheral tissues (Lambert et al., 1991, 1993).

The observation that urinary excretion of dopamine and its metabolites exceeds that of norepinephrine and its metabolites was originally thought to reflect inefficient conversion of dopamine to norepinephrine in sympathetic nerves (Kopin, 1985). In fact, conversion of dopamine to norepinephrine by dopamine β-hydroxylase in sympathetic nerves is about 90% efficient (Eisenhofer et al., 1996b). A substantial amount of the dopamine produced in the body is therefore derived from sources independent of the sympathetic nerves, the adrenal me-

dulla, or the brain; close to a half is produced in mesenteric organs.

Dietary constituents have repeatedly been shown to influence plasma and urinary levels of dopamine metabolites and thus represent one peripheral source of these metabolites (Weil-Malherbe and van Buren, 1969; Hoeldtke and Wurtman, 1974; Davidson et al., 1981; Eldrup et al., 1997; Goldstein et al., 1999). Dietary sources, however, cannot account for the substantial amounts of dopamine metabolites produced after prolonged fasting (Eisenhofer et al., 1997, 1999a).

Emerging evidence now suggests the presence of a third type of peripheral catecholamine system—besides the well known sympathetic nervous and adrenomedullary hormonal systems—involving dopamine as an autocrine or paracrine substance (Goldstein et al., 1995). Such a system unquestionably operates in the kidneys, where dopamine is produced by uptake and decarboxylation of circulating dopa and functions as a natriuretic factor (Lee, 1993; Wolfovitz et al., 1993). The conversion of circulating dopa to dopamine in the kidney accounts for virtually all the free dopamine in urine, which is excreted in about 10 times larger amounts than norepinephrine. Urinary excretion of free dopamine therefore reflects a local renal dopaminergic-natriuretic system and is derived from circulating dopa, not filtration of circulating dopamine.

In contrast to the free dopamine excreted in urine, dopamine sulfate is mainly produced in the gastrointestinal tract from both dietary and locally synthesized dopamine (Eisenhofer et al., 1999a). The substantial contribution of mesenteric organs to the production of the sulfate conjugates of catecholamines and their metabolites is consistent with the high concentrations of the sulfotransferase isoenzyme, SULT1A3, in the gastrointestinal tract (Rubin et al., 1996; Goldstein et al., 1999). In humans, a single amino acid substitution confers on this isoenzyme a high affinity for metabolism of monoamines, particularly dopamine (Dajani et al., 1998, 1999). The importance of this enzyme for metabolism of dietary amines is consistent with the classic observations of Richter (1940), who showed that most orally administered epinephrine is excreted in urine as a conjugated sulfate ester.

Production of sulfate conjugates in the digestive tract therefore appears to reflect an enzymatic "gut-blood barrier" for detoxifying dietary biogenic amines. Additionally, sulfate conjugation in gastrointestinal tissues may be important for limiting the physiological effects of locally produced dopamine. Gastrointestinal tract cells, including gastric parietal cells, pancreatic acinar cells, lamina propria cells, and other "APUD" (amine precursor uptake and decarboxylation) cells, all can produce dopamine or express components of dopamine signaling pathways, including specific dopamine receptors and transporters (Mezey et al., 1996, 1998, 1999). The dopamine so produced appears to have a number of physiological functions, including modulation of sodium transport, gastrointestinal motility, and bicarbonate secretion (Finkel et al., 1994; Flemstrom and Safsten, 1994; Haskel and Hanani, 1994; Glavin and Hall, 1995).

III. Clinical Implications

Correct understanding about the disposition and metabolism of catecholamines is important for avoiding confusion about the physiology of catecholamine systems and facilitating advances in understanding the roles of these systems in health and disease. This understanding includes how components of catecholamine systems are normally regulated in a coordinated fashion, how changes in catecholamine metabolism can be used to interpret physiological and pathophysiological processes, how different clinical conditions may affect catecholamine metabolism, and how disturbances in the disposition and metabolism of catecholamines may contribute to disease processes.

A. Neurodegenerative Processes

Among the disease processes where catecholamines have established roles are those involving neurodegeneration of central and peripheral catecholamine neuronal systems. Foremost among these is Parkinson's disease, featuring loss of dopamine-producing cells and terminals of the central nigrostriatal system. In this and other related neurodegenerative diseases, the catecholamines themselves are hypothesized to play a role as endogenous neurotoxins (Graham, 1978; Stokes et al., 1999). Such a role is suggested by the well established neurotoxicity of 6-hydroxydopamine and is supported by experimental evidence in vitro of catecholamine-induced toxicity in cell culture systems (Graham et al., 1978; Rosenberg, 1988; Michel and Hefti, 1990; Masserano et al., 1996; Shinkai et al., 1997; Velez-Pardo et al., 1997), and in vivo, after intrastriatal injections of dopamine in rats (Filloux and Townsend, 1993; Hastings et al., 1996b).

Mechanisms of catecholamine-induced neurotoxicity are usually ascribed to nonenzymatic auto-oxidation of catecholamines to form reactive quinones (Graham, 1978; Hastings et al., 1996a), associated production of cytotoxic free radicals (Cohen, 1983; Masserano et al., 1996; Offen et al., 1996), and condensation reactions leading to formation of neurotoxic alkaloid derivatives, such as tetrahydroisoquinoline (Nagatsu and Yoshida, 1988; Naoi et al., 1993; Moser et al., 1995; Nagatsu, 1997). Only relatively recently has it been considered that catecholamine-induced neurotoxicity might also involve MAO-catalyzed formation of highly reactive deaminated catecholaldehyde metabolites (Eisenhofer et al., 2000) (Fig. 6).

DOPAL, the deaminated aldehyde metabolite of dopamine, and DOPEGAL, the corresponding metabolite of norepinephrine and epinephrine, are highly toxic in cell

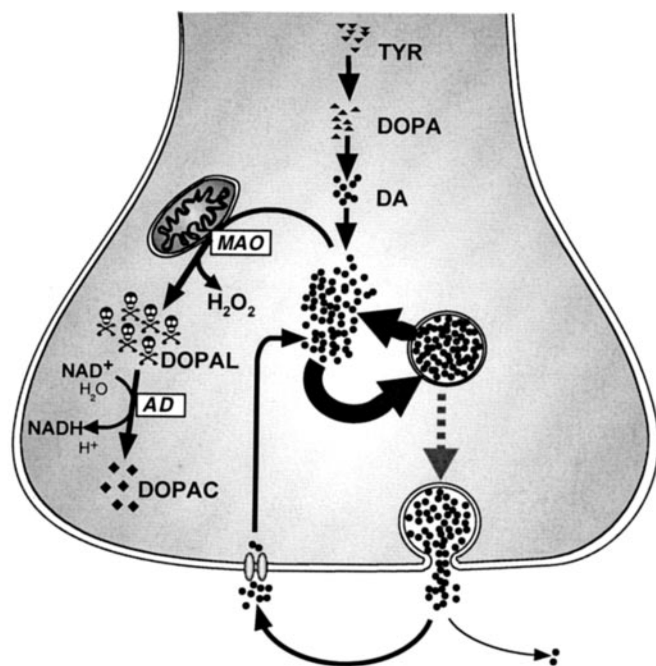

FIG. 6. Model illustrating the processes determining production of the endogenous toxin DOPAL within a dopaminergic nerve terminal. MAO catalyzed deamination of dopamine (DA) to DOPAL and production of hydrogen peroxide (H_2O_2) depend on concentrations of DA within the cytoplasm. These concentrations depend mainly on the vesicular axoplasmic exchange process. Reuptake of released transmitter and synthesis of new transmitter from tyrosine (TYR) and 3,4-dihydroxyphenylalanine (DOPA) are other determinants of cytosolic DA. Production of DOPAL may be increased by disturbances that facilitate leakage of DA from vesicles or inhibit sequestration of DA from the cytoplasm into vesicles. Such disturbances include disruptions in synaptic vesicle function (e.g., dysfunctional α-synuclein), decreased production of ATP required for ATP-dependent vesicular sequestration (e.g., metabolic stress, inhibition of mitochondrial complex I), and decreased cytosolic pH (e.g., ischemia/hypoxia) or alkalization of the vesicular interior (e.g., amphetamine), which disrupt the cytoplasmic-vesicular proton gradient driving the storage process. Increased cytosolic levels of DOPAL might also result from impaired metabolism to DOPAC by aldehyde dehydrogenase (AD). However, the presence of a redundant, but normally inactive pathway, involving reduction of DOPAL to 3,4-dihydroxphenylethanol by aldose or aldehyde reductase (not shown in model), helps ensure conversion of DOPAL to inert deaminated metabolites.

culture systems (Mattammal et al., 1995; Burke et al., 1996; Lamensdorf et al., 2000b; Li et al., 2001), and in vivo in experimental animals (Burke et al., 2001). Since most of the monoamine transmitter produced in catecholaminergic nerves is deaminated within the neuronal cytoplasm, the intraneuronal production of DOPAL and DOPEGAL has considerable potential for a significant role in neurodegenerative processes affecting central and peripheral catecholamine neuronal systems. This potential role, however, remains largely unrecognized, probably due to confusion about several important aspects of catecholamine metabolism. First, it is rarely recognized that the deamination reaction is a two-step process, involving MAO-catalyzed formation of highly reactive aldehyde intermediates, followed by production of more stable and inert acid or alcohol deaminated metabolites. Usually the latter metabolites are considered to be the direct products of deamination. Second, it is rarely appreciated that the bulk of catecholamine metabolism takes place in the same cells where the amines are synthesized. Instead, this is usually thought to mainly occur at sites distant from where the catecholamines are synthesized and released. Finally, because it is rarely understood that most catecholamine deamination occurs secondary to transmitter leaking from storage vesicles, a possible contribution of the vesicular-axoplasmic monoamine exchange process to neurodegeneration remains generally overlooked.

In the vesicular-axoplasmic monoamine exchange process, vesicular monoamine transporters function not only to concentrate monoamines in storage vesicles for release, but also to safeguard neurons against high toxic axoplasmic concentrations of monoamines, and presumably also their deaminated aldehyde metabolites (Fig. 6). Such a function is supported by phylogenetic considerations that vesicular monoamine transporters belong to a family that includes transporter proteins for multidrug resistance in bacteria and tumor cells (Schuldiner et al., 1995). A detoxification and neuroprotective function of vesicular monoamine transporters is further supported by experimental findings that over-expression of these transporters enhances resistance against neurotoxins (Liu et al., 1992, 1994; Kilbourn et al., 1998), whereas under-expression or inhibition potentiates toxicity (Fumagalli et al., 1999; Staal and Sonsalla, 2000). In this way, reduced functional activity of vesicular monoamine transporters has been postulated to contribute to neuronal injury and the development of Parkinson's disease (Liu et al., 1994; Miller et al., 1999).

More broadly, any disturbance in vesicular-axoplasmic monoamine exchange favoring enhanced intraneuronal deamination may contribute to production of neurotoxic catecholaldehydes (Fig. 6). Such disturbances may involve reductions in cytosolic pH or ATP production, which would then interfere with the function of the ATP-dependent vesicular membrane proton pump responsible for maintaining the H^+ electrochemical gradient between cytoplasm and granule matrix, and which provides the driving force for vesicular monoamine transport (Henry et al., 1998). Thus, when energy sources are depleted and intracellular pH is lowered—as in ischemia, anoxia, or cyanide poisoning—there is rapid and massive loss of catecholamines from storage vesicles into the neuronal cytoplasm (Silverstein and Johnston, 1984; Schömig et al., 1987). These same situations also produce injury to catecholamine neuronal systems under a variety of experimental paradigms, including models of cerebral ischemia (Weinberger et al., 1983), developmental hypoxic-ischemic brain injury (Johnston, 1983; Johnston et al., 1984; Silverstein and Johnston, 1984; Burke et al., 1992; Oo et al., 1995), and cyanide poisoning (Kanthasamy et al., 1994).

The corpus striatum is particularly prone to neuronal injury after a hypoxic-ischemic insult (Johnston, 1983). Damage is most acute upon reperfusion and is usually ascribed to oxidative stress (Flaherty and Weisfeldt,

1988; Christensen et al., 1994; Kuroda and Siesjo, 1997). The usually suggested mechanisms involve MAO-induced formation of hydroxyl radicals and autooxidation of the large amounts of catecholamines released from stores during the initial insult (Jewett et al., 1989; Damsma et al., 1990; Simonson et al., 1993; Suzuki et al., 1995; Kunduzova et al., 2002; Bianchi et al., 2003). Although rarely considered, MAO-catalyzed production of toxic catecholaldehydes during reoxygenation could also contribute to the injury. Support for this possibility is provided by findings of large increases in production of deaminated catecholamine metabolites during the reperfusion phase (Gordon et al., 1990; Kumagae et al., 1990; Chahine et al., 1994; Akiyama and Yamazaki, 2001). The extent of injury is predicted by the extent of increase in metabolites (Silverstein and Johnston, 1984). Findings that inhibition of MAO attenuates the hypoxia-ischemia-induced tissue loss of catecholamines (Lamontagne et al., 1991) and minimizes or prevents striatal neuronal necrosis (Matsui and Kumagae, 1991) further support involvement of the catecholamine deamination process in the mechanism of injury.

Although DOPAL and DOPEGAL are highly reactive metabolites, any associated toxicity is normally minimized by rapid conversion of the aldehydes to deaminated acids by aldehyde dehydrogenase or to deaminated alcohols by aldose or aldehyde reductase. The redundant nature of available pathways is consistent with the importance of this step for minimizing toxic effects of the aldehyde metabolites. It may be further surmised that blocking conversion of the aldehyde intermediates to the inactive alcohols or acids would enhance the associated toxicity. This concept is supported by findings that combined inhibition of aldehyde dehydrogenase and aldose or aldehyde reductase in PC12 cells increases production of DOPAL and potentiates the neurotoxic actions of rotenone (Lamensdorf et al., 2000b). Inhibition of MAO blocks this effect, confirming the dependence of the enhanced toxicity on the deamination process.

Although DOPAL and DOPEGAL are toxic and can give rise to a wide variety of toxic products, including isoquinoline condensation products, this does not preclude mechanisms of toxicity involving interactions with other established processes such as oxidative stress or production of free radicals and exogenous toxins. Chronic exposure to the pesticide, rotenone, causes nigrostriatal neurodegeneration in rats, with pathology that resembles that of Parkinson's disease (Betarbet et al., 2002). The toxic mechanism appears to involve metabolic stress, associated with dysfunction of the mitochondrial respiratory chain and inhibition of complex 1. The toxin also increases dopamine turnover, resulting in increased production of deaminated dopamine metabolites, particularly DOPAL (Lamensdorf et al., 2000a; Thiffault et al., 2002). This effect appears to result from disturbance of the vesicular-axoplasmic monoamine exchange process, which leads to increased leakage of dopamine from storage vesicles. Inhibition of complex 1-mediated oxidation of NADH to NAD, and the NAD dependence of aldehyde-dehydrogenase appears to contribute to the accumulation of DOPAL.

The potential involvement of processes regulating the intraneuronal disposition of catecholamines in the development of clinical neurodegenerative processes has received support by findings that a familial form of early-onset Parkinson's disease is due to mutation of the α-synuclein gene (Polymeropoulos et al., 1997; Cabin et al., 2002; Lotharius and Brundin, 2002; Volles and Lansbury, 2002). The α-synuclein protein appears important for maintaining the functional integrity of synaptic vesicles (Cabin et al., 2002). The presence of mutant forms of the α-synuclein protein leads to destabilization and permeabilization of vesicular membranes, loss of vesicular monoamine contents and impaired dopamine storage (Lotharius and Brundin, 2002; Volles and Lansbury, 2002). The resulting disturbance in vesicular-axoplasmic monoamine exchange would be expected to increase intraneuronal deamination and production of neurotoxic catecholaldehydes that could contribute to the pathology in this familial form of Parkinson's disease (Fig. 6).

Generalized disturbances of vesicular monoamine storage or catecholamine metabolism might be expected to produce more wide ranging pathological effects on catecholamine systems than the nigrostriatal degeneration commonly considered to characterize Parkinson's disease. Although the movement disorder of Parkinson's disease is the most prominent clinical feature, other clinical features appear to reflect generalized disturbances of catecholamine systems. In the retina, dopamine is the predominant catecholamine and functions as a neurotransmitter and neuromodulator involved in light-adaptive retinal processes (Denis et al., 1993). Abnormalities in visual processing in Parkinson's disease, including deficient contrast sensitivity and abnormalities in visually evoked potentials, appear linked to deficiencies in retinal dopamine systems (Nightingale et al., 1986; Harnois and Di Paolo, 1990; Price et al., 1992; Djamgoz et al., 1997). Disturbances of digestive function, such as constipation, are common in Parkinson's disease and may be related to defects in gastrointestinal dopamine systems now also identified in the disorder (Edwards et al., 1992; Singaram et al., 1995). Similarly, orthostatic hypotension is also common in patients with Parkinson's disease and is now recognized to reflect sympathetic denervation (Goldstein et al., 2002). Sympathetic denervation in Parkinson's disease involves loss of post-ganglionic noradrenergic but not cholinergic nerves (Sharabi et al., 2003). Loss of sympathetic innervation is most pronounced in the heart and occurs in both familial and sporadic forms of the disease (Taki et al., 2000; Goldstein et al., 2000, 2001).

It is now clear that Parkinson's disease involves more extensive abnormalities of catecholamine systems than

previously recognized. The neurodegenerative process probably involves pathways or mechanisms common to these systems. The high catecholamine contents and particularly high rates of vesicular-axoplasmic exchange of dopamine in central nervous system dopaminergic neurons (Floor et al., 1995) and of norepinephrine in cardiac sympathetic nerves (Eisenhofer et al., 1992a) may predispose these systems to disturbances leading to greater production of toxic catecholaldehydes.

B. Neurocirculatory Physiology and Pathophysiology

Turnover of catecholamines, representing ongoing loss and replenishment by synthesis, is usually considered to be driven by catecholamine release in response to increased nerve impulse activity. This view follows the generally accepted notion that catecholamine systems are by and large "emergency" systems, with little or no tonic activity or roles in body processes under normal resting conditions. The large contribution of leakage of catecholamines from vesicular storage granules to catecholamine metabolism and turnover, although rarely appreciated, has important implications for understanding how catecholamine systems are normally regulated and altered in cardiovascular disease states.

The substantial and irreversible loss of catecholamines by metabolism, following leakage of the amines from storage vesicles into the cytoplasm, might seem inconsistent with cellular economy. In fact, this contribution provides an important mechanism for reducing the requirement for relative increases in catecholamine synthesis to match those in catecholamine release (Eisenhofer et al., 1991c, 1992b, 1998b). Since increases in tyrosine hydroxylase-regulated catecholamine synthesis are limited, this mechanism provides sympathetic nerves with a capacity for a more extended range of sustainable release rates than would otherwise be possible.

The practical advantage of this "gearing down" mechanism may be appreciated by consideration of situations requiring sustained increases in cardiovascular work and sympathetic nerve firing, such as occur with long-distance running. During exercise at 50% of maximum work capacity, norepinephrine release by cardiac sympathetic nerves increases about 10-fold (Fig. 7). This 10-fold increase in release exceeds the maximum 3- to 4-fold increase in norepinephrine synthesis allowed by acute activation of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis. Consequently, if norepinephrine turnover depended exclusively on transmitter release, the inability of norepinephrine synthesis to keep pace with release would rapidly lead to a depletion of transmitter stores, decreased release, and an inability to maintain cardiovascular work. Catecholamine turnover, however, also depends crucially on leakage of transmitter from storage vesicles into the cytoplasm. The large contribution of the leakage process to catecholamine turnover remains constant during increases in release. Thus, during exercise at 50% of maximum work capacity, the 10-fold increase in norepinephrine release by cardiac sympathetic nerves results in only a 3-fold increase in norepinephrine turnover. Transmitter stores can thereby be maintained constant by a relatively modest 3-fold increase in norepinephrine synthesis.

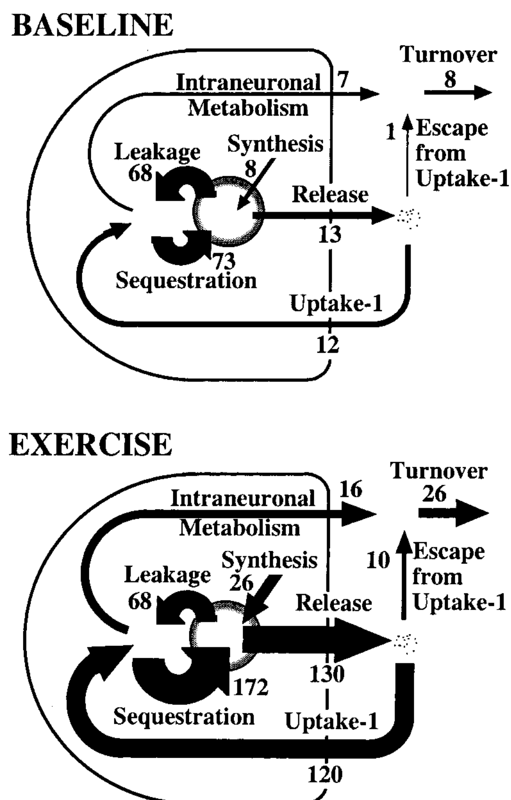

FIG. 7. Schematic diagram showing the determinants of norepinephrine (NE) turnover and synthesis in sympathetic nerves during baseline conditions (upper panel) and during exercise (lower panel). Numbers with each arrow show the relative rates of each process. Norepinephrine turnover in sympathetic nerves is dependent on two inputs: 1) intraneuronal metabolism of norepinephrine leaking from storage vesicles or recaptured after release; and 2) escape from reuptake after release, with subsequent loss by extraneuronal uptake and metabolism or entry into the bloodstream. Due to the large and constant impact on turnover of norepinephrine leakage from vesicular stores, a 10-fold increase (130/13) in norepinephrine release during exercise results in only a 3.2-fold increase (26/8) in norepinephrine turnover. Thus, norepinephrine synthesis need only increase by 3.2-fold to maintain norepinephrine stores constant, despite a much larger 10-fold increase in release.

The above considerations of how synthesis, release, metabolism, turnover, and stores of catecholamines are regulated in a coordinated fashion is strikingly illustrated by functional changes in catecholamine neurons in mutant mice lacking cell membrane norepinephrine or dopamine transporters (Jones et al., 1998; Jaber et al., 1999; Xu et al., 2000). As expected from the roles of these transporters for clearance and recycling of released transmitter, lack of a reuptake mechanism leads to large increases in extracellular concentrations and extraneuronal metabolism of catecholamines, and severely depleted neuronal catecholamine stores. Despite the accelerated loss of catecholamines following exocytotic release, levels of tyrosine hydroxylase are not in-

creased, but are decreased, and synthesis of catecholamines is increased minimally. The severely reduced catecholamine stores and the consequently reduced contribution of vesicular leakage to catecholamine turnover explain the minimal increase in catecholamine synthesis. In effect, the changes represent attainment of a new steady-state, where increased extracellular concentrations, accelerated loss, and demand for increased synthesis of catecholamines are buffered by a reduction in catecholamine stores and resulting decreases in the rates of catecholamine release and neuronal turnover.

A clinical correlate of the above findings in mutant mice has been identified in patients with impaired function of the plasma membrane norepinephrine transporter resulting from a mutation of the gene encoding the transporter (Shannon et al., 2000). Patients with this mutation have excessive increases in heart rate and plasma concentrations of norepinephrine during standing. Another distinctive neurochemical feature is low plasma levels of the intraneuronal norepinephrine metabolite, DHPG. This finding is usually considered to reflect reduced intraneuronal metabolism of recaptured norepinephrine. However, based on the data obtained in mice lacking catecholamine transporters (Jones et al., 1998; Xu et al., 2000), it seems likely that the low plasma levels of DHPG could also reflect decreased vesicular stores of norepinephrine. This would be expected to result in reduced rates of leakage of norepinephrine from vesicular stores and, consequently reduced production of DHPG from this source. Reduced exocytotic release of the transmitter, due to reduced vesicular stores, would also explain why patients with the transporter defect do not have the predicted phenotype of norepinephrine-induced hypertension (Rumantir et al., 2000).

Congestive heart failure represents another condition where impaired neuronal uptake of norepinephrine has been confirmed in experimental models (Liang et al., 1989; Himura et al., 1993; Backs et al., 2001) and clinical studies (Beau and Saffitz, 1994; Bohm et al., 1995; Imamura et al., 1995; Eisenhofer et al., 1996b; Merlet et al., 1999). Impaired transporter function in heart failure is associated with divergent alterations in synthesis, release, metabolism, and sympathoneuronal stores of norepinephrine (Eisenhofer et al., 1996b). The deficiencies of norepinephrine transporter function appear confined to the heart and are relatively mild compared to those associated with the mutations of the transporter gene described above. Another difference is that heart failure is associated with sympathetic nerve activity-dependent increased release of transmitter (Leimbach et al., 1986). The resulting increase in cardiac sympathetic drive is augmented by local impairment of norepinephrine reuptake (Eisenhofer et al., 1996b; Rundqvist et al., 1997).

Similar to findings in mutant mice with deficient transporter function, heart failure also results in marked depletion of cardiac norepinephrine stores (Chidsey and Braunwald, 1966; Pool et al., 1967; DeQuattro et al., 1973; Siltanen et al., 1982; Pierpont et al., 1987; Port et al., 1990). Findings of associated decreases in cardiac tissue levels of tyrosine hydroxylase were initially suggested to contribute to the depletion of norepinephrine stores (Pool et al., 1967; DeQuattro et al., 1973). More recent considerations of the true nature of catecholamine metabolism indicate that, as in mutant mice lacking catecholamine transporters, depletion of norepinephrine stores in heart failure may reflect attainment of a new steady-state and a coordinated adaptive response to chronic alterations in sympathetic neuronal function (Eisenhofer et al., 1996b). According to this view, the depletion of cardiac norepinephrine stores results primarily from increased loss of transmitter associated with increased transmitter release, compounded by impaired transmitter recycling due to reduced efficiency of reuptake and storage. The depleted stores of norepinephrine result in decreased leakage of the transmitter from vesicles and a reduction in DHPG production from this source. This offsets the impact of sympathetic activation on norepinephrine turnover. Thus, the overall increase in cardiac norepinephrine turnover and catecholamine synthesis is relatively small.

C. Catecholamine-Producing Tumors

Correct understanding of catecholamine metabolism is particularly important for the diagnosis of catecholamine-producing tumors, including the choice of appropriate biochemical tests and their implementation and interpretation (Eisenhofer et al., 2001). Nevertheless, texts covering this area invariably provide misleading information about how catecholamines are metabolized. In particular, most metabolism of catecholamines produced by pheochromocytomas is usually considered to occur after the amines are released into the bloodstream. This view is inconsistent with the large body of experimental evidence, discussed above, establishing that most metabolism occurs before catecholamines reach the circulation.

Although intratumoral metabolism of catecholamines is well recognized to occur in neuroblastomas (LaBrosse et al., 1976), such metabolism is not widely appreciated to occur in pheochromocytomas, where signs and symptoms clearly can be attributed to release of catecholamines by the tumors. The possibility that pheochromocytomas contain COMT and produce the *O*-methylated metabolites of catecholamines was first suggested by Sjoerdsma et al. (1957), when these investigators isolated normetanephrine from tumor tissue and established the presence of the enzyme responsible for *O*-methylation. Three years later, Kopin and Axelrod (1960) confirmed that pheochromocytomas contain high concentrations of metanephrine and MHPG. Shortly thereafter, Crout et al. (Crout et al., 1961; Crout and Sjoerdsma, 1964) concluded that in many patients with

pheochromocytoma, most of the increased urinary levels of catecholamine metabolites result from metabolism directly within tumors, before catecholamines ever reach the circulation.

Over the intervening decades the significance of the above early studies to the utility of available tests for diagnosis of pheochromocytoma seems to have been largely forgotten. More recently, however, the concepts established by Sjoerdsma, Crout, Kopin, and others in the 1950s and 60s have been revived by development of improved methods for measuring catecholamine metabolites, particularly plasma concentrations of free (unconjugated) normetanephrine and metanephrine (Lenders et al., 1993). Preliminary application of these measurements for diagnosis of pheochromocytoma by Lenders et al. in 1995 (Lenders et al., 1995) yielded encouraging results with elevated plasma levels of free normetanephrine, metanephrine, or both in every one of 52 patients with pheochromocytoma (i.e., 100% diagnostic sensitivity). The extraordinarily high diagnostic sensitivity of these measurements was explained by the usually low rates of extraneuronal catecholamine *O*-methylation and the relatively large contribution of adrenal medullary cells to the normally low plasma levels of free metanephrines (Eisenhofer et al., 1995b,c). The diagnostic signal produced by catecholamine metabolism within pheochromocytoma tumor cells is therefore most effectively amplified by intratumoral formation of free metanephrines.

The large contribution of intratumoral catecholamine metabolism to elevated levels of plasma free metanephrines in patients with pheochromocytoma was later confirmed in a study showing that the tumors contain unusually high concentrations of membrane-bound COMT, the enzyme isoform most important for *O*-methylation of catecholamines (Eisenhofer et al., 1998a). Concentrations of free metanephrines in tumor tissue were over 10,000 times higher than the elevated levels in plasma. Also, increases in plasma free metanephrines during intravenous infusions of catecholamines were less than 8% of the increases in the parent amines. This indicated that most of the increases in plasma metanephrines in patients with pheochromocytoma could not possibly result from metabolism of catecholamines after release by tumors. Intratumoral metabolism was thus calculated to account for over 94% of the elevated plasma levels of free metanephrines in patients with pheochromocytoma. Findings from vena caval blood sampling studies of particularly high plasma concentrations of free metanephrines in the venous effluent of pheochromocytomas further support the considerable production of metanephrines within tumor tissue (DeQuattro et al., 1980; Pacak et al., 2001).

Presumably production of metanephrines within pheochromocytoma tumor cells occurs by a process similar to that for production of DHPG in sympathetic nerves, where vesicular stores of catecholamines exist in a highly dynamic equilibrium with the surrounding cytoplasm, with passive outward leakage counterbalanced by inward active transport by vesicular monoamine transporters. According to this concept, production of metanephrines within pheochromocytoma tumor cells and normal adrenal medullary cells occurs continuously and independently of fluctuations in catecholamine release. This conclusion is supported by findings that plasma concentrations of free metanephrines show negligible or relatively small increases compared with the parent amines during paroxysmal attacks or tumor manipulation (Eisenhofer et al., 1998a; Raber et al., 2000). Similarly, sympathoadrenal activation in disease-free individuals produces relatively small increases in plasma free metanephrines compared with the parent amines (Eisenhofer et al., 1995b, 1998a).

Another consequence of the dependence of metanephrine production on leakage of catecholamines from vesicular stores is that tumor size is strongly positively correlated with increases in plasma concentrations of metanephrines (Lenders et al., 1995; Eisenhofer et al., 1999b). In contrast, because catecholamine secretion is highly variable, increases in plasma or urinary catecholamines are poorly correlated and cannot be used to predict tumor size.

In addition to COMT, pheochromocytoma tumor cells contain MAO and therefore also produce deaminated catecholamine metabolites, such as DHPG and MHPG. However, the substantial production of DHPG from norepinephrine in sympathetic nerves obscures the relatively small increases in DHPG due to the tumor, making these insensitive markers of pheochromocytoma (Brown, 1984; Duncan et al., 1988; Lenders et al., 1992). Substantial production of VMA from hepatic uptake and metabolism of circulating DHPG and MHPG also explains the poorer utility of measurements of urinary VMA compared with the metanephrines for diagnosis of pheochromocytoma (Peaston and Lai, 1993; Gerlo and Sevens, 1994; Mannelli et al., 1999; Lenders et al., 2002).

Urinary metanephrines are usually measured after an acid-hydrolysis step that liberates the free from the sulfate-conjugated metabolites, the latter the main form excreted in urine. Although the sulfate-conjugated metabolites are formed from free metanephrines, it is likely that conjugation occurs in tissues with high expression of the SULT1A3 sulfotransferase isoenzyme responsible for this step. The gastrointestinal tract is particularly rich in SULT1A3. Sulfate conjugation in the gastrointestinal tract is influenced by diet and local catecholamine production. This may provide an explanation for findings that diagnosis of pheochromocytoma using measurements of urinary fractionated metanephrines is hindered by high rates of false-positive results (Lenders et al., 2002).

Measurement of plasma free metanephrines for diagnosis of pheochromocytoma therefore offers advantages over other tests, including measurements of urinary

metanephrines. Plasma free metanephrines are particularly useful for detection of pheochromocytomas in patients with normal plasma or urinary catecholamines, where tumors may be either nonsecretory or secrete catecholamines episodically. Findings from three independent centers have now established that plasma free metanephrines provide a more sensitive test for detecting pheochromocytoma than measurements of the parent amines or other metabolites (Raber et al., 2000; Lenders et al., 2002; Sawka et al., 2003).

These findings and clarification of the correct pathways of catecholamine metabolism are leading to a more efficient and cost-effective approach for diagnosis of pheochromocytoma. In particular, the high diagnostic sensitivity of measurements of plasma free metanephrines means that a negative test result virtually excludes the tumor, so that no immediate further biochemical testing is necessary. In about 80% of patients with pheochromocytoma, increases in plasma free metanephrines are high enough to confirm the tumor unequivocally. The immediate task in these patients is to locate the tumor. In other patients with mildly elevated plasma levels of free metanephrines new approaches are now available that effectively distinguish true-positive from false-positive results (Eisenhofer et al., 2003).

IV. Future Perspectives

Much of the early work on catecholamines and catecholamine systems was directed at understanding the disposition and metabolism of catecholamines. In most textbooks and reviews on the subject, the metabolic schemes depicting routes of catecholamine metabolism have been based on these early studies. Subsequent studies led to a more refined and correct understanding of the disposition and metabolism of catecholamines. Much of this understanding and the concepts arising from this understanding are not new. Nevertheless, they have yet to be considered in modern texts about catecholamines, catecholamine systems, or diseases featuring catecholamines. As a result, the correct pathways of catecholamine metabolism and associated concepts about catecholamine systems are not as widely appreciated as they should be.

As outlined here, a correct understanding of catecholamine metabolism can be useful for facilitating a clearer appreciation of the function and dysfunction of catecholamine systems in health and disease. This includes how components of catecholamine systems are normally regulated in a coordinated fashion, how changes in catecholamine metabolism can be used to interpret physiological processes and pathological states, and how disturbances in the disposition and metabolism of catecholamines may contribute to disease processes. New and improved diagnostic and therapeutic approaches are then likely to follow.

Development of new and improved methods for diagnosis of catecholamine-producing tumors is one advance that has already followed clarification of the correct pathways of catecholamine metabolism, particularly the large contribution of the vesicular-cytoplasmic monoamine exchange process and the resulting intratumoral formation of *O*-methylated catecholamine metabolites. Further dissemination of the correct understanding of pathways of catecholamine metabolism can be expected to facilitate continuing advances in understanding and treating diseases featuring disturbances of catecholamines systems, including Parkinson's disease, depression, heart failure, hypertension, and other neurocirculatory disorders.

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