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Neurotoxicity and Metabolism of the Catecholamine-Derived 3,4-Dihydroxyphenylacetaldehyde and 3,4-Dihydroxyphenylglycolaldehyde: The Role of Aldehyde Dehydrogenase

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***Abstract*——Aldehydes are highly reactive molecules formed during the biotransformation of numerous endogenous and exogenous compounds, including biogenic amines. 3,4-Dihydroxyphenylacetaldehyde is the aldehyde metabolite of dopamine, and 3,4-dihydroxyphenylglycolaldehyde is the aldehyde metabolite of both norepinephrine and epinephrine. There is an increasing body of evidence suggesting that these compounds are neurotoxic, and it has been recently hypothesized that neurodegenerative disorders may be associated with increased levels of these biogenic aldehydes. Aldehyde dehydrogenases are a group of NAD(P)$^+$-dependent enzymes that catalyze the oxidation of aldehydes, such as those derived from catecholamines, to their corresponding carboxylic acids. To date, 19 aldehyde dehydrogenase genes have been identified in the human genome. Mutations in these genes and subsequent inborn errors in aldehyde metabolism are the molecular basis of several diseases, including Sjögren-Larsson syndrome, type II hyperprolinemia, γ-hydroxybutyric aciduria, and pyridoxine-dependent seizures, most of which are characterized by neurological abnormalities. Several pharmaceutical agents and environmental toxins are also known to disrupt or inhibit aldehyde dehydrogenase function. It is, therefore, possible to speculate that reduced detoxification of 3,4-dihydroxyphenylacetaldehyde and 3,4-dihydroxyphenylglycolaldehyde from impaired or deficient aldehyde dehydrogenase function may be a contributing factor in the suggested neurotoxicity of these compounds. This article presents a comprehensive review of what is currently known of both the neurotoxicity and respective metabolism pathways of 3,4-dihydroxyphenylacetaldehyde and 3,4-dihydroxyphenylglycolaldehyde with an emphasis on the role that aldehyde dehydrogenase enzymes play in the detoxification of these two aldehydes.**

I. Introduction

Aldehyde species are generated during numerous physiological processes from a wide variety of endogenous and exogenous precursors. They are known to be highly reactive and cytotoxic and are involved in processes such as enzyme inactivation, protein modification, and DNA damage (Lindahl, 1992; O'Brien et al., 2005). In the central nervous system (CNS[1]), the catecholamines, dopamine, norepinephrine, and epinephrine, are intraneuronally metabolized to their respective aldehyde metabolite by monoamine oxidase (MAO). Dopamine is deaminated to 3,4-dihydroxyphenylacetaldehyde (DOPAL), and both norepinephrine and epinephrine are deaminated to form 3,4-dihydroxyphenylglycolaldehyde (DOPEGAL). Increasing evidence suggests that these catecholamine-derived aldehydes may in fact be neurotoxins, and their intraneuronal accumulation has been theorized as one mechanism that may be involved in cell death associated with neurodegenerative conditions, including Parkinson's disease (PD) and Alzheimer's disease (AD) (Mattammal et al., 1995; Burke et al., 2003). Aldehydes, including DOPAL and DOPEGAL, are detoxified by various enzyme systems including aldehyde dehydrogenase (ALDH), which is exclusively responsible for their oxidative metabolism (Fig. 1). This article presents a comprehensive review of the role of DOPAL and DOPEGAL in the CNS. First, a general overview of the biological significance and reactivity of aldehydic compounds is given, followed by an introduction to biogenic aldehydes. Second, the intraneuronal formation of DOPAL and DOPEGAL, their possible transport systems, and their identification and quantification in biological samples will be reviewed in detail. Third, the neurotoxicity of DOPAL and DOPEGAL will be examined (including possible mechanisms of toxicity), including their potential role in cell death and neurodegeneration. Finally, the metabolism of DOPAL and DOPEGAL by various enzyme systems will be comprehensively examined with an emphasis on the role of the human ALDH isozymes and the impact of ALDH dysfunction.

[1] Abbreviations: CNS, central nervous system; MAO, monoamine oxidase; DOPAL, 3,4-dihydroxyphenylacetaldehyde; DOPEGAL, 3,4-dihydroxyphenylglycolaldehyde; PD, Parkinson's disease; AD, Alzheimer's disease; ALDH, aldehyde dehydrogenase; 4-HNE, 4-hydroxy-2-nonenal; MDA, malondialdehyde; GSH, glutathione; SN, substantia nigra; VTA, ventral tegmental area; LC, locus ceruleus; RVLM, rostral ventral lateral medulla; DAT, dopamine transporter; MPP$^+$, 1-methyl-4-phenylpyridine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; THP, tetrahydropapaveroline; L-dopa, L-3,4-dihydroxyphenylalanine; MHPG, 3-methoxy-4-hydroxyphenylglycol; HVA, homovanillic acid; LDH, lactic acid dehydrogenase; TH, tyrosine hydroxylase; ROS, reactive oxygen species; H_2O_2, hydrogen peroxide; PT, permeability transition; SNpc, substantia nigra pars compacta; AR, aldose reductase; ADH, alcohol dehydrogenase; ALR, aldehyde reductase; COMT, catechol-*O*-methyltransferase; PST, phenolsulfotransferase; UGT, UDP-glucuronosyltransferase; DOPAC, 3,4-dihydroxyphenylacetic acid; DOPET, 3,4-dihydroxyphenylethanol; DOPEG, 3,4-dihydroxyphenylglycol; DOMA, 3,4-dihydroxymandelic acid; VMA, vanillylmandelic acid; MOPEGAL, 3-methoxy-4-hydroxyphenylglycolaldehyde; AASA, α-aminoadipic semialdehyde; AKR, aldo-keto reductase.

II. Aldehydes

A. Biological Significance and Reactivity

Aldehydes are organic compounds containing terminal carbonyl groups. They can be divided into four general carbonyl classes: 1) saturated alkanals, such as formaldehyde, acetaldehyde, and hexanal; 2) unsaturated alkenals, such as acrolein, 4-hydroxy-2-nonenal (4-HNE), and crotonaldehyde; 3) aromatic aldehydes, such as benzaldehyde, DOPAL and DOPEGAL; and 4) dicarbonyls such as glyoxal and malondialdehyde (MDA).

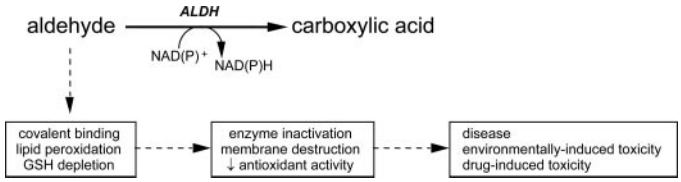

FIG. 1. Consequences of aldehyde toxicity and the general detoxification reaction catalyzed by ALDH.

These compounds are widespread in nature and ubiquitous in the environment. Various aldehydes, including formaldehyde, acetaldehyde, and acrolein, are produced during combustion and are present in smog and cigarette smoke (Rickert et al., 1980; Destaillats et al., 2002). Motor vehicle exhaust represents a major source of environmental aldehydes in air both through direct emission of aldehydes and through the emission of hydrocarbons, which can give rise to aldehydes. Cigarette smoke is also an important source of aldehydes; interestingly, second-hand smoke can contain significantly higher levels of aldehydes than first-hand smoke. Aldehydes are also used or generated in a wide variety of industrial applications (O'Brien et al., 2005). Formaldehyde is used in the production of resins, polyurethane, and polyester plastics and as a fumigant and a preservative in animal feed. Acetaldehyde also has many industrial uses including use in alkyd resin production. Drugs and environmental agents are also important aldehyde precursors. The hepatotoxins allyl alcohol and ethanol are directly metabolized to their corresponding aldehydes, acrolein, and acetaldehyde, respectively. Moreover, acrolein, ethanol, and other agents, such as carbon tetrachloride, also can induce the formation of lipid peroxidation-derived aldehydes (O'Brien et al., 2005). Many drugs, including the anticancer drugs cyclophosphamide and ifosfamide, are also metabolized to aldehyde intermediates (Maki and Sladek, 1993).

A range of aliphatic and aromatic dietary aldehydes, including citral, benzaldehyde, acetaldehyde, and formaldehyde, exist naturally in various foods, particularly in fruits and vegetables, to which they impart flavor and odor (Lindahl, 1992). Cooking fumes also contain a variety of aldehydes. Similarly, aldehydes including hexenal and cinnamaldehyde are approved by the U.S. Food and Drug Administration for use as flavoring additives and spices. In animals, aldehydes, including acrolein, benzaldehyde, and hexanal, act as communication molecules, having roles in attraction or defense (Schauenstein et al., 1977). Likewise, plant species produce aldehydes, including various hexenals, as part of a natural pesticide system against animals and insects. Interestingly, some insects have evolved to feed on these toxic plants and, therefore, can exploit plant-derived aldehydes for their own use in the chemical defense against predators (Williams et al., 2001).

Aldehydes are also generated as physiologically derived intermediates during the biotransformation of many endogenous compounds, including lipids, amino acids, neurotransmitters, and carbohydrates. For example, more than 200 aldehyde species arise from the oxidative degradation of cellular membrane lipids (lipid peroxidation), including 4-HNE and MDA (Esterbauer, 1993). Amino acid catabolism generates several aldehyde intermediates including glutamate γ-semialdehyde, produced during proline and arginine metabolism, and malonate semialdehyde, produced during valine catabolism (Vasiliou et al., 2004). Neurotransmitters, such as γ-aminobutyric acid (GABA), serotonin, norepinephrine, epinephrine, and dopamine, also give rise to aldehyde metabolites (Duncan and Sourkes, 1974; Gibson et al., 1998). Carbohydrate metabolism and ascorbate auto-oxidation generate glycolaldehyde and the dicarbonyl, glyoxal (O'Brien et al., 2005).

Aldehydes play vital roles in normal physiological and therapeutic processes. For example, the aldehyde retinal is essential for vision and its ALDH-dependent oxidation product, retinoic acid, is critical for embryonic development (Siegenthaler et al., 1990). Betaine, the ALDH-oxidation product of betaine aldehyde, is an osmolyte and methyl donor that has been shown to protect cells and organs from osmotic stress-induced toxicity (Horio et al., 2001). Other critical processes involving aldehydes include the biosynthesis of neurotransmitters. The inhibitory neurotransmitter GABA can be formed through the ALDH-dependent oxidation of its aldehyde precursor, γ-aminobutyraldehyde (Ambroziak and Pietruszko, 1987). In addition, the excitatory neurotransmitter, glutamate, is formed by the ALDH-induced oxidation of glutamate γ-semialdehyde. In terms of a therapeutic role, aldehyde intermediates can mediate the efficacy of certain drugs. The antineoplastic agent cyclophosphamide, through its aldehyde intermediate aldophosphamide, gives rise to phosphoramide mustard and acrolein, which are responsible for its tumor-cell killing effects (Sladek et al., 1989).

Although some aldehydes are essential for normal biological processes, many are cytotoxic and even carcinogenic (Yokoyama et al., 1996; Feng et al., 2004) (Fig. 1). Aldehydes are strong electrophilic compounds with terminal carbonyl groups, making them highly reactive. In fact, the aldehyde group is the most reactive among the functional groups of biomolecules. In addition to the electrophilic carbonyl carbon, α and β-unsaturated aldehydes, considered bifunctional aldehydes, such as 4-HNE and acrolein, contain a second electrophile at the β-carbon. Furthermore, 4-hydroxylalkenel aldehydes, including 4-HNE, contain a hydroxyl group that can also participate in reactions. Unlike free radicals, aldehydes are relatively long-lived and, therefore, they not only react with targets in the same vicinity of their formation but also can diffuse or be transported to reach sites that are some distance away (Esterbauer et al., 1991).

Because of their electrophilic nature, aldehydes form adducts with various cellular nucleophiles, resulting in impaired cellular homeostasis, dramatically reduced enzyme activity, and even DNA damage (Sayre et al., 2001; Schaur, 2003). Aldehydes readily form adducts with glutathione (GSH) (Esterbauer et al., 1975), nucleic acids (Basu et al., 1988), and protein amino acids (Nadkarni and Sayre, 1995). Furthermore, the ability of aldehydes

to cross-link proteins and DNA and to even form DNA-protein cross-links (through various adduction mechanisms) has been reported (Nair et al., 1986; Brooks and Theruvathu, 2005). Aldehyde-protein adducts typically involve sulfhydryl groups of cysteine residues and amino groups of lysine residues but can also involve other amino acid side chains including histidine and arginine (Sayre et al., 2001). Aldehydes adduct proteins by various mechanisms including Michael addition-type reactions and Schiff base-type condensation reactions. Aldehyde-nucleic acid adducts primarily involve amino groups of both purines and pyrimidines (Brooks and Theruvathu, 2005). Aldehydes have also been shown to be involved in the adduction of coenzymes, leading to their inactivation and depletion by mechanisms such as Knoevenagel condensation (Farrant et al., 2001). Accordingly, the adduction of aldehydes with various cellular components is believed to be the primary mechanism underlying their toxicity. Subsequent biological effects of aldehyde adduction can be cytotoxic, mutagenic, and even carcinogenic (Krokan et al., 1985; Esterbauer et al., 1991), involving the rapid depletion of GSH and protein thiols and the inactivation of enzymes (Chio and Tappel, 1969) with subsequent alterations in signal transduction pathways (Leonarduzzi et al., 2004), gene expression (Kumagai et al., 2000) and DNA repair (Feng et al., 2004).

Although many enzyme systems exist to detoxify aldehydes, perturbations in aldehyde metabolism do occur and contribute to a variety of disease states. Indeed, the accumulation of aldehydes from inborn errors of aldehyde metabolism has been associated with many pathological conditions (Vasiliou and Pappa, 2000). For example, the impaired metabolism of various endogenous aldehydes is causally associated with many diseases, including Sjögren-Larsson syndrome (Rizzo and Carney, 2005), type II hyperprolinemia (Valle et al., 1976), γ-hydroxybutyric aciduria (Pearl et al., 2003), pyridoxine-dependent seizures (Mills et al., 2006), and hyperammonemia and hypoprolinemia (Baumgartner et al., 2000). In addition, lipid-derived aldehydes, such as 4-HNE, acrolein, and MDA, have been implicated in alcohol-related diseases, including alcoholic liver disease, fibrosis, and atherosclerosis (Poli, 2000; Sun et al., 2001), and neurological diseases, such as PD and AD (Yoritaka et al., 1996; Lovell et al., 2001). Similarly, impaired metabolism of the ethanol metabolite acetaldehyde has been implicated in many alcohol-related diseases, including cirrhosis (Enomoto et al., 1991; Chao et al., 1994) and numerous head and neck cancers (Muto et al., 2000; Yokoyama et al., 2001), and late onset AD (Kamino et al., 2000).

B. Biogenic Aldehydes

Oxidative deamination of various biogenic amines (including indoleamines and catecholamines) results in the formation of "biogenic aldehydes." The indoleamines serotonin and tryptamine generate the biogenic aldehydes 5-hydroxyindole-3-acetaldehyde and indole-3-acetaldehyde, respectively. The catecholamines, dopamine, norepinephrine, and epinephrine, also give rise to biogenic aldehydes upon deamination (Fig. 2). Dopamine generates DOPAL, whereas both norepinephrine and epinephrine are deaminated to form DOPEGAL. Despite Blaschko's hypothesis in the early 1950s that indoleamine- and catecholamine-derived aldehydes may be toxic (Blaschko, 1952), biogenic aldehydes were originally believed to be innocuous intermediates in biogenic amine metabolism. However, early studies investigating their possible role in the pharmacological actions of ethanol (levels of biogenic aldehydes may increase during ethanol metabolism) and their ability to form isoquinoline-derived condensation products with their parent amine (Deitrich and Erwin, 1975; Tipton et al., 1977) led to the discovery that biogenic aldehydes actually have distinct physiological properties of their own. In fact, the indoleamine-derived aldehydes 5-hydroxyindole-3-acetaldehyde and indole-3-acetaldehyde have been shown to illicit many biological effects including neurotransmitter-like actions in the CNS (Sabelli et al., 1969; Palmer et al., 1986) and the inhibition of various enzymes (Tabakoff, 1974; Erwin et al., 1975).

As mentioned, DOPAL and DOPEGAL represent catecholamine-derived biogenic aldehydes. The major catecholamine neurotransmitters found in the human brain are dopamine, norepinephrine, and epinephrine. Dopamine is synthesized by neurons in the substantia nigra (SN), ventral tegmental area (VTA) and hypothalamus, whereas synthesis of norepinephrine and epinephrine takes place primarily in neurons of the locus ceruleus (LC) and rostral ventral lateral medulla (RVLM), respectively (Nestler et al., 2001). Many important functions of the brain including memory, learning, movement, and behavior are thought to be mediated by catecholamines. Accordingly, the loss of specific populations of catecholaminergic neurons and subsequent deficits in related brain function are the basis of various neurodegenerative pathological conditions, including PD and AD. The specific vulnerability and death of these neurons have been hypothesized to involve toxic catecholamine metabolites or compounds that are selectively produced by, or accumulated in, catecholamine neurons such as DOPAL and DOPEGAL (Li et al., 2001; Burke et al., 2004). Indeed, there is an increasing body of evidence demonstrating the neurotoxic properties of the catecholamine-derived aldehydes DOPAL and DOPEGAL by various cytotoxic mechanisms including the generation of free radicals and initiation of apoptosis (Burke et al., 1998; Li et al., 2001). Along those lines, it has been suggested that DOPAL and DOPEGAL represent endogenous neurotoxins that may play a significant role in cell death associated with neurodegenerative diseases (Kristal et al., 2001; Eisenhofer et al., 2004).

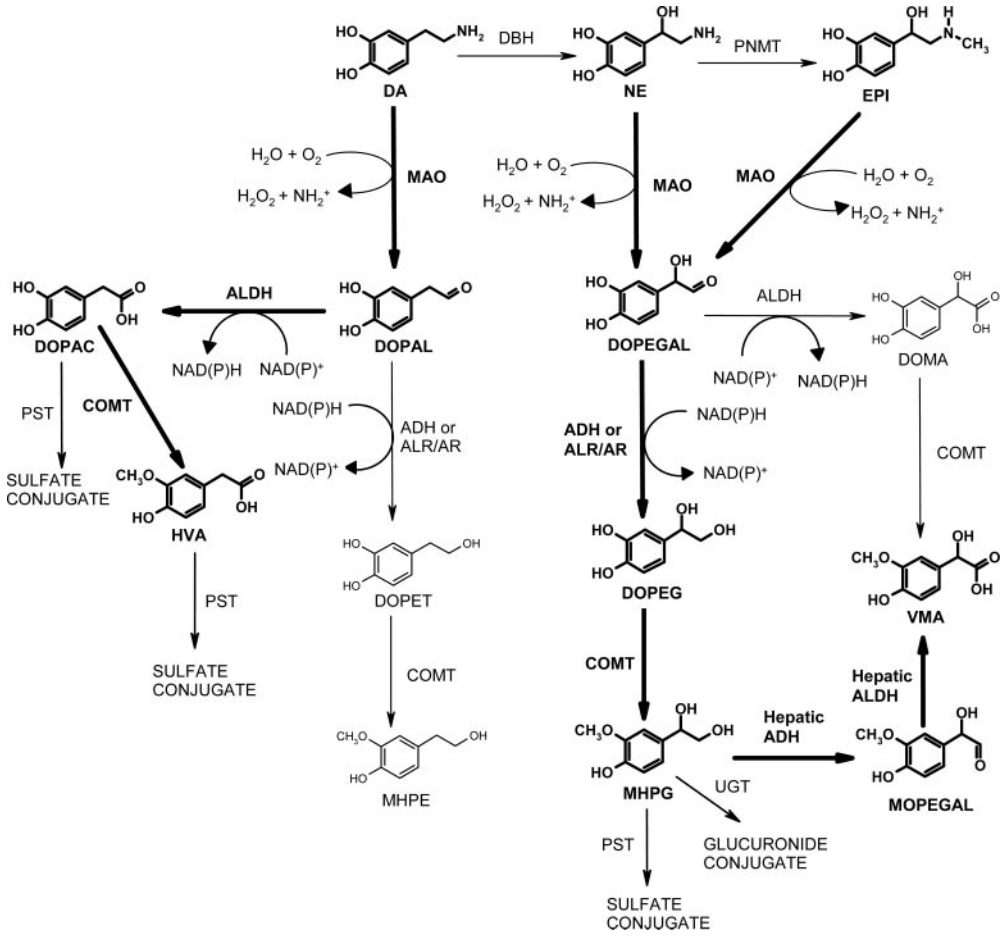

FIG. 2. Formation and metabolism of DOPAL and DOPEGAL. DOPAL is formed from dopamine, whereas DOPEGAL is derived from both norepinephrine and epinephrine. They are metabolized by various enzymes including ALDH, ADH, and ALR/AR. DOPAL is predominantly metabolized by ALDH in the oxidative pathway, whereas DOPEGAL is predominantly metabolized by ADH or ALR/AR in the reductive pathway. Predominant pathways of DOPAL and DOPEGAL metabolism pathways are shown in **bold.** Free HVA is the major brain metabolite of dopamine. Free MHPG is the major brain metabolite of both norepinephrine and epinephrine and is also the major precursor to VMA formation in the liver. DA, dopamine; NE, norepinephrine; EPI, epinephrine; DBH, dopamine-β-hydroxylase; MHPE, 3-methoxy-4-hydroxyphenylethanol.

III. 3,4-Dihydroxyphenylacetaldehyde and 3,4-Dihydroxyphenylglycolaldehyde in the Central Nervous System

A. Intraneuronal Formation

Oxidative deamination of catecholamines to form aldehyde metabolites was first described in the mid-1930s (Richter, 1937). Dopamine is deaminated to form DOPAL, whereas norepinephrine and epinephrine are both deaminated to form DOPEGAL (Fig. 2). A common misconception is that catecholamines are metabolized at sites distant from those of their synthesis and release (for a review of common fallacies about catecholamine metabolism, see Eisenhofer et al., 2004). Rather, most catecholamine metabolism has been shown to take place in the same cells in which they are produced and without prior release (Kopin, 1964; Maas et al., 1970). Accordingly, the formation of DOPAL and DOPEGAL is believed to occur primarily in the cytoplasm of the neurons that synthesize their parent catecholamines (Eisenhofer et al., 1992). This process is thought to occur primarily after the passive leakage of catecholamines into the cytoplasm from storage vesicles or, as a minor pathway, following their reuptake into the nerve terminal (Kopin, 1964; Eisenhofer et al., 1992). Monoamine transporters are responsible for sequestering catecholamines into storage vesicles within neurons, but it is estimated that ≈10% of catecholamines escape into the neuronal cytoplasm and are subsequently metabolized (Eisenhofer et al., 1992). Indeed, 70 to 75% of norepinephrine turnover seems to occur from intraneuronal metabolism of norepinephrine leaking from storage vesicles, with the remainder made up by intraneuronal metabolism after reuptake, extraneuronal uptake and metabolism or loss of norepinephrine to the circulation (Eisenhofer et al., 1996b; Eisenhofer et al., 1998). Numerous in vitro and in vivo studies in animals and humans have demonstrated that leakage of catecholamines from storage vesicles is the primary pathway leading to catecholamine catabolism (Goldstein et al., 1988; Halbrugge et al., 1989; Tyce et al., 1995). The drug reserpine, which blocks the sequestration of catecholamines into storage vesicles, has been shown to cause depletion of catecholamine stores

due to the rapid metabolism of unsequestered catecholamines (Kopin and Gordon, 1962). Studies with rat brain synaptic vesicles have shown dopamine turnover to be more rapid than that of norepinephrine (Floor et al., 1995), suggesting that leakage of dopamine from storage vesicles into the neuronal cytoplasm may be even more significant than it is for norepinephrine.

Intraneuronal formation of DOPAL and DOPEGAL is catalyzed by the actions of MAO (Fig. 2). Whereas other enzymes are involved in the degradation of catecholamines, MAO metabolism seems to be the principal intraneuronal pathway (Rivett et al., 1982). MAO is a flavin-containing, particle-bound enzyme localized primarily in the outer mitochondrial membrane (Schnaitman et al., 1967). In the brain, the enzyme is almost exclusively localized in nerve terminals (Westlund et al., 1985, 1993) where it catalyzes the oxidative deamination of dopamine, norepinephrine, and epinephrine to their respective aldehydes. The MAO reaction first involves the formation of an imine, which subsequently undergoes a nonenzymatic conversion to the corresponding aldehyde. MAO exists as two distinct genetic isoforms, MAO-A and MAO-B, both of which are catalytically active with catecholamines (Youdim et al., 2006). However, studies using preferential inhibitors of MAO-A and MAO-B have indicated that the production of DOPAL and DOPEGAL from dopamine, norepinephrine, and epinephrine can be attributed primarily to MAO-A (Waldmeier et al., 1976; Fowler and Benedetti, 1983; Fornai et al., 2000). The CNS distribution of the two MAO isozymes is consistent with this contention in that catecholaminergic neurons in the LC, RVLM, and SN primarily contain MAO-A whereas MAO-B is localized within serotonergic neurons in the dorsal raphe nucleus and superior central nucleus (Westlund et al., 1985).

B. Transport Mechanisms

After intraneuronal formation, DOPAL and DOPEGAL may leave nerve terminals by simple diffusion and reenter cells through various transport processes. DOPAL is taken up by rat neostriatal synaptosomes, and studies using mazindol, a selective dopamine uptake inhibitor, have suggested that the aldehyde reenters dopaminergic nerve terminals via the dopamine transporter (DAT) (Mattammal et al., 1995). The DAT is expressed in presynaptic terminals of SN neurons where it primarily mediates the reuptake of neuronally released dopamine (Reith et al., 1997; Jones et al., 1998). The DAT is also responsible for the uptake of 1-methyl-4-phenylpyridine (MPP^+), the active metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an exogenous dopaminergic neurotoxin that induces parkinsonian symptoms in affected individuals and is used as a model of PD in animal studies (Javitch et al., 1985). DOPEGAL is taken up by catecholaminergic PC-12 cells and studies using desipramine, which blocks the neuronal catecholamine uptake transporter, have suggested that DOPEGAL is actively transported by this mechanism (Burke et al., 2001).

C. Identification and Quantification in Biological Samples

The presence and activity of biogenic aldehydes in biological tissues was first supported by the finding that tetrahydropapaveroline (THP), the tetrahydroisoquinoline alkaloid condensation product of a Pictet-Spengler condensation reaction between dopamine and DOPAL (Fig. 3), was present in the urine of L-3,4-dihydroxyphenylalanine (L-dopa)-treated PD patients (Sandler et al., 1973). Since then, other studies have confirmed the presence of both DOPAL and DOPEGAL in various tissues, including human brain. In normal human tissues, these aldehydes have been quantitated using chemically synthesized and purified standards (Burke et al., 1999a). In this study, DOPAL and DOPEGAL were separated from 12 catecholamines (and other metabolites), and their levels were analyzed in normal plasma, urine, and postmortem human brain regions. The concentration of DOPEGAL in normal postmortem LC was estimated to be 1.4 μM, a level $\approx$50% of that of 3-methoxy-4-hydroxyphenylglycol (MHPG), a major metabolite of norepinephrine and epinephrine (Burke et al., 1999a, 2004). Normal postmortem human brain SN levels of DOPAL were estimated to be 2.3 μM, a level $\approx$25% higher than that of homovanillic acid (HVA), a major dopamine metabolite. Nominal levels were seen in urine for both DOPAL and DOPEGAL. Plasma levels of DOPAL were minor, whereas those of DOPEGAL were similar to that of epinephrine. Other studies have examined the presence of DOPAL and DOPEGAL in animal tissues. DOPAL has been identified in vivo in rat striatum using *trans*-striatal microdialysis in freely moving rats (Colzi et al., 1996), and DOPEGAL has been detected in pulverized rat adrenal glands (Burke et al., 1995). These studies demonstrate physiological production of DOPAL and DOPEGAL at levels approaching or exceeding those observed for more well-established catecholamine metabolites.

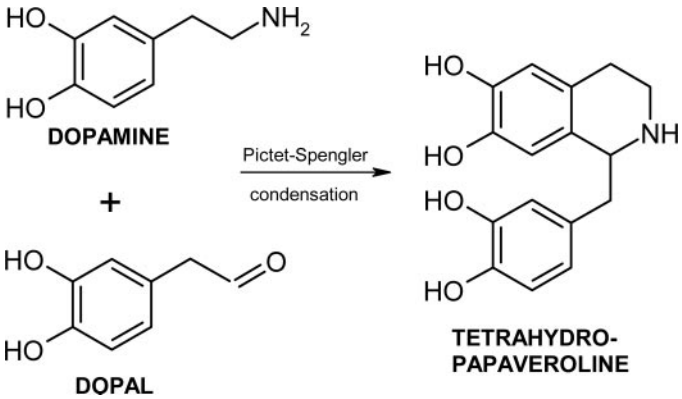

FIG. 3. Formation of tetrahydropapaveroline (THP), the Pictet-Spengler condensation product of DOPAL and dopamine.

IV. Toxicity of 3,4-Dihydroxyphenylacetaldehyde and 3,4-Dihydroxyphenylglycolaldehyde in the Central Nervous System

Although their toxicity was predicted in the 1950s, researchers initially believed that aldehyde metabolites derived from biogenic amines were innocuous intermediates (Renson et al., 1964). Since then, these aldehydes have been shown to be active compounds with physiological properties distinct from those of their parent amines (Palmer et al., 1986). The body of research reporting on investigations of the biogenic aldehydes DOPAL and DOPEGAL that began in the 1950s is not as large as one would expect considering the many molecular and genomic advances that have occurred in the ensuing period. This is due, in part, to the unstable and reactive properties of these catecholamine-derived compounds, making them difficult to synthesize (either chemically or enzymatically) in pure form and/or in large enough quantities to be useful for experimental purposes. Recent advances, however, in the chemical synthesis of DOPAL and DOPEGAL (Narayanan et al., 2003) and the growing number of studies indicating that they may be important neurotoxins will no doubt lead to increased interest in and focus on these compounds in future investigations. Nonetheless, a substantial and convincing body of relatively recent evidence does exist, which indicates the toxicity and reactivity of these compounds in the CNS. On the basis of these studies, a role for DOPAL and DOPEGAL in neurodegenerative diseases has been proposed (Mattammal et al., 1995; Lamensdorf et al., 2000b; Burke et al., 2004).

A. Cytotoxicity

Concentrations of DOPAL and DOPEGAL >6 μM induce dose-dependent toxicity in various cell lines, including differentiated PC-12 cells (Mattammal et al., 1995; Burke et al., 1996; Kristal et al., 2001). Levels of the aldehydes identified as being toxic in vitro have been reported to be close to physiological levels found in normal human postmortem brain (Burke et al., 1999a; Kristal et al., 2001). For example, DOPAL at 6.6 μM, a concentration close to the physiological levels reported in normal human autopsy specimens of the SN (Burke et al., 1999a), induced significant cytotoxicity in PC-12 cells and reduced viability by ≈30% (Kristal et al., 2001). DOPAL at 66 μM caused cell death in ≈67% of neuronally differentiated PC-12 cells after 72 h, whereas cultures incubated with equivalent concentrations of dopamine, HVA, or THP were indistinguishable from controls (Kristal et al., 2001). These results are consistent with previous findings demonstrating that PC-12 cells are resistant to dopamine concentrations <1 mM (Cantuti-Castelvetri and Joseph, 1999). On the basis of these findings, it has been suggested that DOPAL is ≈100-fold more toxic to PC-12 cells than is dopamine (Kristal et al., 2001).

DOPAL-induced PC-12 cell damage elicits concentration-dependent lactic acid dehydrogenase (LDH) release, a measure of cytotoxicity. PC-12 cells incubated with 33 μM DOPAL for 8 h produced a 6-fold increase in LDH (Mattammal et al., 1995). In the same study, incubation for 24 h with 6.5 μM DOPAL induced both significant degeneration of the neuritic processes and a decrease in the number of viable cells. Incubation for 24 h with 33 μM DOPAL resulted in almost no cell survival. Short term (5 min) incubation of PC-12 cells with DOPAL (100 μM) also results in significant LDH release (Hashimoto and Yabe-Nishimura, 2002).

DOPAL is also toxic to neuroblastoma SK-N-SH and SH-SY5Y cells, fetal rat mesencephalic cultures and rat neostriatal synaptosomal preparations. In SK-N-SH cells, exposure to DOPAL (1–500 μM) for 24 h produced a concentration-dependent increase in LDH leakage into the cell medium with toxicity being pronounced at 100 μM (Lamensdorf et al., 2000a). In catecholaminergic SH-SY5Y cells, early increased levels of DOPAL induced by dopamine treatment and ALDH inhibition produced delayed cell toxicity and cell losses that increased with time (Legros et al., 2004a). Dopaminergic cultures, prepared from the ventral mesencephalon of rat embryos, exposed to 1 to 5 μM DOPAL for 24 h showed no toxicity as measured by the disappearance of tyrosine hydroxylase (TH) immunoreactivity (Mattammal et al., 1995). Between 7.5 and 20 μM DOPAL, a gradual reduction in dopamine uptake was seen without a reduction in the number of TH-immunoreactive cells. However, treatment of cultures with 33 μM DOPAL resulted in the disappearance of TH immunoreactivity, with the surviving TH-immunoreactive cells showing rounded cell bodies and highly fragmented fiber networks. These morphological changes were specific to dopaminergic neurons and were not evident in other CNS cells. In rat neostriatal synaptosomes, treatment with DOPAL for 30 min (10–100 μM), produced a concentration-dependent decrease in the number of living cells and a concomitant increase in the release of LDH (Mattammal et al., 1995).

DOPEGAL is also toxic in vitro. DOPEGAL concentrations of 5.9 and 59.5 μM decreased PC-12 cell viability (by 23 and 61%, respectively), with most of the cell loss occurring after 2 days of exposure (Burke et al., 1996). Epinephrine itself was also slightly toxic to these cells, reducing viability by 17%, but no other oxidative or methylated metabolite of epinephrine (aside from DOPEGAL) was toxic to PC-12 cells. The results of this study also underscored the importance of the terminal carbonyl group in the toxicity of DOPEGAL. Converting the terminal carbonyl moiety of DOPEGAL to a hydroxyl group to form its tautomer, 2′,3,4-trihydroxyacetophenone, diminished toxicity significantly.

In vivo cytotoxicity of DOPAL has been reported in neurons and glia in the SN and VTA (Burke et al., 2003). DOPAL, dopamine, and oxidative, reduced, and methylated metabolites of dopamine were injected into rat SN

and VTA. Five days after treatment, these regions were evaluated by Nissl preparation and cell-specific immunoreactivities. At doses of 100 ng, DOPAL was most toxic to SN neurons, followed by VTA neurons and, finally, glial cells. Neurons of the SN were consistently more affected, indicating the selective toxicity of DOPAL toward dopaminergic nerves. Neither dopamine nor its other metabolites elicited evidence of neurotoxicity, even when injected at 5-fold higher doses than that shown for DOPAL to cause toxicity. In contrast, rats treated systemically for 30 days with L-dopa, which is enzymatically converted to dopamine (by L-dopa decarboxylase), exhibited a 3-fold increase in the levels of DOPAL in the brain but showed no evidence of nigrostriatal dopaminergic cytotoxicity as measured by striatal dopamine content (Legros et al., 2004b).

In vivo toxicity of DOPEGAL has also been demonstrated—in rat RVLM (Burke et al., 2001). DOPEGAL was injected into adrenergic neurons in the RVLM and apoptosis of these adrenergic neurons (identified immunohistochemically by their content of the epinephrine-synthesizing enzyme phenylethanolamine *N*-methyltransferase) was evaluated. Fifty nanograms of DOPEGAL caused apoptotic loss of epinephrine neurons after 18 h, as evaluated by in situ terminal deoxynucleotidyl-transferase mediated dUTP nick-end label staining. The degree of neurotoxicity was both dose- and time-dependent. Ten-fold higher doses of DOPEGAL produced necrosis of these neurons. Neither epinephrine nor MHPG was shown to be cytotoxic in this study.

Various mechanisms have been suggested to explain the observed cytotoxicity of DOPAL and DOPEGAL. These include protein adduction, isoquinoline formation, and free radical generation.

B. Protein Adduction

It is well known that aldehydes react with proteins to form various adducts that can disrupt protein function and cause cellular damage. It has been suggested that biogenic aldehydes react with proteins to form both unstable and stable adducts (Esterbauer et al., 1991). Evidence of this formation has been illustrated in reactions between DOPAL and the protein hemoglobin (Helander and Tottmar, 1989). DOPAL initially reacts with hemoglobin to form Schiff bases involving its aldehyde group and the free amino groups of lysine, tyrosine, or valine residues (Fig. 4). Schiff bases are inherently unstable and the binding that involves them can be reversed or further converted into more stable products by physiological reducing agents, such as glutathione or ascorbate (Tuma et al., 1984). Stable adducts formed between DOPAL and hemoglobin rendered the aldehyde unable to serve as a substrate for ALDH, whereas the DOPAL that was more loosely bound (i.e., Schiff base) could. Interestingly, at concentrations exceeding 5 μM, DOPAL actually inactivated ALDH by interacting with the surface of the protein through irreversible covalent modification in areas deemed important for enzyme activity (MacKerell and Pietruszko, 1987). Exhaustive dialysis and reducing agents did not reverse the inactivation. The substrate analog, chloral, protected ALDH against DOPAL-mediated inactivation. The authors suggested that DOPAL inactivates ALDH by formation of a covalent bond involving its catechol ring rather than its aldehyde group. A recent study supports this observation of substrate-mediated inhibition of ALDH by DOPAL, although the mechanism was not investigated (Florang et al., 2006).

C. Isoquinoline Formation

Carbonyl compounds can react with β-arylethylamines, such as dopamine, in a rapid, nonenzymatic Pictet-Spengler condensation reaction to form isoquinoline alkaloid derivatives (Yamanaka et al., 1970; Sandler et al., 1973). For example, the reaction between acetaldehyde and dopamine results in the formation of salsolinol (Fig. 5) (Sandler et al., 1973), whereas that of DOPAL and dopamine generates THP (Davis and Walsh, 1970; Cohen, 1976) (Fig. 3). THP, salsolinol, and other isoquinoline derivatives are structurally related to the selective dopaminergic neurotoxin and PD-inducing agent MPTP/MPP^+ and have been suggested to play a role in dopaminergic cell death characterized by PD (McNaught et al., 1998; Storch et al., 2002). Indeed, THP and salsolinol, have been demonstrated to be selective dopaminergic neurotoxins (Goto et al., 1997; Storch et al., 2002). Salsolinol and THP are found in high concentrations in the urine of PD patients on L-dopa treatment (Sandler et al., 1973) and salsolinol is found in the cerebrospinal fluid of untreated PD patients (Maruyama et al., 1996). The primary mechanism underlying MPTP/

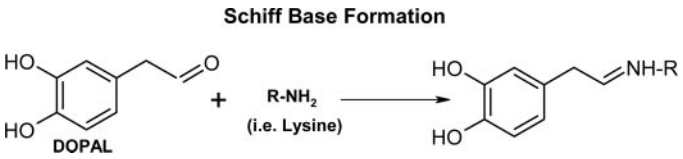

FIG. 4. Proposed Schiff base formation between DOPAL and amino acid residues of proteins, such as lysine.

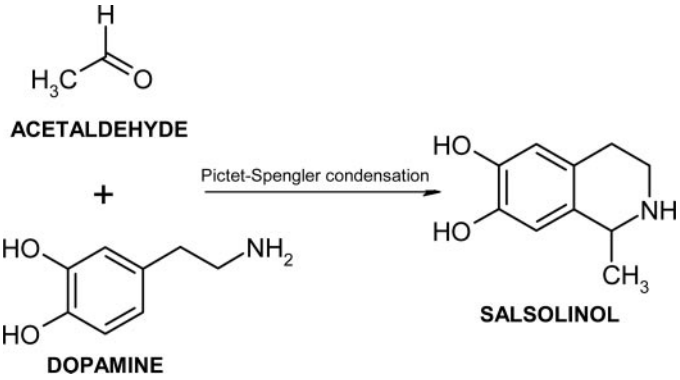

FIG. 5. Formation of salsolinol, the Pictet-Spengler condensation product of acetaldehyde and dopamine.

MPP^+ dopaminergic toxicity (after MPP^+ uptake by the DAT into dopamine neurons) is MPP^+-induced inhibition of complex I of the mitochondrial respiratory chain, leading to depleted ATP, enhanced ROS production and ultimately dopaminergic cell death (Brooks et al., 1989; Cleeter et al., 1992). Similarly, THP is also a complex I inhibitor (Suzuki et al., 1990; McNaught et al., 1995). Inhibition of complex I also leads to the decreased availability of NAD^+, the required cofactor in ALDH-mediated DOPAL metabolism. This could result in the accumulation of DOPAL and increased generation of THP. As mentioned, the dopaminergic specificity of MPTP/MPP^+ relies on the uptake of MPP^+ by the DAT, which transports this toxin selectively into dopaminergic cells. In vitro, THP has been shown to inhibit dopamine uptake through the DAT (Okada et al., 1998), indicating that it may also be a DAT substrate. THP also inhibits dopamine biosynthesis through mechanisms involving increased oxidative stress and the inhibition of TH activity (Kim et al., 2005). Indeed, many isoquinolines exhibit pharmacological actions and can act as false neurotransmitters or inhibitors of physiological mechanisms that regulate the actions of catecholamines (Cohen, 1976). For example, THP has been suggested to be responsible for some of the hypotensive and β-adrenergic actions of L-dopa (Sandler et al., 1973). Accordingly, it is possible that some of the neurotoxic effects reported for DOPAL may be due, in part, to the generation of THP.

D. Free Radical Generation

Like aldehydes, free radicals and reactive oxygen species (ROS) are known to damage cellular components and interrupt physiological functions, which can lead to various disease states, including cell death and cancer. The reactions catalyzed by MAO to form DOPAL and DOPEGAL produce hydrogen peroxide (H_2O_2), which can generate other ROS and free radicals. Recently, DOPAL itself has been reported to generate free radicals, specifically the hydroxyl radical, in the presence of H_2O_2 (Li et al., 2001). This free hydroxyl radical production was not observed with DOPEGAL, dopamine, or other dopamine metabolites. The authors speculated that DOPAL may act as a cofactor in the generation of hydroxyl radicals. In support of this hypothesis, DOPAL did not produce the hydroxyl radical in the absence of H_2O_2 or in the presence of Fe^{2+} alone. However, H_2O_2 in the presence of either Fe^{2+} or DOPAL did result in hydroxyl radical formation. Thus, DOPAL may have a role similar to that of Fe^{2+} in the formation of hydroxyl radicals from H_2O_2 wherein DOPAL functions as a reducing agent and is oxidized in the process. In addition to its electrophilic carbonyl carbon, DOPAL contains two easily oxidizable phenolic groups. Auto-oxidation of DOPAL to the DOPAL-*o*-quinone, a process reported for dopamine (Hasegawa et al., 2006), may also produce free hydroxyl radicals in the presence of H_2O_2, similar to that reported for the dopamine derivative, 6-hydroxydopamine (Cohen and Heikkila, 1974). Furthermore, it was suggested that the ability of DOPAL to produce the hydroxyl radical may be due to its lower redox potential in relation to DOPEGAL or other dopamine metabolites (Cohen and Heikkila, 1974; Liu and Mori, 1993).

DOPEGAL has also been reported to generate a free radical in the presence of H_2O_2. In this case, the free radical seems to be a DOPEGAL radical that may involve the side-chain β-hydroxyl group (Burke et al., 1998). According to electron paramagnetic resonance analyses, this radical elicited a signal different from that for the hydroxyl radical, leading the authors to suggest that DOPEGAL itself has a free radical form (Li et al., 2001). This observation was not seen with norepinephrine. It is intriguing that DOPAL and DOPEGAL have the capacity to generate free radicals, and they seem to do so by different pathways. Generation of ROS by DOPAL and DOPEGAL could have serious functional significance arising from GSH depletion and increased cellular oxidative stress. Further study of these processes is necessary to elucidate the exact mechanisms involved.

E. Mechanisms of Apoptosis

Apoptosis is a form of programmed cell death associated with a disruption in Ca^{2+} homeostasis (Orrenius and Nicotera, 1994) and the activation of caspase protease proteins (Scarlett and Murphy, 1997). Mitochondria are known to play a role in apoptotic neuron death (Wallace, 1999), and recent evidence has associated apoptosis with the induction of the mitochondrial membrane permeability transition (PT). Indeed, mitochondrial PT activation is believed to be a critical factor in the development of neurotoxicity and neurodegeneration (Bachurin et al., 2003), and it has been linked to the apoptotic type of catecholaminergic neuron death seen in PD and other neurodegenerative diseases (Lassmann et al., 1995; Anglade et al., 1997). Induction of the PT is characterized by matrix swelling, outer membrane rupture, release of apoptotic signaling molecules from the intermembrane space, and a collapse of the mitochondrial membrane potential due to increased permeability of the inner membrane (Bachurin et al., 2003; Fiskum et al., 2003). PT activation is thought to be caused by the opening of PT pores on the inner mitochondrial membrane. These pores control the transport of Ca^{2+} ions and small compounds (up to 1.5 kDa) in and out of the mitochondria and thereby function to maintain Ca^{2+} homeostasis in the cell. PT induction also seems to be involved in releasing the apoptotic initiation factors, cytochrome *c* and Apaf-1, which activate downstream caspases, leading to apoptosis (Kluck et al., 1997; Scarlett and Murphy, 1997). Various reactive species, such as free radicals, ROS, and aldehydes, are known to activate the mitochondrial PT (Kristal et al., 1996; Packer et al., 1997). DOPAL and DOPEGAL, both of which are

generated by MAO on the outer mitochondrial membrane in close proximity to mitochondrial PT pores, have been reported to induce the Ca^{2+}-mediated activation of the mitochondrial PT (Burke et al., 1998; Kristal et al., 2001). In isolated, energetically compromised liver mitochondria, very low DOPAL concentrations ($\geq$0.125 μM) increased the rate of PT induction at physiological Ca^{2+} concentrations, as measured by mitochondrial swelling (Kristal et al., 2001). In contrast, dopamine at high concentrations (up to 500 μM) had no effect on PT induction. DOPAL activation of mitochondrial PT was blocked by specific PT inhibitors, including cyclosporine A and trifluoperazine. These inhibitors also protected differentiated PC-12 cells from DOPAL-induced cytotoxicity, indicating that mitochondrial PT activation is one mechanism involved in DOPAL-induced cell death (Kristal et al., 2001). In contrast, actively respiring mitochondria were shown to be highly resistant to PT induction by DOPAL, suggesting that mitochondrial dysfunction may need to precede DOPAL-mediated toxicity.

DOPEGAL at low concentrations (6 μM) has also been reported to induce the mitochondrial PT in isolated liver mitochondria, whereas norepinephrine had no such effect (Burke et al., 1998). DOPEGAL may also disrupt Ca^{2+} homeostasis. Exposure of PC-12 cells to 0.5 μM DOPEGAL produced a 12-fold increase in cytosolic Ca^{2+} shortly after exposure (Burke et al., 2000). PC-12 cells cultured in the presence of very high concentrations of DOPEGAL ($\geq$30 mM) showed increased activity of caspase 3, a key protease in the apoptotic process. In the same study, boc-aspartate-fluor-*O*-methyl-ketone, a caspase inhibitor, blocked DOPEGAL-induced (3 μM) cytotoxicity in cultured sympathetic neurons. DOPEGAL (20 μM) was also shown to trigger the release of Ca^{2+} from isolated mitochondria. Based on these findings, it was suggested that DOPEGAL may induce apoptotic cell death in neurons through the disruption of calcium homeostasis (Burke et al., 1997). Indeed, DOPAL and DOPEGAL may induce apoptotic cell death by activating the mitochondrial PT, resulting in the release of Ca^{2+} and the activation of caspases. Activation of the mitochondrial PT by DOPAL and DOPEGAL could be a result of the free radical species generated by these compounds, as has been reported for other aldehyde species (Ka et al., 2003) or, alternatively, by mechanisms involving their inherent reactivity as aldehydes, as was reported for 4-HNE (Kristal et al., 1996). Another mechanism of apoptosis that may be pertinent is complex I inhibition. THP, like the PD-inducing agent MPTP/MPP^+(Cleeter et al., 1992), is a complex I inhibitor and has been shown to induce apoptosis in vitro in dopaminergic cell lines (Seaton et al., 1997). Further investigation of cell death mechanisms initiated by DOPAL and DOPEGAL is warranted.

F. Potential Role in Neurodegeneration

Given the increasing evidence demonstrating the neurotoxic properties of DOPAL and DOPEGAL, it is not surprising that their role as endogenous neurotoxins and involvement in selective neuron death associated with neurodegenerative diseases, such as PD and AD, have been proposed (Mattammal et al., 1995; Eisenhofer et al., 2000; Burke et al., 2004). PD is a progressive neurodegenerative disease characterized by bradykinesia, rigidity, resting tremor, and ataxia. Although all catecholaminergic neuron subtypes are lost in PD (Gai et al., 1993; Lang and Lozano, 1998), the major symptoms are associated primarily with the selective loss of dopaminergic neurons in the SN pars compacta (SNpc) region and norepinephrine neurons in the LC region. PD is also characterized by the formation of Lewy bodies in the SN, which are cytoplasmic inclusions made up of ubiquitin and α-synuclein proteins (Piao et al., 2000). The mechanisms involved in these processes are not well established. Many hypotheses on the etiology of PD exist, including alterations in α-synuclein (Zarranz et al., 2004), increased free radicals and oxidative stress (Przedborski and Ischiropoulos, 2005), DAT dysfunction (Storch and Schwarz, 2000), mitochondrial dysfunction (Cassarino and Bennett, 1999), and environmental toxins (Betarbet et al., 2000). More recently, it has been suggested that endogenous neurotoxins produced specifically by catecholaminergic neurons, such as DOPAL and DOPEGAL, may contribute to the selective vulnerability of these cells to degeneration in the development of PD (Kristal et al., 2001; Burke et al., 2004).

In PD, SNpc neurons are more vulnerable to neurodegeneration than VTA and hypothalamic arcuate dopaminergic neurons (Storch and Schwarz, 2000). Likewise, DOPAL has been shown to be significantly more toxic to dopaminergic SN neurons than dopaminergic VTA neurons and glia in vivo (Burke et al., 2003; Burke et al., 2006). Furthermore, these studies demonstrate that DOPAL is significantly more toxic to these neurons than dopamine and other metabolites. Stereotactic injections of DOPAL, dopamine, and other metabolites into the SN of Sprague-Dawley rats demonstrated that only DOPAL, at concentrations within the physiological range of 2 to 3 μM, causes neurodegeneration. Physiological concentrations of dopamine and other metabolites did not cause cytotoxicity. However, dopamine at 200-fold greater doses than DOPAL did produce lesions in the SN and VTA.

As mentioned, the selective dopaminergic toxicity of MPTP is due to DAT-mediated uptake of MPP^+ and, similarly, dopaminergic neurodegeneration in PD has been hypothesized to involve the cellular accumulation of dopamine-like molecules into dopamine neurons by the presynaptic DAT (Lee et al., 2001). DOPAL has been implicated as a substrate for the DAT and could accumulate in dopamine neurons by this mechanism (Mat-

tammal et al., 1995). Moreover, the DAT is expressed in higher concentrations in SNpc neurons than in other dopaminergic neurons, and this mechanism may explain the increased sensitivity of SNpc neurons to DOPAL (Storch and Schwarz, 2000). As mentioned, THP, may also serve as a DAT substrate. The preferential uptake of DOPAL and/or THP into dopaminergic neurons by the DAT may enhance dopaminergic neurotoxicity.

Compared with other neuronal subpopulations, SNpc neurons may have increased cytosolic Ca^{2+} concentrations and be more susceptible to free radical damage (Hirsch et al., 1997). As mentioned, both DOPAL and DOPEGAL have been suggested to initiate apoptosis through Ca^{2+}-mediated processes (Burke et al., 1998; Kristal et al., 2001) and to generated free radical species (Burke et al., 1998; Li et al., 2001). The specific vulnerability of SNpc neurons coupled with these mechanisms could also lead to enhanced cytotoxicity.

Excess dopamine release from dopaminergic neurons is another mechanism thought to be involved in PD neurodegeneration. Indeed, the PD-inducing agent MPTP/MPP^+ is a potent dopamine-releasing agent (Obata, 2002). DOPAL is also reported to increase dopamine release in PC-12 cells and rat striatal synaptosomes. In PC-12 cells, DOPAL (10 μM) caused a >2-fold increase in the release of dopamine (Hashimoto and Yabe-Nishimura, 2002). This effect was shown to be Ca^{2+}-independent. Other reactive aldehydes tested (i.e., 4-HNE) did not initiate dopamine release, suggesting that the effect was specific to DOPAL. In rat striatal synaptosomes, 33 μM DOPAL caused a significant increase in dopamine release (>55%) (Mattammal et al., 1995). This effect was specific to dopamine-containing terminals as opposed to those containing GABA. Excess DOPAL-induced dopamine release may be one mechanism underlying the neurotoxicity of DOPAL and may be a factor in dopaminergic neurodegeneration characterized by PD.

Central to the hypothesis that endogenous neurotoxins, such as DOPAL and DOPEGAL, are involved in neurodegenerative diseases is the requirement that they accumulate to levels that become toxic to catecholamine neurons. Many mechanisms could lead to increased intraneuronal concentrations of these aldehydes including, as mentioned, the preferential uptake of DOPAL by the DAT. In addition, the increased synthesis of DOPAL and DOPEGAL could influence their levels. Accordingly, age-related increases in MAO have been reported (Oreland and Gottfries, 1986), which could lead to increased formation of these aldehydes. Furthermore, pharmacological treatment with the dopamine precursor, L-dopa, used to treat PD, has been shown to elevate rat brain DOPAL levels by as much as 18-fold (Fornai et al., 2000; Legros et al., 2004b).

Impaired metabolism of DOPAL may also affect its accumulation. Indeed, in catecholaminergic neuroblastoma SH-SY5Y cells, significant accumulation of DOPAL was achieved by treatment with dopamine (1 mM) along with the inhibition of DOPAL metabolism by the ALDH inhibitor disulfiram (10 μM) (Bonnet et al., 2004; Legros et al., 2004a). Under these conditions, increased cytotoxicity was also demonstrated. Similarly, mitochondrial dysfunction of complex I and the resulting decreased availability of NAD^+, the required cofactor for ALDH-mediated oxidation of aldehydes, may lead to increased levels of both DOPAL and DOPEGAL. Indeed, complex I inhibition by rotenone leads to significant DOPAL accumulation in PC-12 cells (Lamensdorf et al., 2000a). Under these conditions, the further inhibition of DOPAL metabolism by specific inhibitors of ALDH and aldose reductase (AR) increased DOPAL levels even more (12-fold that of control) (Lamensdorf et al., 2000b). DOPAL-mediated substrate inhibition of ALDH (MacKerell and Pietruszko, 1987; Florang et al., 2006) and age-related ALDH deficiencies (Chen and Yu, 1996) may also play a role in DOPAL accumulation in the CNS.

Mitochondrial dysfunctions, including complex I inhibition, are associated with neurodegenerative diseases (Robinson, 1998; Olanow and Tatton, 1999). Complex I inhibitors, including rotenone, MPP^+, isoquinoline, and THP, induce apoptosis in dopaminergic cell lines (Seaton et al., 1997). A mitochondrial complex I deficit has been identified in the SN of PD patients (Schapira et al., 1990) and hypothesized to result from genetic mutations and/or environmental toxins (Bachurin et al., 2003; Fiskum et al., 2003). Chronic complex I inhibition by rotenone is used to create a model of PD in rats that involves a selective loss of SN and LC neurons, as well as Lewy bodies in the SN (Betarbet et al., 2000). Accumulation of DOPAL along with preexisting mitochondrial dysfunctions may act synergistically, leading to enhanced neurotoxicity. Indeed, at minimally toxic concentrations, rotenone significantly increased DOPAL-induced cytotoxicity and death in nerve growth factor-differentiated PC-12 cells (Kristal et al., 2001). Likewise, accumulation of DOPAL by both complex I inhibition (rotenone) and ALDH/AR inhibition potentiated rotenone-induced toxicity in PC-12 cells (Lamensdorf et al., 2000b). Both the accumulation of DOPAL and the enhancement of rotenone-induced toxicity were abrogated by inhibiting the formation of DOPAL with the MAO inhibitor, clorgyline. These observations suggest that the MAO-catalyzed formation of DOPAL and its accumulation by various mechanisms may be important processes that aggravate the neurotoxicity associated with mitochondrial dysfunction. Accordingly, there is substantial documentation of a neuroprotective effect of irreversible MAO inhibitors in vitro and in animal models of PD (Tabakman et al., 2004). Furthermore, the MAO inhibitors selegiline and the newly available, rasagiline, are both currently approved by the U.S Food and Drug Administration to treat PD and, used alone or as an adjunct to L-dopa therapy, seem to be promising treat-

ments for patients with both early and advanced PD (Henchcliffe et al., 2005). Central to the activity of MAO inhibitors is their capacity to boost dopamine levels by blocking dopamine metabolism. It is, therefore, conceivable that the concomitant blockade of the production of DOPAL and DOPEGAL may also contribute to their therapeutic effects. In support of this contention, it has been postulated that increased dopamine metabolism, occurring after an initial loss of dopamine neurons, plays a role in the progression of nigrostriatal degeneration in PD (Graham, 1978; Cohen et al., 1997).

Oxidative stress is believed to be a critical factor in neurodegenerative diseases (Sayre et al., 2001). SNpc neurons have been reported to be particularly sensitive to oxidative stress and may have increased levels of H_2O_2 (Hirsch et al., 1997). H_2O_2 is generated during the formation of DOPAL and DOPEGAL and has been shown to enhance formation of DOPAL- and DOPEGAL-mediated free radicals (Burke et al., 1998; Li et al., 2001). These processes could exacerbate oxidative stress in the SNpc and be a factor in neurodegeneration of these neurons in disease states. Similarly, the formation of Lewy bodies in PD has been suggested to involve increased oxidative stress. Aggregation of α-synuclein is enhanced in the presence of free hydroxyl radicals (Hashimoto et al., 1999). Free hydroxyl radicals generated by DOPAL have also been hypothesized to be involved in the oxidative modification of α-synuclein and the formation of Lewy bodies in dopaminergic SN neurons (Li et al., 2001). Physiological concentrations of DOPAL (1.5–3 μM) cause aggregation of α-synuclein in catecholaminergic SH-SY5Y and dopaminergic MN9D cells (Burke et al., 2006). Moreover, it has been postulated that α-synuclein may actually contribute to the neurotoxicity of DOPAL (Burke et al., 2004) in a mechanism similarly described for dopamine-induced neurotoxicity. Accordingly, α-synuclein has been shown to bind to the DAT, enhancing both dopamine uptake and dopamine-induced apoptosis (Lee et al., 2001) and these processes may affect DOPAL neurotoxicity. α-Synuclein also catalyzes the formation of H_2O_2 (Turnbull et al., 2001), which could contribute to the production of DOPAL-generated free hydroxyl radicals and subsequent aggregation of α-synuclein into Lewy bodies (Li et al., 2001). Recently, it has been demonstrated that α-synuclein and oxidized catechol metabolites may work synergistically to potentiate each other's toxic effects (Hasegawa et al., 2006). α-Synuclein was shown to exacerbate apoptotic cell death induced by *o*-quinone metabolites of dopamine and L-dopa, and it was suggested that oxidized catechol metabolites may form adducts with α-synuclein, leading to enhanced aggregation. Furthermore, it was suggested that the mitochondrial membrane may represent an initial target for these compounds in dopaminergic neurodegeneration. As mentioned, DOPAL may also be oxidized to its *o*-quinone form, and this process may facilitate the formation of free radicals and the activation of the mitochondrial PT. Therefore, it is possible that DOPAL-*o*-quinone may participate in reactions with α-synuclein similar to those described for oxidized catechol metabolites.

AD is primarily a late-onset, progressive, age-dependent neurodegenerative disorder characterized clinically by the impairment of cognitive functions and changes in behavior and personality (Robert et al., 2005). The disease is associated with the presence of intracellular neurofibrillary tangles and extracellular β amyloid plaques and the apoptotic degeneration of neuronal subpopulations, specifically norepinephrine neurons of the LC and epinephrine neurons of the C-1 area of the RVLM (Bondareff et al., 1982; Lassmann et al., 1995). As for PD, the molecular mechanisms of AD are not fully understood, and many hypotheses have been advanced to explain the neuron loss underlying its pathological changes. Mitochondrial dysfunction and increased oxidative stress (Hirai et al., 2001), defective axonal transport (Younkin et al., 1986; Burke et al., 1999b), and free radicals, especially those generated by amine oxidation (Sano et al., 1997), have all been implicated in AD. The neurofibrillary tangles associated with AD consist of a phosphorylated form of tau protein (Lee et al., 1991). Hyperphosphorylated tau protein and/or neurofibrillary tangles can cause mitochondrial dysfunction and inhibition of axonal transport in AD, which can lead to the accumulation of neurotoxins (Younkin et al., 1986; Mandelkow et al., 2003). Accordingly, accumulation of DOPEGAL, along with enzymes involved in its synthesis, including dopamine-β-hydroxylase and MAO-A, has been demonstrated in norepinephrine cell bodies in the LC in AD (Burke et al., 1999b). Accumulation of DOPEGAL in noradrenergic cells may also proceed from many of the same processes described above for DOPAL, including increased uptake and synthesis and/or decreased metabolism. Relatively limited studies of the role of DOPEGAL in AD exist. However, it has been hypothesized that DOPEGAL may play a role in AD by mediating apoptotic neuron death (Burke et al., 1997, 2001) through mechanisms involving the activation of the mitochondrial PT through its reactivity as an aldehyde or its reported free radical form (Burke et al., 1998). Adrenergic neurons in AD become atrophic (Burke et al., 1994) and demonstrate morphological characteristics similar to those seen in adrenergic neurons exposed to DOPEGAL in vivo (Burke et al., 2000). Further investigation is necessary to determine the possible role, if any, of DOPEGAL in neurodegenerative processes of AD.

In summary, experimental evidence suggests that both DOPAL and DOPEGAL can be neurotoxic and may act as neuronal death messengers in the CNS. However, although an attractive theory, the significance of the role, if any, of DOPAL and DOPEGAL in neurodegen-

eration and associated diseases has yet to be elucidated. To date, evidence of their possible role in these neuropathological conditions is compelling but remains to be further substantiated. The fact that they are synthesized intraneuronally and seem to be selectively neurotoxic to the distinct populations of catecholamine neurons that are most affected in these disease states is suggestive. Likewise, their accumulation in the CNS by mechanisms associated with these disease states has been demonstrated. They also have been reported to generate free radical species and to activate the mitochondrial PT, processes believed to be involved in the apoptotic neurodegeneration characterized by PD and AD. In the case of PD, mitochondrial impairment resulting from genetic or environmental sources may serve to lower the threshold for DOPAL-mediated toxicity in catecholaminergic neurons. Likewise, high local levels of DOPAL and DOPEGAL at the mitochondrial level (where they are formed) may be a causal or contributing factor in the mitochondrial dysfunction associated with neurodegenerative diseases. Whether the suggested neurotoxicity of DOPAL and DOPEGAL reflects a primary causal event or merely a secondary contribution or potentiation of disease conditions that are intrinsically toxic for neurons is unknown. To date, the neurotoxicity of DOPAL and DOPEGAL and, specifically, their potential role in neurodegenerative diseases have been investigated in a relatively limited number of studies. More investigation is needed to show whether any relationship exists between these aldehyde species and neurodegeneration, including more study to elucidate their precise mechanisms and actions of neurotoxicity.

V. Metabolism of 3,4-Dihydroxyphenylacetaldehyde and 3,4-Dihydroxyphenylglycolaldehyde in the Central Nervous System

A. Overview

Several enzymes are known to be involved in the metabolism of both DOPAL and DOPEGAL, including primarily ALDH, alcohol dehydrogenase (ADH), and aldehyde/AR reductases (ALR/AR) (Fig. 2). Downstream pathways also involve catechol-*O*-methyltransferase (COMT), phenolsulfotransferase (PST), and UDP-glucuronosyltransferase (UGT). Given the potential toxicity of these compounds, it is not surprising that this level of metabolic redundancy exists. These enzyme systems are discussed in detail in designated subsections below. Herein will be found an overview of DOPAL and DOPEGAL metabolism.

As mentioned, misconceptions of catecholamine metabolism exist, specifically, that involving norepinephrine and epinephrine. It is generally agreed that DOPAL and DOPEGAL are formed from the MAO-catalyzed oxidative deamination of their parent catecholamines, which represents the primary pathway of catecholamine metabolism (Fig. 2). However, two different respective pathways exist for the direct metabolism of DOPAL and DOPEGAL, namely an oxidative pathway catalyzed by ALDH or a reductive pathway catalyzed by either ADH or ALR/AR. The presence of these two divergent pathways has led to erroneous reports on the metabolism of these aldehydes. Although both pathways are represented to some degree under normal biological conditions, DOPEGAL metabolism predominantly proceeds by the reductive pathway, whereas DOPAL metabolism proceeds mainly by the oxidative pathway. It is now accepted that the presence or absence of the β-hydroxyl group on the respective aldehyde metabolite determines which pathway will predominate (Breese et al., 1969; Tabakoff et al., 1973; Duncan and Sourkes, 1974). Thus, DOPAL, lacking the β-hydroxyl group, is preferentially metabolized by the ALDH-catalyzed oxidative pathway to 3,4-dihydroxyphenylacetic acid (DOPAC) (Tank et al., 1981; Lamensdorf et al., 2000a). DOPAC is then predominantly *O*-methylated by COMT to HVA, the major CNS metabolite of dopamine (Dedek et al., 1979). Formation of the sulfate conjugates of DOPAC and HVA represent minor metabolic pathways. The reduction of DOPAL to the corresponding alcohol, 3,4-dihydroxyphenylethanol (DOPET), by ADH or ALR/AR, followed by *O*-methylation of DOPET to 3-methoxy-4-hydroxyphenylethanol, is considered a minor pathway of DOPAL metabolism (Kopin, 1985). Whereas DOPAL metabolism is relatively simple and the predominant pathways involved are widely accepted, DOPEGAL metabolism has proven to be more complex, leading to the confusion that exists in earlier reports in the literature. This confusion persists to some degree today despite the fact that a significant body of definitive evidence from the past four decades has conclusively demonstrated the following:

1. Because of the presence of the β-hydroxyl group on DOPEGAL, reduction of DOPEGAL to the corresponding alcohol, 3,4-dihydroxyphenylglycol (DOPEG), by ADH or ALR/AR is favored over the oxidation pathway (Duncan and Sourkes, 1974) (Fig. 2). Accordingly, the ALDH oxidation of DOPEGAL to the corresponding carboxylic acid, 3,4-dihydroxymandelic acid (DOMA) represents a minor pathway (Kopin, 1985) that only becomes significant upon inhibition of ALR/AR (Kawamura et al., 1997).
2. MHPG (free, unconjugated) produced by the *O*-methylation of DOPEG represents the major CNS metabolite of norepinephrine and epinephrine (Karoum et al., 1977).
3. MHPG enters the systemic circulation and is the major intermediate in the systemic production of vanillylmandelic acid (VMA) (Mardh et al., 1983; Mardh and Anggard, 1984; Eisenhofer et al., 1996a). In this process, MHPG is oxidized by hepatic class I ADH isozymes to the aldehyde, 3-methoxy-4-hy-

droxyphenylglycolaldehyde (MOPEGAL) (Mardh et al., 1985), which is then oxidized by hepatic ALDH to VMA (Messiha, 1978). As such, the production of VMA from the *O*-methylation of DOMA represents a minor pathway. Much confusion in the literature exists regarding VMA formation from norepinephrine and epinephrine, and it was erroneously reported in earlier studies that VMA is principally produced by the *O*-methylation of DOMA. Despite substantial evidence to the contrary, including the fact that DOMA is only a minor metabolite of DOPEGAL, this view persists >40 years later.

4. VMA is the major systemic end-product of norepinephrine and epinephrine metabolism, followed by the sulfate and glucuronide conjugates of MHPG (Mardh et al., 1983; Mardh and Anggard, 1984; Eisenhofer et al., 1996a).

Impaired metabolism of DOPAL and DOPEGAL by a dysfunction in any of the pathways described above could potentially contribute to their accumulation and enhanced toxicity in disease states. Many polymorphic ALDH alleles that display phenotypes of decreased catalytic activity and reduced metabolism of aldehyde substrates exist. In support of this notion, mutations in ALDH genes and subsequent errors in aldehyde metabolism are the molecular basis of several known disease states, most of which are characterized by neurological abnormalities. Furthermore, several pharmaceutical agents and environmental toxins are also known to disrupt or inhibit ALDH function, and these processes play a role in impaired metabolism and enhanced toxicity of aldehydes, which could also be a factor in DOPAL and DOPEGAL toxicity.

B. Aldehyde Dehydrogenase

In the 1960s, the involvement of ALDH in the oxidation of catecholamine-derived aldehydes in mammalian brain was postulated (Erwin and Deitrich, 1966). Since then, biogenic aldehydes, including DOPAL and DOPEGAL, have been shown to be specific physiological substrates for ALDH in the human brain (Tabakoff and Gelpke, 1975; Ryzlak and Pietruszko, 1989; Ambroziak and Pietruszko, 1991). In the CNS, ALDH catalyzes the irreversible oxidation of DOPAL to DOPAC, which can then be excreted as its sulfate conjugate or further metabolized by COMT to HVA (Dedek et al., 1979; Lamensdorf et al., 2000a) (Fig. 2). In a minor metabolic pathway of VMA formation, ALDH oxidizes DOPEGAL to its carboxylic acid, DOMA, which is further metabolized by COMT (Kopin, 1985). Hepatic ALDH is involved in the major pathway of VMA formation as it oxidizes MOPEGAL, the aldehyde metabolite of MHPG, to VMA (Messiha, 1978).

1. Human Aldehyde Dehydrogenases. The human ALDH gene superfamily consists of 19 putatively functional genes with distinct chromosomal locations (Fig. 6) (Vasiliou and Nebert, 2005). The ALDH enzymes catalyze the $NAD(P)^+$-dependent irreversible oxidation of a wide spectrum of aliphatic and aromatic aldehydes generated during the metabolism of endogenous and exogenous compounds (Table 1). The ALDH proteins are found in all subcellular regions including cytosol, mitochondria, endoplasmic reticulum, and nucleus with some found in more than one compartment (e.g., ALDH3A1 and ALDH7A1). In addition, most of the ALDHs have a wide tissue distribution, and some display distinct substrate specificity (Vasiliou et al., 2004). Generally regarded as detoxification enzymes, ALDHs serve to protect cells from the effects of aldehydes by converting them to their respective carboxylic acids (Fig. 1). This is evident from several experimental settings in which an ALDH protects against aldehyde-induced cytotoxicity or apoptosis (Pappa et al., 2005). However, the most compelling evidence relies on the observation that mutations in ALDH genes (leading to loss of function) are associated with distinct phenotypes in humans and rodents (Table 1) (Vasiliou and Pappa, 2000; Vasiliou et al., 2004). A genetic polymorphism in the *ALDH2* gene, frequent in and confined to individuals of Asian origin (Goedde et al., 1992), results in the catalytic inactivation of ALDH2, leading to the accumulation of acetaldehyde (Yoshida et al., 1984). Although this *ALDH2* polymorphism confers protection against the development of alcoholism, it seems to be associated with an increased risk for esophageal, stomach, colon, lung, and head cancers (Yokoyama et al., 1996, 2001) and is identified as a risk factor for late-onset AD (Kamino et al., 2000). Mutations in *ALDH3A2* result in impaired metabolism of medium- and long-chain fatty aldehydes derived from

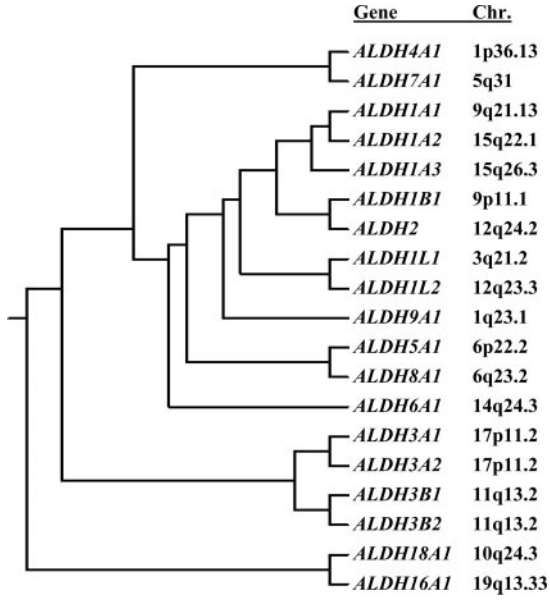

FIG. 6. Clustering dendrogram showing the evolutionary relationship of the nineteen human ALDH genes and their chromosomal (Chr.) locations.

TABLE 1
Human ALDH proteins

ALDH	Subcellular Location	Present in CNS?	Preferred Substrate	Mutational Phenotype[a]
ALDH1A1	Cytosol	Yes	Retinal	Alcohol sensitivity; alcoholism
ALDH1A2	Cytosol	Yes	Retinal	Increased risk for neural tube defects
ALDH1A3	Cytosol	Yes	Retinal	(?) *Aldh1a3*(−/−) mice die after birth
ALDH1B1	Mitochondria	Yes	Aliphatic aldehydes	Unknown
ALDH1L1	Cytosol	Yes	10-Formyltetrahydrofolate	(?) *Aldh1l1*(−/−) mice have low fertility
ALDH1L2	Unknown	Unknown	Unknown	Unknown
ALDH2	Mitochondria	Yes	Acetaldehyde	Decreased risk for alcoholism; increased risk for various cancers; increased risk for late onset AD
ALDH3A1	Cytosol, nucleus[b]	No	Aromatic and medium chain aliphatic	(?) *Aldh3a1*(−/−) mice develop cataracts
ALDH3A2	Microsomes	Yes	Fatty aldehydes	Sjögren-Larsson syndrome
ALDH3B1	Cytosol[c]	Yes[c]	Unknown	Unknown
ALDH3B2	Unknown	Unknown	Unknown	Unknown
ALDH4A1	Mitochondria	Yes	Glutamate γ-semialdehyde	Type II hyperprolinemia
ALDH5A1	Mitochondria	Yes	Succinate semialdehyde	γ-Hydroxybutyric aciduria
ALDH6A1	Mitochondria	Yes	Malonate semialdehyde	Developmental delay
ALDH7A1	Cytosol, nucleus[d]	Yes	α-Aminoadipic semialdehyde	Pyridoxine-dependent seizures
ALDH8A1	Cytosol	Yes	Retinal	Unknown
ALDH9A1	Cytosol	Yes	γ-Aminobutyraldehyde	Unknown
ALDH16A1	Unknown	Unknown	Unknown	Unknown
ALDH18A1	Mitochondria	Yes	Glutamic γ-semialdehyde	Hyperammonemia, hypoprolinemia

[a] (?) denotes evidence of phenotype in animals, but not yet demonstrated in human studies.
[b] Although predominantly cytosolic, ALDH3A1 is also found in the nucleus.
[c] S. Marchitti and V. Vasiliou, unpublished data.
[d] N. Lassen, M. Cantore, P. A. Weston, T. Estey, and V. Vasiliou, unpublished data.

fatty alcohols, phytanic acid, ether glycerolipids, and leukotriene B_4 (Kelson et al., 1997) and are the molecular basis of Sjögren-Larsson syndrome (Rizzo and Carney, 2005). This is a rare autosomal recessive neurocutaneous disorder characterized by mental retardation, diplegia or tetraplegia, and congenital ichthyosis (Willemsen et al., 2001). ALDH4A1 oxidizes glutamate γ-semialdehyde, a proline metabolite that exists in equilibrium with Δ^1-pyrroline-5-carboxylate. Mutations in *ALDH4A1* cause type II hyperprolinemia, an autosomal recessive disorder characterized by neurological manifestations, such as seizures and mental retardation, and plasma accumulation of proline and Δ^1-pyrroline-5-carboxylate (Valle et al., 1976). The latter seems to be a vitamin B_6 antagonist (Mills et al., 2006). ALDH5A1 metabolizes succinic semialdehyde, a GABA metabolite (Chambliss and Gibson, 1992). Mutations in *ALDH5A1* cause γ-hydroxybutyric aciduria, a rare autosomal recessive disorder in GABA metabolism that is characterized by neurodevelopmental effects, including mental retardation and epilepsy (Gibson et al., 1998) and associated with GABA and 4-hydroxybutyrate accumulation in blood serum and cerebrospinal fluid (Pearl et al., 2003). ALDH6A1 catalyzes the oxidative decarboxylation of malonate and methylmalonate semialdehyde to acetyl- and propionyl-CoA, respectively (Vasiliou and Pappa, 2000). Loss of ALDH6A1 function is associated with an inborn metabolic disorder that results in developmental delay (Roe et al., 1998). Mutations in *ALDH7A1*, which encodes α-aminoadipic semialdehyde (AASA) dehydrogenase (also known as antiquitin), have recently been identified as the cause of pyridoxine-dependent seizures through a defect in the cerebral lysine degradation pathway (Mills et al., 2006). As in the case of ALDH4A1, lack of ALDH7A1 leads to accumulation of piperideine-6-carboxylic acid, which exists in equilibrium with AASA and has been shown to inactivate pyridoxalphosphate by a Knoevenagel condensation (Farrant et al., 2001). Pipecolic acid and AASA are markedly elevated in urine, plasma, and cerebrospinal fluid in patients with pyridoxine-dependent seizures and thus can be used as biomarkers of the disease (Mills et al., 2006; Plecko et al., 2006). Recently, three polymorphisms in the human *ALDH1A2* gene have been associated with increased risk for neural tube defects such as spina bifida (Deak et al., 2005). ALDH1A2 is important in the conversion of retinal to retinoic acid and seems vital in normal spinal cord development (Niederreither et al., 2002). In addition to clinical phenotypes associated with mutations in ALDH genes, transgenic knockout mice suggest the pivotal role of ALDHs in physiological functions and processes. For example, *Aldh1a2* (also known as Raldh2)-null mice are embryonic-lethal (Niederreither et al., 1999), *Aldh1a3* (also known as Raldh3)-null mice die shortly after birth (Dupe et al., 2003), and *Aldh3a1*- and *Aldh1a1*-null mice develop cataracts (Lassen et al., 2006).

TABLE 2
ALDH isozymes involved in the metabolism of DOPAL

ALDH Isozyme	Reported Isozyme Description	Source[a]	Subcellular Location of Activity[b]	K_m for DOPAL	References
ALDH1A1	ALDH-E1	H liver	Cytosol	0.4 μM	MacKerell et al. (1986); Ambroziak and Pietruszko (1991)
ALDH2	ALDH2	H recombinant[c]	Mitochondria	N.D.	Florang et al. (2006)
ALDH2	ALDH-E2	H Liver	Mitochondria	1.0 μM	MacKerell et al. (1986); Ambroziak and Pietruszko (1991)
ALDH2	Low K_m MT ALDH	H brain	Mitochondria (MX)	4.2 μM	Maring et al. (1985)
ALDH3A1	ALDH3A1	H recombinant[c]	Cytosol	15 μM	S. Marchitti and V. Vasiliou, unpublished data
ALDH9A1	ALDH-E3	H liver	Cytosol	2.6 μM	Ambroziak and Pietruszko (1991)
ALDH1A1	ALDH-II	H liver	Cytosol	17 μM	Harada et al. (1982)
Unknown	Similar to liver ALDH-E1	H brain	Cytosol	3 μM	Ryzlak and Pietruszko (1987)
Unknown	Low K_m cytosolic ALDH	H brain	Cytosol	1.5 μM	Maring et al. (1985)
Unknown	Cytosolic ALDHs (5 isozymes)	R liver	Cytosol	Range: 5 μM–3 mM	Tank et al. (1981)
Unknown	Similar to liver ALDH-E2	H brain	Mitochondria	$<$1 μM	Ryzlak and Pietruszko (1987)
Unknown	Similar to liver ALDH-E2	H Brain	Mitochondria	0.5 μM	Ryzlak and Pietruszko, 1987
Unknown	Low K_m MT ALDH	R Brain	Mitochondria (MX)	0.3 μM	Pettersson and Tottmar (1982)
Unknown	High K_m MT ALDH	R Brain	Mitochondria (MX)	31 μM	Pettersson and Tottmar (1982)
Unknown	High K_m MT ALDH	H Brain	Mitochondria (MB)	197 μM	Maring et al. (1985)
Unknown	MT ALDH	B Brain MT	Mitochondria	10 μM	Erwin and Deitrich (1966)
Unknown	MT ALDHs (3 isozymes)	R Liver	Mitochondria	Range: $<$1–180 μM	Tank et al. (1981)
Unknown	MC ALDH	R Liver	Microsomes	450 μM	Tank et al. (1981)
Unknown	ALDH-II	H Brain	Cytosol, mitochondria	MT 2 mM; C 2 mM	Hafer et al. (1987)
Unknown	High-affinity ALDH	T Brain	Cytosol, mitochondria	3.7 μM[d]	Nilsson (1988)
Unknown	Low-affinity ALDH	T Brain	Cytosol, mitochondria	150 μM[c]	Nilsson (1988)
Unknown	ALDH-I and ALDH-II	H brain SN	C, MC, MT, N	N.D.	Agarwal et al. (1982)
Unknown	ALDH-I	H brain	C, MC, MT	MT 0.3 mM; MC 35 mM C 0.6 mM	Hafer et al. (1987)
Unknown	Low-affinity ALDH	R Brain	N.D.	60 μM	Nilsson (1988)
Unknown	High-affinity ALDH	R Brain	N.D.	0.5 μM	Nilsson (1988)

[a] The source of ALDH protein is abbreviated as follows: human (H), rat (R), or rainbow trout (T).
[b] The subcellular location of DOPAL-oxidizing activity is abbreviated as follows: N.D., not detected; MT, mitochondrial; MC, microsomal; C, cytosolic; MX, mitochondrial matrix; MB, mitochondrial, membrane-bound; N, nucleus.
[c] Recombinant purified protein.
[d] A K_m was given, but it was unclear as to which subcellular compartment it referred to.

2. Aldehyde Dehydrogenases Involved in 3,4-Dihydroxyphenylacetaldehyde and 3,4-Dihydroxyphenylglycolaldehyde Metabolism. Although ALDH activity is found in virtually all neural tissues (Raskin and Sokoloff, 1972), the identification and properties of the specific ALDH isozyme(s) in the brain involved in the metabolism of DOPAL and DOPEGAL remain to be resolved with certainty. There have been a number of investigations on the ALDH oxidation of these aldehydes, specifically DOPAL (Table 2). However, some, if not most, early attempts to characterize specific ALDHs involved in the oxidation of these aldehydes were performed using crude homogenates, subcellular fractions and semipurified (rather than purified) ALDH enzymes. In light of the present knowledge of the ALDH family (Table 1) and the multiplicity of ALDH isozymes present in various subcellular regions, the findings of these studies, particularly those concerning ALDH isozyme identities, must be viewed as informative but not conclusive. Likewise, it is not surprising that early reports describe the presence of multiple ALDH isozymes capable of oxidizing DOPAL that were detectable in various brain regions and subcellular fractions in mammalian brain (Harada et al., 1978; Agarwal et al., 1982; Pietruszko et al., 1984; Maring et al., 1985). In post-mortem human brain, the highest ALDH activities for DOPAL have been found in corpus striatum, cerebellum, medulla oblongata, and pons (Agarwal et al., 1982; Harada et al., 1982). ALDH DOPAL-oxidizing activities have been detected in cytosolic, mitochondrial, microsomal, and nuclear fractions of human brain (Agarwal et al., 1982). However, the highest activity was localized primarily in the mitochondrial and cytosolic fractions (Agarwal et al., 1982; Hafer et al., 1987). Ryzlak and Pietruszko (1987) described two distinct mitochondrial ALDH isozymes in the human brain (Ryzlak and Pietruszko, 1987). These isozymes resembled mitochondrial ALDH2 and displayed K_m values for DOPAL of $<$1 μM. Both "high K_m" (human brain, 197 μM; rat brain, 31 μM, 60 μM) and "low K_m" (human brain, 4.2 μM; rat brain, 0.3 μM, 0.5 μM) ALDH isozyme activities for DOPAL have been reported in the mitochondrial fraction of human (Maring et al., 1985) and rat brain (Pettersson and Tottmar, 1982; Nilsson, 1988). In human brain, the high K_m

activity was localized to the mitochondrial membrane, whereas the low K_m activity was found in mitochondrial matrix. In rat brain, however, both high K_m and low K_m activities were found in mitochondrial matrix (Pettersson and Tottmar, 1982), a finding in agreement with investigations of the subcellular location of ALDH DOPAL-oxidizing activity in rat liver (Tank et al., 1981). Total ALDH activity in bovine brain mitochondria, was shown to have a K_m of 10 μM for DOPAL and 3 μM for DOPEGAL (Erwin and Deitrich, 1966). Furthermore, efficient DOPAL metabolism by rat brain mitochondrial lysate and total rat brain mitochondrial ALDH activity has been described recently (Florang et al., 2006). Similarly, rainbow trout brain exhibited low K_m ALDH activity (3.7 μM) and high K_m ALDH activity (150 μM) for DOPAL with the majority of activity being localized in the mitochondrial fraction (60–70%), whereas the remainder seemed to be cytosolic (30–40%) (Nilsson, 1988). This finding is in agreement with studies of ALDH activity with other biogenic aldehyde substrates in rat brain (Erwin and Deitrich, 1966). These studies suggested that DOPAL as well as, possibly, DOPEGAL oxidation by ALDH occurs primarily in mitochondria (Pettersson and Tottmar, 1982). However, other studies have suggested that the cytosol represents the primary location of ALDH DOPAL-oxidizing activity. Ryzlak and Pietruszko (1987) and Maring et al. (1985) described the presence of a cytosolic ALDH isozyme in human brain with a low K_m for DOPAL (3 and 1.5 μM, respectively) (Maring et al., 1985; Ryzlak and Pietruszko, 1987). Moreover, Maring et al. (1985) found a higher percentage of total ALDH activity for DOPAL in the cytosol, and the authors suggested that ALDH-mediated oxidation of biogenic aldehydes may take place primarily in this subcellular compartment., 1985). The presence of a low K_m cytosolic ALDH isozyme has also been demonstrated in rat brain, and a similar hypothesis was suggested (Weiner and Ardelt, 1984). It is important to be aware that the subcellular location of DOPAL-oxidizing ALDH activity could be affected by postmortem decay of the human and animal tissues being studied. Such decay could result in the breakdown of membranes and the increased release of ALDH proteins into the cytosolic compartment, leading to spurious results. Further investigation of the subcellular location of DOPAL-oxidizing ALDH activity in the brain is warranted.

Many studies point to a role for either the cytosol or mitochondria as the site of DOPAL oxidation. However, multiple ALDH isozymes are believed to have cytosolic (eight) and mitochondrial (six) localizations in brain (Table 1). The cytosolic and mitochondrial ALDH isozymes referred to in many early studies may be, but not conclusively, cytosolic ALDH1A1 and mitochondrial ALDH2, respectively (Harada et al., 1982; Ryzlak and Pietruszko, 1987). Both isozymes have been reported to be present in various human brain areas, including the cerebellum, medulla, pons, striatum, substantia nigra, and cortex (Pietruszko et al., 1984; Hafer et al., 1987), with ALDH1A1 usually present in markedly lower concentrations than ALDH2 (Pietruszko et al., 1984). ALDH2 has also been found in numerous rat brain structures, including the LC, SN, and regions of the basal ganglia (Zimatkin and Karpuk, 1996). ALDH1A1 is highly and specifically expressed in human dopaminergic cells of the SN and VTA (Galter et al., 2003a; Grimm et al., 2004). High levels of both ALDH1A1 and ALDH2 have also been demonstrated in cerebral microvessels and postulated to function as an enzymatic blood-brain barrier to biogenic aldehydes (Petersen, 1985). ALDH1A1, purified from human liver, has K_m values of 0.4 and 8 μM for DOPAL and DOPEGAL, respectively (MacKerell et al., 1986; Ambroziak and Pietruszko, 1991). The same study showed that ALDH2 (purified from human liver) has similar affinity for these aldehydes with K_m values of 1.0 and 18 μM for DOPAL and DOPEGAL, respectively. A recent study has shown effective metabolism of DOPAL by human recombinant ALDH2, although no K_m value was determined (Florang et al., 2006). Daidzein, reportedly a selective inhibitor of ALDH2 (Keung, 2001), leads to a significant increase in DOPAL levels in PC-12 cells (Lamensdorf et al., 2000b). In the presence of acetaldehyde, the metabolism of DOPAL and DOPEGAL by ALDH2 has been reported to be significantly impaired, suggesting that these aldehydes compete with acetaldehyde for metabolism by this isozyme (MacKerell et al., 1986). Other studies report that the ALDH-induced oxidation of DOPAL is not dramatically impaired by relatively high concentrations of acetaldehyde (50 μM–1 mM) and suggest that two distinct isozymes of ALDH are responsible for the oxidation of DOPAL and acetaldehyde (Tank and Weiner, 1979; Tank et al., 1981). Metabolism of DOPAL and DOPEGAL by ALDH1A1 was not nearly as affected by acetaldehyde as was ALDH2 and metabolism only began to be significantly impaired in the presence of 100 μM acetaldehyde (MacKerell et al., 1986). These results are in agreement with the well-established observation that ALDH1A1 has a lower affinity for acetaldehyde than ALDH2 and suggest that the opposite may be true in the case of biogenic aldehydes such as DOPAL and DOPEGAL. Further study of ALDH2 and ALDH1A1 isozymes is needed to determine their respective roles in the metabolism of DOPAL and DOPEGAL. Yet to be investigated is whether DOPAL and/or DOPEGAL levels are elevated in individuals lacking functional ALDH2 or who have low ALDH1A1 expression. *Aldh1a1*- and *Aldh2*-null mutant mice have been created and would also be useful to study (Isse et al., 2002; Fan et al., 2003). It is possible that both isozymes participate in the metabolism of these aldehydes in a complementary manner.

Other ALDH isozymes may also be involved in DOPAL and DOPEGAL metabolism. ALDH9A1 participates in an alternate biosynthetic pathway for GABA

in the brain, and this isozyme, purified from human liver, has been shown to efficiently oxidize DOPAL (K_m = 2.6 μM) (Ambroziak and Pietruszko, 1991). Recent association studies of single nucleotide polymorphism combination patterns have linked both ALDH3B1 and COMT with the development of paranoid schizophrenia and have led to the hypothesis that ALDH3B1 may be involved in dopamine metabolism pathways (Xu et al., 2004; Sun et al., 2005). Many other ALDH isozymes have been shown to be present in the CNS and cannot be ruled out as candidates in DOPAL and DOPEGAL oxidation (Table 1). A comprehensive and systematic investigation of the ALDH superfamily is necessary to definitively identify the isozyme(s) responsible for DOPAL and DOPEGAL metabolism in the human brain. This study would serve to clarify the past literature and lead to more precise knowledge of DOPAL and DOPEGAL metabolism.

3. Role of Aldehyde Dehydrogenase Dysfunction. Neuronal accumulation of DOPAL and DOPEGAL has been postulated to be a critical factor in the neurotoxicity of these aldehydes. A potential mechanism that would influence the concentrations of these aldehydes, particularly DOPAL, is a defect or impairment of their metabolism by ALDH. Under normal circumstances, ALDH represents the predominant DOPAL metabolism pathway but is considered a minor enzymatic pathway in DOPEGAL metabolism. However, both oxidative and reductive pathways have been shown to contribute, at least to some extent, to DOPAL and DOPEGAL metabolism (Kawamura et al., 1997) and impairment of minor metabolic pathways is believed to contribute to their accumulation (Lamensdorf et al., 2000b). Moreover, as such, it is also possible that during disease states, the balance between the reductive and oxidative pathways of DOPAL and DOPEGAL metabolism may be disrupted. Mechanisms potentially affecting ALDH function that could influence neuronal concentrations and neurotoxicity of DOPAL and DOPEGAL include genetic and age-related deficiencies, substrate and xenobiotic inhibition, metabolic stress conditions, mitochondrial dysfunctions, and oxidative stress (Fig. 7). As mentioned above, mutations in several ALDH genes resulting in catalytic deficiency have been causally linked to several diseases with neurological abnormalities including Sjögren-Larsson syndrome. In addition, ALDH2 deficiency in humans is a risk factor for late-onset AD (Kamino et al., 2000), and transgenic mice with low activity of ALDH2 exhibit an age-dependent neurodegeneration and memory loss similar to the pathogenesis of AD (Ohta and Ohsawa, 2006). ALDH18A1 deficiency in humans is associated with progressive neurodegeneration (Baumgartner et al., 2005). Age-related decreases in ALDH have also been described in the CNS (Chen and Yu, 1996) and associated with reduced CNS metabolism of aldehydes (Meyer et al., 2004), which could also potentially affect neuronal levels of DOPAL and DOPEGAL and be a factor in age-related neurodegeneration such as idiopathic AD and PD. Indeed, ALDH1A1 mRNA expression is significantly decreased in the SNpc of patients with sporadic PD (Galter et al., 2003a; Mandel et al., 2005). Furthermore, ALDH1A1 protein expression is extremely low within the TH-positive dopaminergic neurons of the SNpc in patients with PD (Mandel et al., 2005).

Substrate inhibition of ALDH has been reported for several aldehydes including DOPAL. As mentioned, DOPAL ($\geq$5 μM) has been reported to impair its own metabolism by directly inhibiting the catalytic function of ALDH (MacKerell and Pietruszko, 1987). Supporting this earlier finding, substrate inhibition of ALDH (specifically ALDH2) by DOPAL ($\geq$19 μM) was recently confirmed and shown to proceed in a dose-dependent manner (Florang et al., 2006). The ability of DOPAL to participate in substrate inhibition of ALDH suggests that this aldehyde can influence its

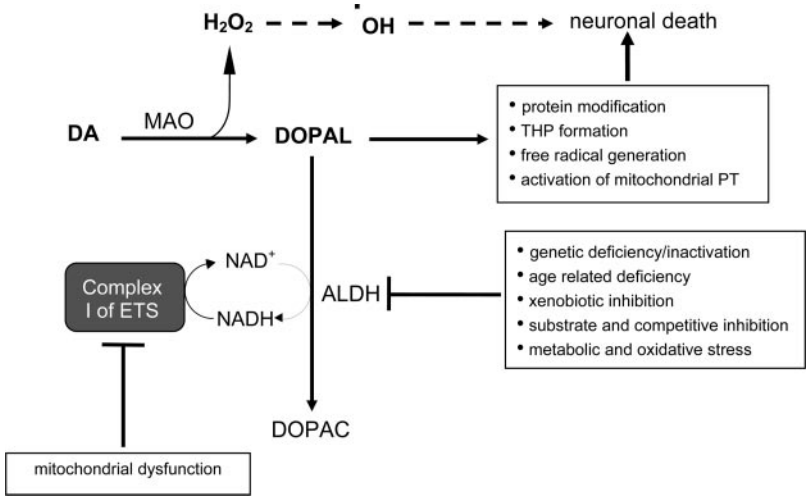

FIG. 7. Potential mechanisms of ALDH dysfunction leading to increased accumulation and neurotoxicity of DOPAL. DA, dopamine; ETS, electron transport system.

own cellular concentration, possibly leading to increased accumulation within neurons.

A striking feature of ALDH isozymes is their sensitivity to in vitro and in vivo inhibition by a number of diverse drugs and environmental agents. Antidipsotropic agents, such as daidzein and disulfiram, are potent inhibitors of ALDH that lead to the accumulation of DOPAL in vitro and in vivo (Lamensdorf et al., 2000b; Legros et al., 2004b). The actions of these inhibitors are not clear, but it seems that in the case of disulfiram the mechanism involves adduction with ALDH (Shen et al., 2001). Disulfiram treatment in humans can be neurotoxic and, in some cases, can lead to basal ganglia damage and even parkinsonism (Laplane et al., 1992), and these effects may be due to ALDH inhibition. Furthermore, it has been suggested that the antidipsotropic effects of daidzein, a selective inhibitor of ALDH2, may be mediated by the accumulation of DOPAL and other biogenic aldehydes (Keung, 2001). Other xenobiotics, including ethanol and several pharmaceuticals can also inhibit the function of ALDH, and it is possible that these agents may influence DOPAL and DOPEGAL levels in the brain. For example, ethanol, through its active metabolite acetaldehyde, has been shown to interfere with the metabolism of biogenic aldehydes including DOPAL and DOPEGAL through the competitive inhibition of ALDH (Davis et al., 1967; Deitrich and Erwin, 1975; MacKerell et al., 1986). A number of ALDH isozymes thought to play a role in acetaldehyde metabolism, namely ALDH2, ALDH1A1, and ALDH1B1, may be affected by this process (Ueshima et al., 1993; Stewart et al., 1995). Acetaldehyde-mediated competitive inhibition of ALDH-DOPAL oxidation has also been shown to result in DOPAL accumulation and increase the generation of THP (Davis et al., 1970). Chlorpropamide, a hypoglycemic drug, undergoes bioactivation to various reactive derivatives that inhibit ALDH (Lee et al., 1992). Haloperidol and pimozide, two medications for Tourette's syndrome, have both been shown to significantly inhibit ALDH activity and may have effects on biogenic amine metabolism (Messiha, 1988). Other drugs including various antineoplastic, antipsychotic, and antibiotic agents also inhibit ALDH activity (Messiha et al., 1983; Vasiliou et al., 1986; Ren et al., 1999). It is therefore highly likely that ALDH inhibition also occurs from various other drugs, dietary substances, and/or environmental agents. For example, toxicological effects associated with the flavoring and fragrance agent, citral, include ALDH inhibition (Boyer and Petersen, 1991).

Metabolic stress, such as glycolysis inhibition, and mitochondrial dysfunction have been shown to adversely affect the activity of ALDH, resulting in a shift in the metabolism of DOPAL that favors its accumulation (Lamensdorf et al., 2000a). Mitochondrial dysfunction, specifically complex I defects, as reported to occur in PD, decrease the availability of the ALDH cofactor NAD^+ and have been shown to affect aldehyde metabolism by ALDH in the CNS (Meyer et al., 2004). Treatment with rotenone, a complex I inhibitor, leads to significant DOPAL accumulation in PC-12 cells, and combined glucose deprivation markedly potentiates this effect (Lamensdorf et al., 2000a,b). Under these conditions, the addition of the ALDH inhibitor daidzein significantly enhanced levels of DOPAL (Lamensdorf et al., 2000b). As mentioned above, the condensation product of DOPAL and dopamine, THP, has also been shown to be a complex I inhibitor, and its formation could further exacerbate ALDH inhibition under conditions of DOPAL accumulation.

Oxidative stress, implicated as a major factor in neurodegenerative diseases (Sayre et al., 2001), may also affect ALDH function. The formation of DOPAL and DOPEGAL involves the generation of H_2O_2, which has been shown to inhibit ALDH activity (Towell and Wang, 1987) and, in the presence of ferrous iron, can give rise to hydroxyl radicals that can dramatically affect cell viability by initiating lipid peroxidation. Levels of 4-HNE and acrolein, highly cytotoxic lipid peroxidation markers, are elevated in AD brain (Lovell et al., 1997, 2001) and increased 4-HNE-protein adducts are found in nigral neurons of PD brain (Yoritaka et al., 1996; Dalfo et al., 2005). The identity of these proteins adducted by 4-HNE has not been elucidated but evidence suggests that ALDH may represent a target. 4-HNE has been shown to be a potent mixed-type inhibitor of ALDH2 with a measured K_i = 0.5 μM (Mitchell and Petersen, 1991). Moreover, 4-HNE, at concentrations within the pathophysiological range (1.5–30 μM) (Lovell et al., 1997), significantly inhibited ALDH2-dependent metabolism of DOPAL in a dose-dependent manner (Florang et al., 2006). 4-HNE (2–50 μM) was also shown to inhibit both rat brain mitochondrial lysate and total rat brain mitochondrial ALDH activity directed toward DOPAL. These findings are especially interesting because these mitochondrial preparations are known to contain a number of other mitochondrial ALDH isozymes, in addition to ALDH2 (Ryzlak and Pietruszko, 1989). Acrolein (30 μM), a lipid peroxidation marker and environmental agent, has been shown to inhibit both mitochondrial and cytosolic ALDH isozymes by a mixed-type inhibition (Mitchell and Petersen, 1988). Therefore, the lipid peroxidation markers, 4-HNE and acrolein, have the potential to significantly affect ALDH function in multiple subcellular regions, providing information about the potential significance of oxidative stress on DOPAL metabolism.

In summary, impaired metabolism of DOPAL and DOPEGAL by a defect in ALDH could lead to the accumulation of these reported neurotoxins and enhance their toxicity by various mechanisms (Fig. 7). Further study of the ALDH proteins involved in the detoxification of these compounds and possible mechanisms disrupting these pathways will further elucidate the role of

this enzyme family in the metabolism and neurotoxicity of DOPAL and DOPEGAL.

C. Alcohol Dehydrogenase

ADHs catalyze the reversible oxidation of alcohols to aldehydes or ketones using NAD^+/NADH as electron acceptor and donor, respectively. These enzymes actually favor (and have higher K_{cat} values for) aldehyde reduction over alcohol oxidation, an observation made for most alcohol/aldehyde pairs (Mardh and Vallee, 1986; Hoog et al., 2001). There are five classes of human ADH enzymes (corresponding to at least seven individual human genes) that differ significantly in substrate specificities and inhibition characteristics (Hoog et al., 2001). Class I ADH is composed of three isozymes, namely ADH1A, ADH1B, and ADH1C, whereas classes II through V each have one isozyme corresponding to the class number (Duester et al., 1999). In the metabolism of DOPAL and DOPEGAL, the corresponding alcohols generated by ADH are DOPET and DOPEG, respectively (Kopin, 1985) (Fig. 2). As mentioned, the reductive pathway by ADH or ALR/AR is favored in DOPEGAL metabolism whereas the oxidative pathway by ALDH is favored in DOPAL metabolism (Kawamura et al., 1997). In DOPEGAL metabolism, hepatic class I ADH isozymes catalyze the conversion of MHPG to the aldehyde, MOPEGAL (Mardh et al., 1985), which is then converted by hepatic ALDH to VMA (Messiha, 1978). Liver class I ADH enzymes have also been reported to efficiently catalyze the reduction of DOPAL (K_m = 8.9 μM), and it has been reported that class I ADH is involved in the metabolism of catecholamines (Mardh and Vallee, 1986; Svensson et al., 1999). The presence of ADH class 1 mRNA has been reported in adult rat brain (Martinez et al., 2001). However, most studies indicate that class I ADH is not present in human brain and, in fact, identify class III ADH as the only brain isozyme (Beisswenger et al., 1985; Galter et al., 2003b). Further investigation into ADH isozyme distribution and function is needed to elucidate the role of the ADH enzyme family in the CNS metabolism of DOPAL and DOPEGAL.

D. Aldehyde and Aldose Reductase

Aldehyde reductase (ALR; AKR1A1) and AR (AKR1B1) are members of the aldo-keto reductase (AKR) superfamily. The AKR family comprises >40 known enzymes and proteins with unique, but broad, substrate specificity. On the basis of their wide substrate specificity, AKRs are thought to be involved in the general detoxification of reactive aldehydes. Human ALR is involved in the reduction of biogenic and xenobiotic aldehydes and is present in virtually every tissue, with the largest amounts being found in kidney cortex and liver (Gabbay and Cathcart, 1974; Wirth and Wermuth, 1985). ALR has been shown to be specifically involved in the metabolism of MAO products of catecholamines, including DOPAL and DOPEGAL (Tabakoff et al., 1973; Tipton et al., 1977), and it is commonly cited as the primary enzyme responsible for the reduction of DOPEGAL. However, it has been proposed on the basis of K_m, which is 1 order of magnitude lower for AR than ALR, and other enzymatic assays, that AR may be the predominant enzyme (Wermuth, 1985; Kawamura et al., 1999). Nonetheless, in the CNS, ALR/AR catalyze the NADPH-dependent reduction of aldehydes to their corresponding alcohols (Tabakoff and Erwin, 1970). In the reduction of DOPAL and DOPEGAL, the corresponding alcohols produced are the same as those produced by ADH, namely DOPET and DOPEG, respectively (Fig. 2). Despite DOPAL being preferentially metabolized by the oxidative pathway, inhibition of ALR/AR has been shown to contribute to the accumulation of DOPAL (Lamensdorf et al., 2000b), indicating that the reductive pathway of DOPAL catabolism is represented to some extent. Similarly, in DOPEGAL metabolism, ALR/AR inhibition has been shown to lead to increased levels of DOMA and decreased levels of DOPEG (Kawamura et al., 1997).

E. Downstream Metabolic Pathways

*1. Catechol-*O*-Methyltransferase.* COMT catalyzes the magnesium-dependent transfer of methyl groups from *S*-adenosylmethionine to the *m*-hydroxyl group of catechol compounds, including catecholamines and their metabolites (Axelrod and Tomchick, 1958). The COMT gene is located on chromosome 22q11 and encodes two transcriptional variants encoding soluble and membrane-bound isoforms of COMT (Craddock et al., 2006). Although these isoforms are present in the brain and share many similarities including reaction mechanisms and structure, they are not identical (Jeffery and Roth, 1984). Membrane-bound COMT is the major brain species and exhibits a higher affinity for catecholamines than does the soluble form (Rivett et al., 1982). In the brain, COMT seems to be predominantly localized in non-neuronal cellular elements, although the presence of small quantities in neurons cannot be excluded. Indeed, one study suggested that membrane-bound COMT is localized predominantly to neurons, whereas soluble COMT is found in glial cells (Rivett et al., 1983). In the DOPAL metabolism pathway, COMT methylates DOPAC to HVA, which, in its free form, is the major human brain metabolite of dopamine released into the blood (Dedek et al., 1979) (Fig. 2). In DOPEGAL metabolism, the *O*-methylation of DOMA to VMA represents a minor pathway, whereas the *O*-methylation of DOPEG to MHPG, followed by sequential hepatic oxidation by ADH and ALDH to produce VMA, predominates (Mardh and Anggard, 1984; Eisenhofer et al., 1996a). Genetic variations in COMT have been associated with a variety of diverse clinical phenotypes, most notably with psychiatric conditions including schizophrenia (Craddock et al., 2006). L-dopa is a substrate for COMT, and high COMT activity has been associated with poor therapeu-

tic management of PD and an increased incidence of drug-induced toxicity (Muller et al., 2006).

2. Phenolsulfotransferase. PSTs are members of a large multigene family of cytosolic enzymes found primarily in the liver, kidney, lung, and brain. They catalyze the sulfate conjugation of many xenobiotics and endogenous substrates using 3′-phosphoadenosine-5′-phosphosulfate as the cofactor. Sulfation is considered to be a detoxification reaction generating highly water-soluble sulfuric acid esters that can be excreted in the urine. PSTs are present in human brain (Rivett et al., 1982), and in the metabolism pathway of DOPAL and DOPEGAL they catalyze the sulfation of DOPAC, HVA, and MHPG (Fig. 2). However, sulfate conjugation of these metabolites occurs primarily in mesenteric organs (Eisenhofer et al., 1996a). There are two human PSTs that can be distinguished by their thermal stability, viz., thermally stable and thermally labile. Thermally labile PST preferentially catalyzes the sulfation of dopamine, epinephrine, and L-dopa, whereas thermally stable PST prefers simple phenols, such as acetaminophen, as substrates (Veronese et al., 1994).

3. UDP-Glucuronosyltransferase. UGTs are microsomal enzymes that catalyze the glucuronidation of a variety of exogenous and endogenous compounds, many of which play important roles in carcinogenesis. Glucuronidation represents a major detoxification pathway that facilitates the elimination of many lipophilic reactive species. To date, 22 human UGT isozymes have been identified (Mackenzie et al., 2005). A wide variety of xenobiotics are known to be glucuronidated and UGT isozymes show broad overlapping substrate specificity toward these chemicals (Miners and Mackenzie, 1991). In contrast, UGT isozymes display distinct substrate specificity toward endogenous compounds such as bilirubin, which is glucuronidated almost exclusively by UGT1A1 (Bosma et al., 1994). Mutations in this gene lead to unconjugated hyperbilirubinemias (Tukey and Strassburg, 2000). Genetic polymorphisms have been identified for almost all UGT isozymes, and many have been associated with various types of cancer (Guillemette et al., 2000; Nagar and Remmel, 2006). Glucuronidation occurs mainly in the liver but can also take place in other organs such as the kidney and gastrointestinal tract. In the metabolism of catecholamines, UGT catalyzes the glucuronidation of MHPG, the major brain metabolite of norepinephrine and epinephrine (Karoum et al., 1977; Boobis et al., 1980) (Fig. 2).

VI. Concluding Remarks

This article is a comprehensive review of the formation, toxicity, and metabolism of DOPAL and DOPEGAL, with particular emphasis on the role ALDH enzymes play in these processes. The presence and activity of DOPAL and DOPEGAL in the human brain have been demonstrated, and their role as neurotoxins in the CNS has been suggested. DOPAL and DOPEGAL seem to induce selective apoptotic cell death in dopaminergic and noradrenergic neurons (respectively) by various mechanisms including free radical generation and activation of the mitochondrial PT. THP, formed from the reaction of DOPAL and dopamine, has been shown itself to be a selective dopaminergic neurotoxin and may prove to be significant in the pathological processes associated with DOPAL. The accumulation of DOPAL and DOPEGAL is believed to be one mechanism underlying their suggested neurotoxicity and may result from their impaired metabolism by various enzyme systems including ALDH. The physiological significance of the ALDH enzyme family is underscored by the fact that impaired metabolism of many endogenous aldehydes, caused by mutations in ALDH genes, is associated with a number of known disease states, many characterized by neurological abnormalities. In addition to genetic deficiencies, many other mechanisms exist that can result in impaired ALDH function and potentially lead to the accumulation and enhanced toxicity of DOPAL and DOPEGAL. The function of the ALDH family may be to provide an initial defense against the toxicity of these aldehydes, particularly DOPAL. However, if this pathway is compromised, elevated levels of DOPAL and/or DOPEGAL may overpower cellular defense mechanisms and eventually lead to neuron death. In this regard, ALDH dysfunction may contribute to the reported neurotoxicity of DOPAL and DOPEGAL and be a factor in the neuropathological conditions with which these compounds may be associated. Further investigation of the mechanisms of neurotoxicity of DOPAL and DOPEGAL and their detoxification by ALDHs is necessary to determine whether any relationship exists between these aldehyde species, the ALDH family, and neurodegenerative diseases.

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