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HYPOTHESIS

Potential Endogenous Epoxides of Tyrosine: Causative Agents in Initiating Idiopathic Parkinson Disease?

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Abstract. The metabolism of tyrosine by tyrosine hydroxylase has the potential for generating reactive and labile intermediates, such as epoxides, with the capacity for acting as neurotoxins upon dopaminergic neurons. Those epoxides that are rapidly hydrolyzed by epoxide hydrolases might not be expected to show toxicity, but those that are shielded and retained might show such properties. Since the types of epoxides produced would be determined genetically, this may explain a genetic component being observed in the causation of Parkinson's disease. Though physiological symptoms observed in Parkinson's disease have been ameliorated through the administration of levodopa, the drug does not prevent the continued metabolism of tyrosine and thereby disease progression. Since metabolism of tyrosine leads to the catecholamines such as dopamine, epinephrine and norepinephrine, it is relevant to examine the biochemical formation of other catechols, such as the catechol estrogens, and determine whether comparable intermediates may be implicated in promoting the disease state.

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Abbreviations used: PD, Parkinson's disease; L-DOPA, levodopa

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1. Hypothesis

Though the cause of idiopathic PD still remains obscure, support for the chemical origins of idiopathic PD, is the fact that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin, formed as a contaminant in the production of an illicit narcotic has the capacity for rapidly and permanently destroying dopaminergic neurons in the *substantia nigra* [1). Though the precise mechanism by which MPTP produces the observed physiological effects remains to be fully clarified, it has been postulated that it is converted within the CNS by MAO-catalyzed oxidation to 1-methyl-4-phenylpyridinium nucleus (MPP+), the ultimate toxin. When individuals ingested MPTP, they experienced severe and rapid end-stage PD.

Another neurotoxin used in laboratory animals to induce Parkinsonism by destroying dopaminergic neurons selectively is 6-hydroxydopamine [2, 3]. Its neurotoxic properties may be due to its oxidation to 2-(2-aminoethyl)-5-hydroxy-1,4-benzoquinone. However, the precise molecular mechanism by which this compound acts neurologically remains to be determined.

These chemical initiators of PD raises an important question, namely, whether some compounds normally produced in intermediary metabolism of tyrosine might possess similar though less neurotoxic properties upon dopaminergic neurons. In essence, this hypothesis proposes that endogenous derivatives produced in some individuals have such a capacity and are the basis for the genetic and progressive nature of PD.

2. Introduction / Background

It has been estimated that in the United States alone there are 1 to 1.5 million Americans who have PD with 60,000 new cases diagnosed each year [4]. Though this disease is usually observed in patients who are older than 60 to 65, it can occur in individuals much earlier in life. Though the exact molecular nature of the disease remains unknown and subject to speculation (5, 6], it has been noted that 15 -20% of PD patients have at least one relative who has demonstrated Parkinson-like symptoms. This observation indicates the possible involvement of genetic factors for some particular families in the etiology of PD.

The clinical and pathophysiological symptoms of those suffering from PD were first described by James Parkinson, an English physician, in 1817 [7]. His detailed exposition of the clinical manifestations of PD have well-served the clinicians since his time. Among the clinical symptoms usually observed are: tremor or trembling of various extremities at rest; rigidity and stiffness of various muscles; slowness of movement known as bradykinesia; and difficulty in walking with impairment in balance and coordination.

Recently, scientists and clinicians begun to understand that the cause of this degenerative and progressive neuromuscular disorder lies with the destruction of dopamine-producing neurons in the brain (CNS), specifically those in the *substantia nigra* and the *locus ceruleus*. By the time symptoms are manifested, it has been estimated that the Parkinsonian patient has lost between 80-90% of these particular nerve cells. It has been suggested that the beginnings of PD may predate symptoms by eight years [8], indicating that with idiopathic PD, disease progression is generally a slow process with progression generally occurring after age 50.

Understandably, clinicians and neuroscientists have focused upon physiological pathology [9] and upon the medicinal therapy regimens [10, 11] required to ameliorate these symptoms in patients. However, the specific biochemical bases by which those nerve cells in the CNS are destroyed remain obscure. In addition to the loss of these dopaminergic neurons, other nondopaminergic neurons are severely affected and this contributes to the observed morbidity. Yet, it is apparent that formation of certain chemical intermediates must predate neuronal destruction and subsequent neurological observations. Gaining needed knowledge as to the structures and metabolites involved might provide a major impetus in understanding the course of PD and ultimately its treatment.

Proof of the importance of the loss of do-

pamine in causing the clinical signs observed was demonstrated with the administration of the dopamine precursor, namely the naturally-occurring amino acid levodopa to both those with idiopathic PD and the disease generated by MPTP and 6-hydroxydopamine. L-DOPA, which is 'the gold standard' for treating PD [12, 13], is capable of crossing the blood-brain barrier (BBB), in contrast with dopamine and dopamine derivatives, where it is decarboxylated to dopamine, reversing the effect on those neurons deprived of dopamine. Carbidopa, an inhibitor of the enzyme dopa decarboxylase, is used [14] in combination with L-DOPA to prevent the latter's premature metabolism before it can penetrate the brain and generate dopamine intraneurally. This remains the most effective form of therapy for PD.

In addition to dopamine replacement strategies, there are dopamine agonists that stimulate the dopamine receptor [15], enhancing and prolonging the effect of dopamine. Other approaches to increase the longevity of dopamine involve the use of monoamine oxidase inhibitors [16] and catechol-O-methyltransferase inhibitors [17] that prevent dopamine metabolism.

It is worth noting that since dopamine is largely the biochemical precursor of both norepinephrine and epinephrine, a reduction in brain dopamine concentration would materially impact the level of these latter two catecholamines in the CNS. And thereby may contribute to the clinical symptoms observed that involve a reduction in their concentrations in nondopaminergic neurons.

There are five important questions that need to be answered in explaining the etiology of idiopathic PD. First, what possible endogenous structures are formed in intermediary metabolism with the capacity for selectively destroying the dopaminergic neurons in the *substantia nigra* region of the CNS? Epoxides are proposed [18] as potential reactive intermediates from exogenous and endogenous substances but these have not yet been related to tyrosine metabolism. Secondly, why does this condition appear to have a genetic component affecting only a relatively small subset of the population [19-24]? Thirdly, why is the disease progressive in face of effective initial therapy in ameliorating its symptoms? Fourthly, why is there with a long lead time between the disease's beginnings and its full pathological manifestations? And, finally, why has no therapy proven effective in slowing disease progression [25]? Any hypothesis that seeks to explain the cause of idiopathic PD must be congruent in addressing the above observations.

Oxidative stress has been implicated in the pathogenesis of PD and other neurodegenerative diseases through the formation of reactive oxygen species (ROS) [26]. In the case of PD, oxidation of tyrosine into the catecholamines becomes important and obligatory in this transformation. The complete metabolic sequence in catechol formation remains as yet undefined. But in the case of the catechol estrogens, a relevant example, arene oxides have been proposed as intermediates in their formation from estradiol [27, 28]. If such intermediates are formed with catechol estrogens, similar precursors may arise with catecholamines since comparable enzymatic steps could be postulated. These arene oxides may be the ROS involved in oxidative stress.

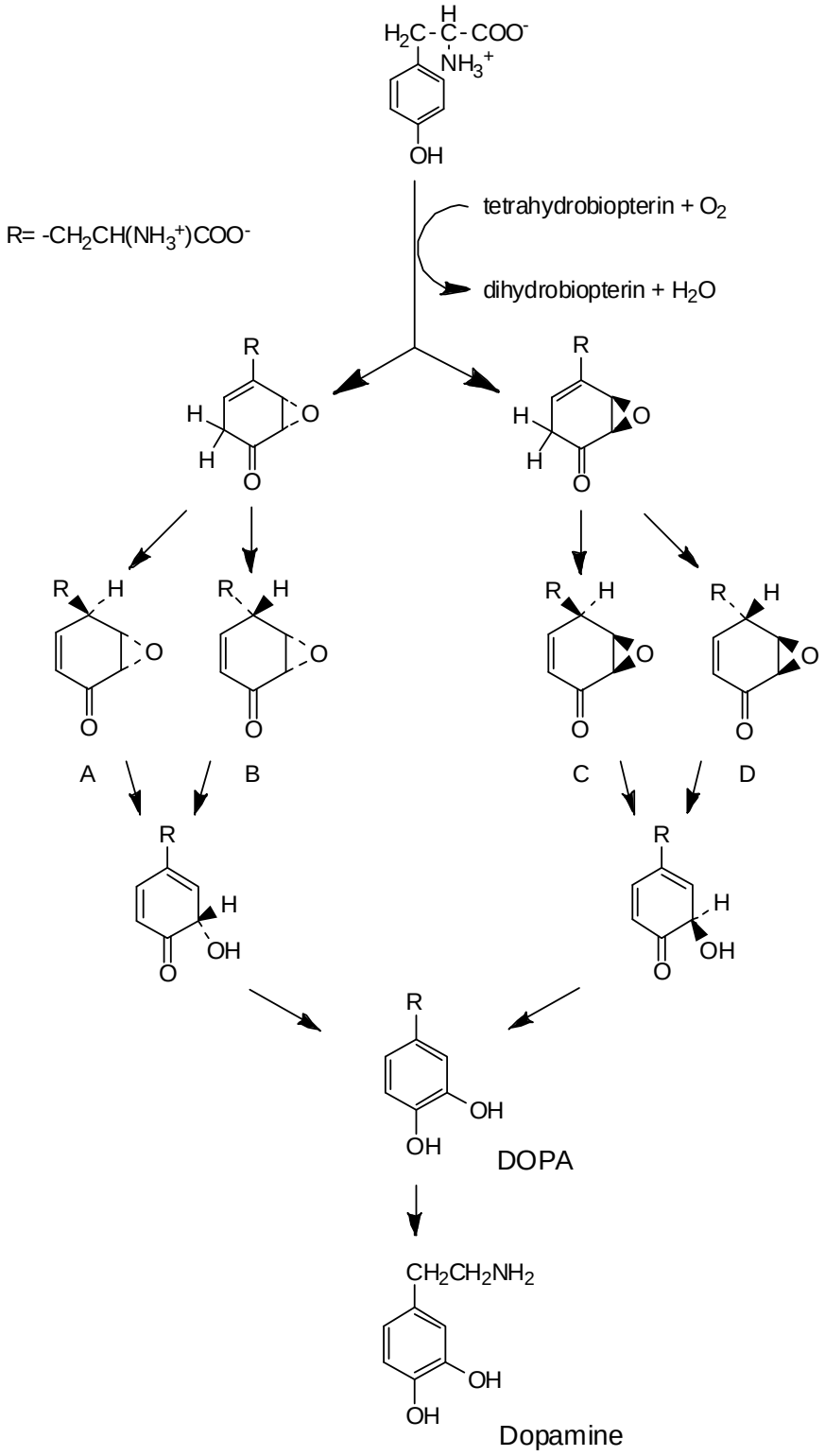
3. Hypothesis / Theory

There is an unproven but implied postulate that the conversion of tyrosine to levodopa involves the direct insertion of a hydroxyl group into tyrosine to produce the catechol amino acid, L-DOPA. No transient intermediates are proposed to be involved and yet the biological conversion of benzene to phenol involves an epoxide [29] and similar structures have been evoked to account for the conversion of estradiol to the catechol estrogens [30, 31].

If such arene oxides are formed in the metabolism of tyrosine, these could react with nucleophilic functionalities on proteins, RNA, and DNA resulting in covalent changes and thereby cellular toxicity. The question is: are such structures produced in the formation of dopa/dopamine? And if so, could they be factors contributing to the pathology observed in PD?

It is known in the case of the metabolism of polycyclic aromatic hydrocarbons that not all arene oxides produce toxic effects. An important

Catechol Formation from Tyrosine: Possible Biochemical Transformations



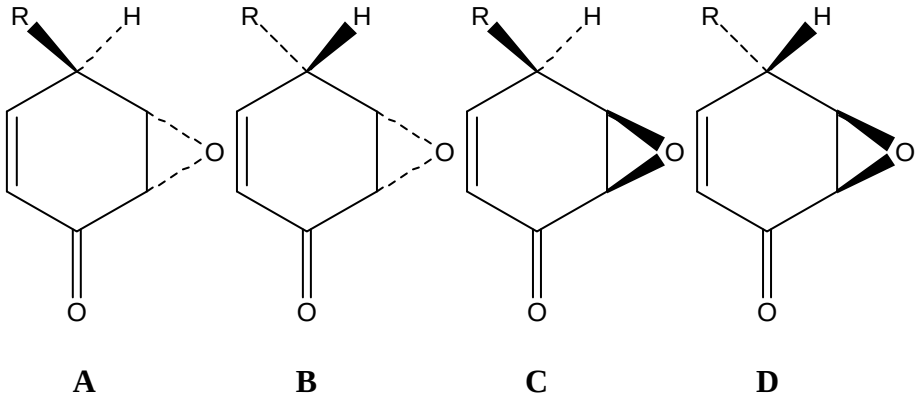


FIGURE 1

factor appears to be the epoxide's biological half-life and the rapidity by which they are hydrolyzed by epoxide hydrolases. When the epoxide is shielded stereochemically and their retention time is sufficiently long, they appear to behave as carcinogens [32]. In the case that such epoxides are formed in tyrosine metabolism, they could behave as neurotoxins toward dopaminergic neurons in the *substantia nigra,* and thereby adversely affect the synthesis of dopa/dopamine.

Thus, it is relevant to examine fully the metabolism involved in the conversion of tyrosine to L-DOPA and determine whether labile intermediates formed in this sequence could be contributing factors in causing idiopathic PD. One of the essential problems in intermediary metabolism has been a primary focus on stable metabolites because of a previous inability to determine the complete metabolic sequence. Yet determining all intermediates in the sequence may be crucial if one is to understand the etiological basis of PD.

Now, with more sophisticated analytical methods, it may be possible to identify transient intermediates and to undertake their syntheses. It is conceivable that such structures are not as labile chemically as previously presumed since potential epoxides derived from estradiol have been synthesized and well characterized [30]. Also in the case of benzene, its arene oxide has now been isolated from biological systems and fully categorized chemically [29].

In the case of tyrosine metabolism, one could expect the four possible epoxides (epoxyenones) as shown in the schematic above (FIG. 1). They are listed as **A**, **B**, **C**, and **D**. None of these have been synthesized chemically and therefore it has not been determined whether any of them are intermediates in the formation of L-DOPA or what, if any, are their effects on dopaminergic neurons. Of the four potential metabolites, compound **A** is probably the more shielded one stereochemically and may not be readily hydrolyzed by epoxide hydrolases to L-DOPA as other epoxides. However, that is speculative and remains to be determined. The rate of hydrolysis may be a crucial factor in determining which, if any, of these structures, have neurotoxic properties. It may be expected that the formation of **A** through **D** would be genetically determined.

In FIG. 1 the stereochemical representations of the four possible arene oxides (epoxyenones] derived from tyrosine are shown. They are **A**, **B**, **C**, and **D**.

All of these structures might be expected to be at least as stable chemically as that of benzene arene oxide [29]. Their preparation would permit one to determine whether these structures are intermediates in the formation of L-DOPA. And if so, what are their effects upon dopaminergic neurons? One can assume that the extent of their formation might be expected to be genetically determined by those enzymes responsible for epoxidation.

The more stable epoxides are those which

might be shielded stereochemically and hindered from hydrolysis by epoxide hydrolases. These epoxides, reacting analogous to suicide substrates, could contribute to the cause of PD by their continuous formation and neurotoxic action upon dopaminergic neurons. And this may explain why the disease has both a genetic component and for which no therapy appears to slow disease progression. Those less stable epoxyenones may be hydrolyzed more readily to L-DOPA with subsequent conversion to dopamine. However from a biological standpoint, more metabolically stable compounds may be the causative factor in the disease.

4. Evaluation of the Hypothesis / Idea

The epoxides shown in FIG. 1 are compounds **A**-**D**. None of which have been synthesized to determine whether any or all are intermediates in the metabolism of tyrosine. Each will have to be prepared and fully characterized chemically before any biochemical studies can be undertaken. Then, it will be necessary to find out whether any of these compounds are formed in the metabolism of tyrosine by means of trapping experiments using either radiolabeled or deuterium-labeled tyrosine in which the label will not be removed in tyrosine's metabolism. This is a critical first step.

If any of these arene oxides (epoxyenones) are intermediates in tyrosine's metabolism to L-DOPA, then their individual neurotoxic properties could be determined. None of these would be expected as neurotoxic as MPTP/MPP+ or 6-hydroxydopamine but the metabolism of tyrosine forming these epoxides may serve to provide a continuous source of endogenous neurotoxins, despite their low potency. In effect, this would serve to explain why L-DOPA therapy, though initially effective, is unable to prevent the further deterioration of a patient's symptoms. Since the formation of the various epoxyenones would be expected to be controlled genetically, this could account for why only a small subset of the population is subjected to idiopathic PD. In essence, there could be endogenous structures produced that are responsible for the disease's progression and these must be recognized before an effective therapeutic regimen can be initiated. The hypothesis' main thesis is that metabolic intermediates in tyrosine's metabolism may be the causative factors that are the responsible entities for idiopathic PD.

5. Consequences of the Hypothesis and Discussion

The failure of L-DOPA to ameliorate completely idiopathic PD may be due to the continued metabolism of tyrosine. If that were the case, then effective therapy must not only focus on the replacement of L-DOPA in the CNS and a suitable concentration level of dopamine therein but in blocking the continued metabolism of tyrosine to produce certain arene epoxide precursors of L-DOPA. In essence a two-pronged approach may be essential for the treatment of idiopathic PD.

Without understanding the biochemical causes, proposing a treatment regimen remains incomplete and unfortunately flawed. It may be understandable that the focus on the metabolism of tyrosine has been only on those chemical intermediates that are stable and readily isolatable. However, it is apparent that labile and genetically-controlled reactive intermediates in any biochemical sequence may be of crucial importance in probing disease causes. Now, with more sophisticated analytical instrumentation than heretofore, it is becoming increasingly possible to measure reactive and transient intermediates *in vivo*. These compounds may be important and contributing factors in disease progression. Once a full understanding of the biochemical causes of idiopathic PD have been determined, then rational and effective therapy can be designed and initiated. It may be that certain arene oxides (epoxyenones) produced in the normal metabolism of tyrosine have been the critical and unknown factors responsible for both the initiation and subsequent progression of this neurodegenerative disease.

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