Intracyclization Rates of 6-Hydroxydopamine and 6-Aminodopamine Analogs under Physiological Conditions†

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It is agreed that the neurotoxic action of 6-hydroxydopamine and 6-aminodopamine is related to their ease of oxidation. The initial oxidation products, the *p*-quinone and *p*-quinone imine, readily undergo 1,2-intracyclization. These reactions could represent an important loss of active neurotoxic agent available for uptake. A variety of substituted 6-aminodopamine analogs was prepared and their formal potentials and cyclization rates were measured accurately. The effect of the balance of ease of oxidation vs. rate of cyclization on their neurotoxicity was examined. The results are in general accord with in vivo lifetimes for 6-hydroxydopamine and 6-aminodopamine in rat caudate nucleus.

Since the discovery of the neurotoxicity of 6-hydroxydopamine (6-OHDA), a great deal of effort has gone into attempts to understand the molecular mode of action of this remarkable compound. Only a few close analogs of 6-OHDA, such as the corresponding α-methyl-6-OHDA and 6-aminodopamine (6-ADA), have similar neurotoxicities.[1] It appears generally agreed that the selective neurotoxicity of 6-OHDA-like compounds is related to specific uptake into catecholamine neurons.

Almost all workers agree that the distinctive action of 6-OHDA is directly correlated with its ease of oxidation. Beyond this point, the picture is less clear. Either the 6-OHDA oxidation products or generated H_2O_2 and/or superoxide ion may be responsible for the neuronal destruction. The current status of this problem is unresolved.[2,3]

However, it is clear that if 6-OHDA and its analogs are to be selective, the damaging oxidation process, whatever its exact nature, occurs intraneuronally following uptake. But 6-OHDA-like compounds, when injected into nervous tissue, rapidly react with oxygen and other endogenous oxidants and, hence, extraneuronal oxidation cannot be prevented. Indeed, in vivo electrochemical measurements have already shown that ca. 20% of the injected 6-OHDA is converted to its oxidized *p*-quinone (6-OHQ) form within a few minutes after injection into rat caudate nucleus.[4,5]

The 6-OHQ is, of course, the conjugate oxidant of 6-OHDA and in redox equilibrium with it. In that sense, the *p*-quinone is still available for selective uptake, unless it undergoes further chemical reactions which remove it from the redox equilibrium. It is just such fast chemical reactions of the *p*-quinone (or the *p*-quinone imine from 6-ADA) that are the subject of this study. Earlier work showed that both 6-OHDA and 6-ADA upon oxidation undergo a 1,2-intracyclization, leading first to the *p*-quinoidal oxidized indoline, rearranging to the same 5,6-dihydroxyindole.[6] Thus, this intracyclization could rapidly remove the *p*-quinone or quinone imine from the redox equilibria. (Other rapid nucleophilic reactions with endogenous compounds can lead to generalized extraneuronal damage but are not the concern of the present studies.)

Although very difficult to do by other methods, electrochemical measurements provide accurate rates for these intracyclizations at physiological pH. By synthesis manipulation of the ethylamine side chain, as well as the "6"-substituent, it is possible to vary the intracyclization rates over a wide range—with and without significant variations in the initial ease of oxidation of the 6-OHDA or 6-ADA analogs. The present studies investigate the balance between ease of oxidation and intracyclization and the effect of this balance on the neurotoxic properties of 6-OHDA-like compounds. The rates of formation of the cyclized products have also proven to be of significance in interpreting the results of inhibition of catechol *O*-methyltransferase activity by 6-OHDA and 6-ADA derivatives as reported in this issue by Borchardt and coworkers.[7]

Results and Discussion

Table I summarizes the results. The ease of oxidation of each compound to the corresponding *p*-quinone or *p*-quinone imine is indicated in the second column. The value $E^{0'}$ (7.4) is the formal reduction potential measured in pH 7.4 buffer. These values are within a few millivolts of the classical standard reduction potential. The more negative the value of $E^{0'}$ (7.4), the more easily is the compound oxidized. The next two columns show the observed rate constant for intracyclization, k_{obsd}, and the half-life, $t_{1/2}$, of the *p*-quinone or *p*-quinone imine. The values of $t_{1/2}$ are rounded off for easy comparison.

That a balance between ease of oxidation and rate of intracyclization may indeed be important to the uptake availability is seen by comparing 6-OHDA and 6-ADA. While both are easily oxidized by the brain environment, it is considerably easier to oxidize 6-OHDA. Its *p*-quinone intracyclizes only slowly ($t_{1/2}$ = 38 min) and hence its availability to the redox equilibrium is only slightly altered. With 6-ADA, any *p*-quinone imine rapidly disappears via intracyclization with a $t_{1/2}$ of only 28 sec. One might conclude that if this rapid conversion occurred extraneuronally, it would seriously limit the effective uptake of 6-ADA. But if one "corrects" the $t_{1/2}$ for the relative differences in ease of oxidation of 6-OHDA and 6-ADA, it is next shown that the two compounds would be approximately equally effective.

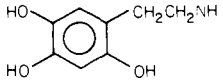
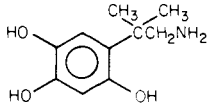
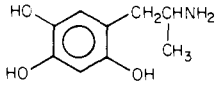
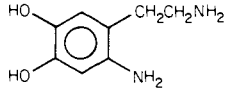
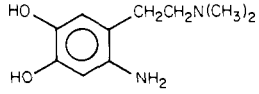
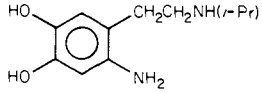
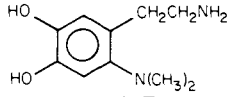
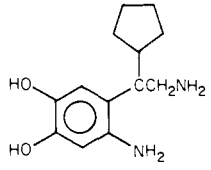
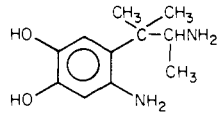
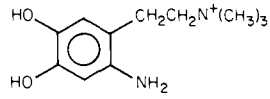
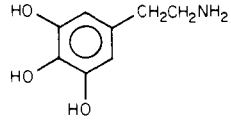
McCreery established that an active redox buffer system in rat brain (very likely ascorbate–dehydroascorbate) interacts rapidly with 6-OHDA-like compounds to establish their redox equilibrium and that the potential of this brain system is −0.200 V vs. SCE.[8] From this potential and the $E^{0'}$ (7.4) for 6-OHDA and 6-ADA, one can calculate directly from the Nernst equation (with constants for 37°C), the ratio C_{ox}/C_{red} for 6-OHDA and 6-ADA in brain tissue. If these ratios are expressed as

$$R = C_{ox}/(C_{ox} + C_{red})$$

(i.e., for 6-OHDA, R is equivalent to the concentration of 6-OHQ divided by the sum of the concentrations of 6-OHQ

† This paper is dedicated to the memory of Big Ed, who was always there when we needed him. His enthusiasm and drive contributed much to this work.

Table I. Electrochemical Rate Data of 6-OHDA-Like Compounds

No.	Reductant	$E^{0'}$ (7.4) (V vs. SCE)[a]	Intracyclization rates	
			k_{ox}, sec^{-1}	$t_{1/2}$[b]
	6-OHDA Derivatives			
1		−0.210	$2.98 \pm 0.21 \times 10^{-4}$	38 min
2		−0.210	0.07 ± 0.02[c]	10 sec
3		−0.210	$4.53 \pm 0.32 \times 10^{-4}$	25 min
	6-ADA Derivatives			
4		−0.135	0.0244 ± 0.007	28 sec
5		−0.130	0.0153 ± 0.0003	46 sec
6		−0.122	0.0117 ± 0.0002	59 sec
7		−0.051	1.429 ± 0.008	500 msec
8		−0.11	6.90 ± 0.22[c]	100 msec[c]
9		−0.12	15.35 ± 2.3[c]	45 msec
10		−0.12	0	
	5-OHDA			
11		−0.029	29 ± 10[c,d]	20 msec

[a] $E^{0'}$ (7.4) is the formal potential at pH 7.4 and 25°C in V vs. SCE, as measured on a hanging mercury drop electrode. [b] Half-life, rounded to two significant figures for ease of comparison. [c] Measured at 25°C; too fast to measure by existing techniques at 37°C or compound too unstable. [d] This reaction is not the usual intracyclization but a second-order reaction; by analogy to the reactions of pyrogallol, probably dimerization.

and 6-OHDA), one finds from the $E^{0'}$ (7.4) data

$R_{6\text{-OHDA}} = 0.33$

$R_{6\text{-ADA}} = 0.025$

Assuming that intracyclization of the oxidized form in vivo is the predominant pathway of extraneuronal disappearance of both initially oxidized and reduced forms, we can derive the following rate expression

$$-d(C_{ox} + C_{red})/dt = k_{ox}C_{ox}$$

where k_{ox} is the electrochemically measured intracyclization rate (Table I). This expression can be rewritten to express the total concentration of oxidized and reduced forms

$$-d(C_{ox} + C_{red})/dt = k_{ox}R(C_{ox} + C_{red})$$

From this we can calculate the half-life of the total concentration of oxidized and reduced forms in vivo

$$t_{1/2\ obsd} = 0.693/k_{ox}R$$

Utilizing the values of k_{ox} in Table I and the R values above one finds

for 6-OHDA, $t_{1/2\ obsd} = 1.9$ hr

for 6-ADA, $t_{1/2\ obsd} = 0.3$ hr

Although gross assumptions are involved, these calculated $t_{1/2\ obsd}$ are satisfying because they are the same orders of magnitude as experimentally determined values for in vivo lifetimes of 6-OHDA and 6-ADA, respectively. Using in vivo injection techniques, McCreery et al.[4,5] found electrochemically detectable 6-OHDA had an in vivo lifetime of ca. 30 min and 6-ADA ca. 90 min. This agreement is considered quite satisfactory in view of the fact that the intracyclization is assumed to be the only reaction of the oxidized forms. Similar calculations for compound **3** indicate a $t_{1/2\ obsd}$ of 1.3 hr, a result in agreement with its established neurotoxicity and our hypotheses.

Pursuing this argument for the other 6-ADA derivatives (compounds **5–9**) suggests that all of them would be considerably less effective neurotoxins. They are oxidized within 10–15 mV as easily as 6-ADA; yet, the lifetimes of their oxidation products are extremely short. This is in accord with experimental results—none of the compounds when injected intracerebrally into mouse brain caused any long-term norepinephrine depletion.[9] However, this does not validate the above hypothesis. For obvious structural reasons, it is almost certain that these compounds are not taken up effectively by catecholamine neurons. Hence, there is no effective way of testing the arguments for these 6-ADA derivatives. Similarly, compound **10**, the side-chain quaternary compound, oxidized readily yet cannot intracyclize. It should be an effective compound by the arguments above. Obviously, however, it is not taken up by catecholamine neurons.

It is interesting, in this light, to compare the behavior of the so-called 5-hydroxydopamine, compound **11**. Its quinone undergoes very rapid chemical reaction ($t_{1/2} \sim$ 20 msec) and, at first glance, might be considered a poor candidate for uptake. However, it is considerably more difficult to oxidize than either 6-OHDA or 6-ADA since it can only form an *o*-quinone. Thus, it remains a very effective molecule for uptake and is widely used as a catecholamine neuronal "marker". However, even when it accumulates intraneuronally, it is not neurotoxic because it is too difficult to oxidize.

Experimental Section

Compounds **1** and **11** (Table I) were reagent grade, commercially obtained compounds (Regis). Compound **4** was prepared locally as previously described.[10] The syntheses of compounds **5**, **8**, **9**, and **10** are described in a paper in this journal.[7] Compounds **2** and **3** were prepared by Reid,[11] and compounds **6** and **7** were prepared by Nerland and Smissman.[12]

Conventional electrochemical techniques were used to measure the oxidation potentials and the rates of reaction of the generated quinones. Oxidation potentials (and qualitative rate information) were obtained by cyclic voltammetry (a voltage scanning technique) at scan rates of 2 V/min up to 1000 V/min. In addition to yielding information about the redox properties of the quinone–hydroquinone pair, cyclic voltammetry detects the presence of electroactive products of chemical reactions of the oxidized species. The reported oxidation potentials were measured with a Hg electrode vs. a saturated calomel electrode (SCE). A further description of cyclic voltammetry and other electrochemical techniques and their application to molecules of neuropharmacological interest is available.[13]

To measure the rate of reaction of quinone, the hydroquinone was oxidized at a constant potential and the current–time decays were observed to follow an inverse $\sqrt{t}$ behavior which indicates that any chemical reactions of quinone generate a reducible species. At a later time, the electrode was switched to a potential sufficient to reduce the quinone previously electrogenerated. The measured current at this potential corresponds to the amount of quinone which has not been consumed by a chemical reaction. From a knowledge of the potential switch time (which can vary from 50 msec to 20 sec) and the amont of quinone consumed, one can calculate the reaction rate of the quinone. Further details of this technique are available.[9] Rates were measured at a Hg electrode and a carbon paste electrode with identical results.

Since many of the compounds in Table I are easily air-oxidized at physiological pH, all solutions were deoxygenated with a vigorous stream of argon. Under these conditions the hydroquinone forms were stable for about 30 min at millimolar concentrations.

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Stereochemical Studies of Adrenergic Drugs. Diastereomeric 2-Amino-1-phenylcyclobutanols†

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The synthesis of the *cis*- and *trans*-2-amino-1-phenylcyclobutanols **2** and **3** is described. The results of the potentiation of the action of (−)-norepinephrine by these two compounds are discussed.

The mechanism by which catecholamines, as well as other phenethanolamines, interact with various segments of the adrenergic nervous system has been of great interest in recent years.[1-3]

Almost all of the phenethanolamine derivatives studied thus far have been conformationally flexible molecules. An

† This paper is dedicated to Edward E. Smissman.