Applications of Electrochemistry in Studies of the Oxidation Chemistry of Central Nervous System Indoles

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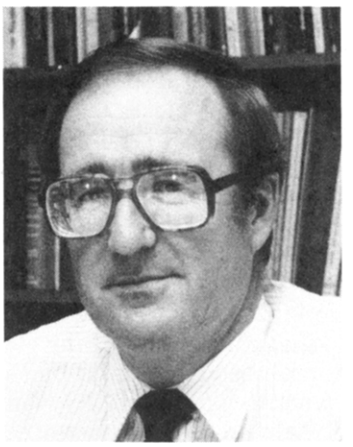
Contents



Glenn Dryhurst was born in Birmingham, England, in 1939. He obtained his Ph.D. in Analytical Chemistry at the University of Birmingham in 1965 and then spent 2 years as a postdoctoral research associate with Professor Philip J. Elving at the University of Michigan. In 1967 Dryhurst joined the Department of Chemistry and Biochemistry at the University of Oklahoma where he currently serves as Chairman of the Department and holds the position of George Lynn Cross Research Professor of Chemistry and Biochemistry. During 1987/1988 he was a Fulbright Senior Professor at Konstanz University in Germany. Dryhurst's research interests have focused on the electrochemistry and interfacial behaviors of biologically significant N-heterocyclic molecules, particularly purines, pteridines, and most recently indoles. His current research is aimed at understanding the in vitro and in vivo oxidation chemistry and biochemistry of indolic neurotransmitters and neurotoxins and the relationships of such chemistry to an understanding of the etiology of neurodegenerative illnesses. Dryhurst currently serves as Chairman of the Organic and Biological Electrochemistry Division of The Electrochemical Society and as a Divisional Editor of the *Journal of the Electrochemical Society*. He is the author or coauthor of four books and more than 140 research papers.

I. Introduction

Oxidation and reduction reactions play a dominant role in energy conversion and substrate metabolism in living organisms. For example, redox processes are involved in the conversion of solar radiation into chemical energy, which takes place in the photosynthetic apparatus of plants. This apparatus serves as the primary source of energy for all living systems. The respiratory electron transport chain is a series of oxidation–reduction reactions by which an initial electron-donor species, e.g., pyruvate or malate, is oxidized and, ultimately, molecular oxygen is reduced. This chain accomplishes the oxidation of NADH to NAD^+ and $FADH_2$ to FAD, which are then employed in oxidative phosphorylation. The metabolism of many naturally occurring substances, drugs, and other xenobiotics proceed by oxidative or reductive pathways. The redox character of such processes suggests that electrochemical techniques should provide useful tools to investigate their thermodynamics, kinetics, and mechanistic pathways. Several recent books and monographs have reviewed much of the electrochemical work that has been carried out to elucidate the oxidation and reduction chemistry and biochemistry of biologically significant compounds.[1-6]

During the past two or three decades the instrumentation for and theory of electrode processes have been developed to a very high level of sophistication.[7] As a result, modern electroanalytical techniques can in principle be employed to probe the most subtle nuances of redox reactions occurring at an electrode surface. There is now much evidence that redox reactions mediated by enzymes can be mimicked at an electrode surface. Hence, much biochemically relevant information can be derived from investigations of electrochemically driven redox reactions. The elegant studies of Savéant and his co-workers on the electrochemistry of vitamin B_{12} and related species provide classic examples of the detail that can be obtained about biological redox systems with use of electrochemical approaches (see ref 8 for a review of these studies). Many enzyme-mediated redox reactions are highly substrate selective and have been designed by nature to proceed under only carefully controlled, but, often, extremely complex conditions. As a result, it is difficult to elucidate mechanistic details of these processes. By contrast, an electrochemically driven reaction can generally be studied over a very wide range of experimental conditions. The effects of potential, pH, buffer, solvent, and structural modifications of substrate are readily studied so that great mechanistic insight into a redox reaction can be obtained. Thus, if an electrochemically driven and enzyme-mediated redox reaction can be shown to yield the same product profile, then a careful investigation of the electrode process can provide profound insight into the chemical mechanisms associated with the biochemical reaction. New electroanalytical techniques provide the ability to detect extremely reactive, short-lived intermediate species formed in an

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electrode reaction. The most recent and, potentially, most important of these is fast-sweep cyclic voltammetry using ultramicroelectrodes.[9]

The fact that many electrochemical and enzymatic redox reactions proceed by *chemically* identical reaction pathways suggests that in instances where little is known about the metabolism of a particular substrate it would be useful to conduct electrochemical investigations of its redox chemistry. These investigations would provide information about reduction and oxidation potentials, formation of reactive intermediate species, reaction pathways, and mechanisms. These in turn might well be of relevance to the biological transformations of the compound.

Electrochemical techniques by themselves, however, cannot be used to elucidate the overall mechanism of an electrode process. It is absolutely essential that the ultimate products of the electrochemical reaction are isolated and identified. This generally requires that chromatographic methods are employed to separate and purify reaction products and that powerful modern spectroscopic techniques (e.g., NMR, MS, IR, UV–visible, and X-ray diffraction) are used for structure elucidation. Attempts should also be made to characterize reaction intermediates by electroanalytical, spectral, and kinetic methods. Many electrochemical investigations are seriously flawed by their failure to support mechanistic conclusions by adequate characterization of reaction products and intermediates. Nevertheless, in those instances where electrochemical studies are coupled with appropriate chromatographic, spectral, and kinetic investigations, a vast amount of information can often quite rapidly be generated about the redox properties of biologically significant compounds. This information will include, inter alia, structures of potential biochemical products and techniques for their detection and characterization. As a result, when investigations of the in vitro or in vivo redox transformations of the compound of interest are undertaken, the search for metabolites and the elucidation of mechanism can be greatly facilitated.

During the past 20 years, work in this laboratory has been concerned with studies of the oxidation chemistry and biochemistry of various classes of nitrogen heterocyclic compounds. We have used electrochemical techniques to explore the fundamental oxidation chemistry of such compounds and to assist in understanding enzyme-mediated oxidation processes. The underlying rationale for such studies, as described earlier, is that the mechanisms and product profiles elucidated in carefully controlled electrochemical investigations can assist in understanding biological oxidation processes.

This review will first focus on recent work on the electrochemical oxidation of some 5-hydroxyindole compounds that are found naturally in the mammalian central nervous system. More than 20 years of biochemical and chemical research failed to yield information beyond the fact that 5-hydroxyindoles can be oxidized. Electrochemical investigations, on the other hand, have allowed considerable progress toward understanding the basic oxidation chemistry of these compounds. In addition, the use of electrochemical approaches to assist in understanding the oxidation chemistry of some indolic neurotoxins will be described. Our investigations into the oxidation chemistry and biochemistry of endogenous 5-hydroxyindoles and of indolic neurotoxins are at an early stage. Nevertheless, it will be shown that electrochemical investigations might provide valuable clues concerning the role of oxidation reactions of endogenous 5-hydroxyindoles in the etiology of some mental illnesses and how the oxidation reactions of indolic neurotoxins play a central role in their neurodegenerative effects.

II. Indoles in the Central Nervous System

The aromatic amino acid L-tryptophan (TPP) is one of a rather select group of compounds that can cross the blood–brain barrier. In the central nervous system (CNS) TPP undergoes the critically important metabolic transformations illustrated in Figure 1.[10–16] TPP is first converted to 5-hydroxytryptophan (5-HTPP), by the enzyme L-tryptophan hydroxylase, and is then decarboxylated, by 5-hydroxytryptophan decarboxylase, to give 5-hydroxytryptamine (5-HT; serotonin). The known catabolic fate of 5-HT proceeds by an initial oxidation reaction, catalyzed by monoamine oxidase, to give 5-hydroxyindole-3-acetaldehyde (5-HIAD). The principal fate of this aldehyde is oxidation by an aldehyde dehydrogenase to 5-hydroxyindole-3-acetic acid (5-HIAA) although a small fraction is reduced (aldehyde reductase) to the alcohol 5-hydroxytryptophol (5-HTOL). In the pineal gland, 5-HT is converted to the hormone melatonin by the route shown in Figure 1.

Following the initial discovery of 5-HT in the brain[17,18] and because of its structural resemblance to certain psychoactive drugs,[19] the indolamine began to be suspected of being the chemical neurotransmitter it is now known to be. Neurons (i.e., nerve cells), which employ 5-HT as a chemical neurotransmitter, are known as serotonergic neurons. Serotonergic pathways have been implicated, in the regulation of body temperature, sleep, moods, and emotional states.[20]

At about the time that 5-HT was first discovered in the brain, suggestions began to appear in the literature that a defect in the normal CNS metabolism of the indolamine (Figure 1) might in some way be related to mental illnesses.[21,22] Subsequently, such hypothetical faulty metabolic pathways were implicated with mental illnesses such as schizophrenia and depression.[23–26] A recurring suggestion was since the catabolism of 5-HT is oxidative in nature, that 5-HT might be converted to more highly hydroxylated indolamines that are toxic in the CNS, hence resulting in neuronal damage.[14,27,28] The fact that 5,6-, 5,7-,[29–35] and 4,5-dihydroxytryptamine[33,36] and more highly hydroxylated indolamines are powerful neurotoxins in mammalian brain lends significant credence to this suggestion. Furthermore, it has been known for many years that 5-HT and other endogenous 5-hydroxyindoles are oxidized in the presence of human serum and ceruloplasmin.[37–43] Hemolysates of erythrocytes also oxidize 5-HT and other 5-hydroxyindoles.[44] It has also been suggested[45] that some melanins found in the CNS might be derived from oxidation reactions of 5-hydroxyindoles. In vitro experiments with 5-HT, 5-HTOL, and 5-HIAA support the latter suggestion.[46] Microsomal melanogenesis from 5-hydroxyindoles has been hypothesized to proceed by oxidation to very reactive quinone imine intermediates that subsequently polymerize.[46] Experiments with

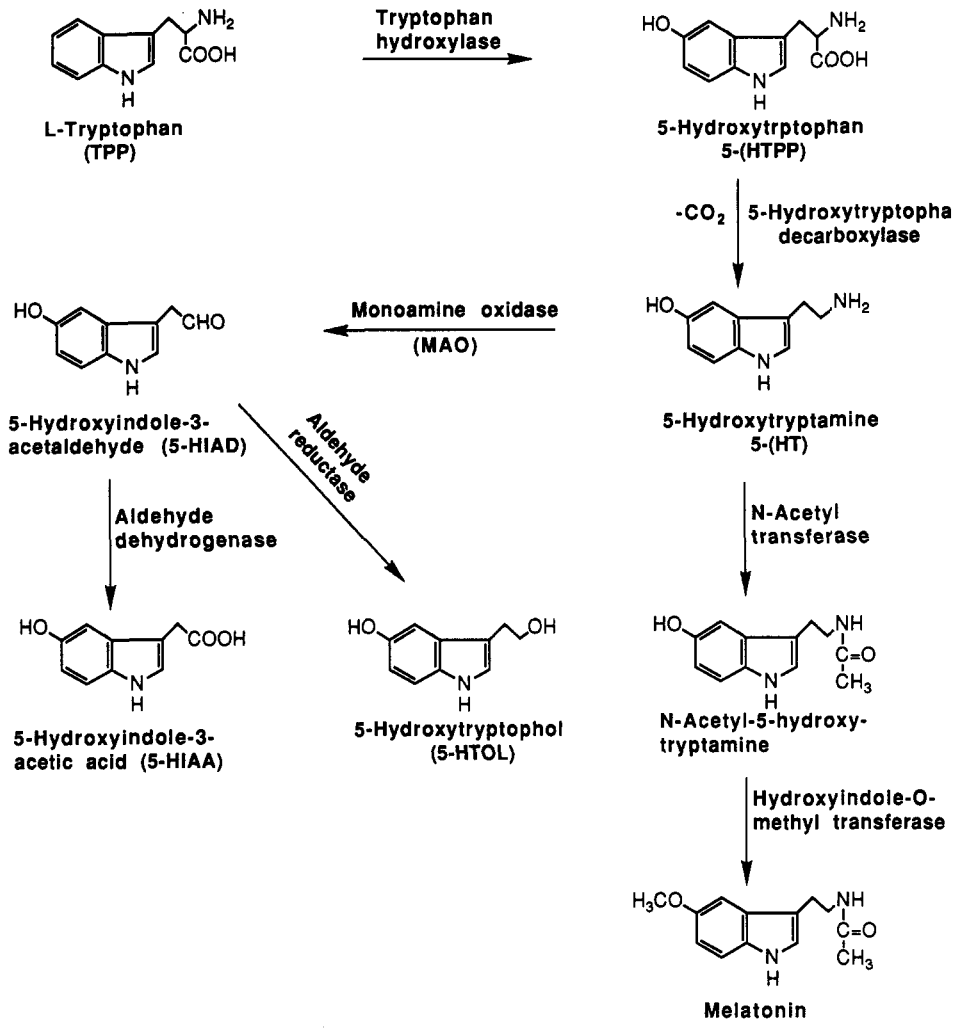


Figure 1. Metabolic pathways for L-tryptophan (TPP) in the central nervous system.[10-16]

molecular oxygen and Ag^+ reveal that 5-HT and other 5-hydroxyindoles are easily oxidized compounds. A relatively long-lived dimeric intermediate has been speculated to be formed in such oxidations.[28] Oxidation of 5-HT by ferricytochrome *c*,[47] alkaline permanganate,[48] autoxidation in basic solution,[49] ceruloplasmin,[50] and during metabolism[51,52] have been claimed to generate radical intermediates although the fate of these radicals has not been investigated.

By the mid-1980s it was clear that endogenous 5-hydroxyindoles are easily oxidized compounds both chemically and biochemically. Suggestions about oxidative intermediates included radicals,[47-52] quinone imines,[46] dihydroxyindoles,[44] and a dimer.[28] Products have been speculated to include uncharacterized melanin-like pigments.[46] However, as a result of numerous studies only one indisputable fact emerged: namely, that the endogenous 5-hydroxyindoles are easily oxidized compounds. Not a single product of such oxidations had been isolated and structurally characterized. Furthermore, nothing of significance was known about the biological role of oxidative intermediates or products in the CNS. In view of the suggestion that anomalous oxidations of 5-HT or other endogenous indoles might play a role in the etiology of mental illnesses, it was clear that a comprehensive study of the oxidation chemistry and biochemistry of these compounds was necessary. Accordingly, a program was initiated in this laboratory to study the oxidation reactions of endogenous indoles. Electrochemical techniques have played a crucial role in these studies.

III. Electrochemical Oxidations of CNS Indoles

The electrochemical oxidation of 5-HT, 5-HTPP, and 5-HTOL in aqueous solution has now been studied extensively. The primary focus of this section will be on the chemical neurotransmitter 5-HT although the behaviors of 5-HTPP and 5-HTOL will be discussed when they differ significantly from 5-HT.

Analyses for 5-HT in various regions of mammalian brain reveal concentrations ranging from about 0.7 to 42 μM in wet tissue.[53,54] However, in both central and peripheral serotonergic neurons, 5-HT is stored in synaptic vesicles at very much higher concentrations.[55] Accordingly, the electrochemistry of 5-HT has been studied at concentrations ranging from $\leq$30 μM to >10 mM.

A representative cyclic voltammogram (CV) of 20 μM 5-HT using a pyrolytic graphite electrode (PGE) at pH 2 is shown in Figure 2. At this very low concentration, two oxidation peaks, I_a and II_a, appear. After scan reversal a reversible couple (peaks III_c/I_a') appears. With increasing concentrations of 5-HT, oxidation peak II_a grows somewhat relative to peak I_a and above about 0.1 mM 5-HT an additional reversible couple (peaks II_c/II_a'; Figure 3) appears at more positive potentials than peaks III_c/I_a'. At very low 5-HT concentrations oxidation peaks I_a is strongly influenced by adsorption effects.[56,57] However, at high 5-HT concentrations ($\geq$6

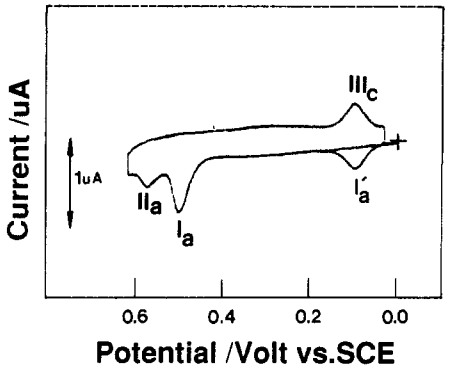


Figure 2. Cyclic voltammogram at the PGE of 20 μM 5-hydroxytryptamine at pH 2.0. Sweep rate 20 mV s^{-1} (reprinted from ref 56; copyright 1987 American Chemical Society).

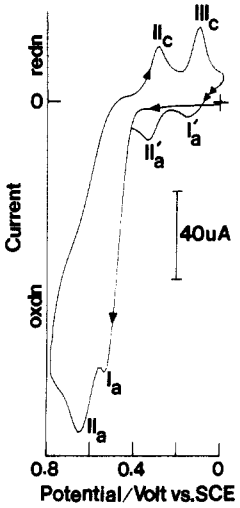
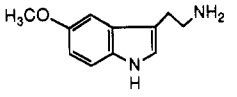


Figure 3. Cyclic voltammogram at the PGE of 11.5 mM 5-hydroxytryptamine at pH 2.0. Sweep rate 200 mV s^{-1} (reprinted from ref 58; copyright 1990 Elsevier).

mM) peak I_a exhibits characteristics close to those expected for a reversible diffusion-controlled one-electron process.[58,59] Furthermore, the effects of sweep rate, 5-HT concentration, and pH on the peak potential (E_p) for peak I_a are in approximate accord for those theoretically predicted for a family of electrochemical dimerization reactions.[60] The CVs shown in Figures 2 and 3, however, clearly reveal that the peak I_a reaction is more complex than a simple dimerization in view of the fact that at least two reversibly reduced oxidation products must be formed that are responsible for the II_c/II_a' and III_c/I_a' couples. The complexities of the overall peak I_a process may be resolved by an analysis of the products formed by electrooxidation of 5-HT.[56-58,61] Such an analysis reveals that the major projects are the simple dimers **9–12** (Scheme I). Thus, on the basis of characteristics of peak I_a and the fact that simple dimers are the major electrooxidation products, it has been proposed[58] that 5-HT is initially oxidized in a reversible one-electron abstraction reaction to the radical cation 5-HT$^{\bullet+}$ which, in a rate-determining step, deprotonates to yield the radical 5-HT$^{\bullet}$ (Scheme I). A most interesting fact about the dimeric products formed (**9–12**) is that they all contain at least one 5-HT residue linked at the C(4) position. Simple dimers are also formed as major electrooxidation products of 5-HTPP[62,63] and 5-HTOL,[64] and each of these similarly contains at least one residue of the parent compound linked at C(4). In no instance have dimers linked at other positions (e.g., 6 → 6′, 2 → 2′, 2 → 6′, 3 → 6′, etc.) been detected. Hence, it may be concluded that in the reactive 5-HT$^{\bullet}$ species the unpaired electron is located at C(4). The odd electron in 5-HT$^{\bullet}$ is almost surely in a p orbital, permitting interaction with the adjacent carbonyl π system, resulting in stabilization. Accordingly, 5-HT$^{\bullet}$ is attacked by 5-HT, i.e., a radical–substrate coupling reaction,[60] to yield dimer radicals 1–4 (Scheme I). A second, reversible one-electron, one-proton oxidation of the latter species then yields dimers **5–8**, which enolize to their more stable, isolated forms **9–12**. The mechanism outlined in Scheme I is based on the assumptions[60] that chemical reactions that follow deprotonation of 5-HT$^{\bullet+}$ are very fast so that a stationary state is established by mutual compensation of the diffusion and rate-determining chemical steps and between the chemical reactions that produce and consume intermediate species. These assumptions imply that voltammetric peak I_a is, in effect, completely irreversible in the sense that no reversible reduction peak coupled to peak I_a should appear in CV. In fact, no such reduction peak can be observed in CVs of 5-HT at pH 2 at sweep rates as high as 100 V s^{-1}, suggesting that the latter assumptions are correct. That a radical–substrate dimerization reaction (i.e., 5-HT$^{\bullet}$ + 5-HT → dimer) probably occurs has been tested by electrooxidation of 5-HT at pH 2 at peak I_a potentials in the presence of 5-methoxytryptamine (**13**).[65] The latter compound is not electrochemically



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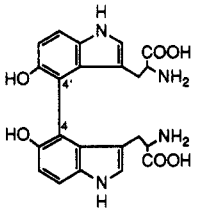
oxidized at peak I_a potentials. Reaction products include dimers **9–12** and at least two additional dimers containing one residue each of 5-HT and **13**. Thus, **13** can act as a substrate and attack radical 5-HT$^{\bullet}$.

Electrochemical oxidations of 5-HTPP[62,63] and 5-HTOL[64] in acidic aqueous solution follow the same general pathway outlined in Scheme I. However, in the case of 5-HTPP only two simple dimers have been isolated as products, 5,5′-dihydroxy-4,4′-bitryptophan (**14**) and 5,5-dihydroxy-4,6′-bitryptophan (**15**). Owing



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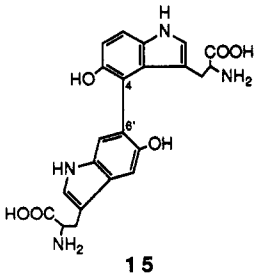

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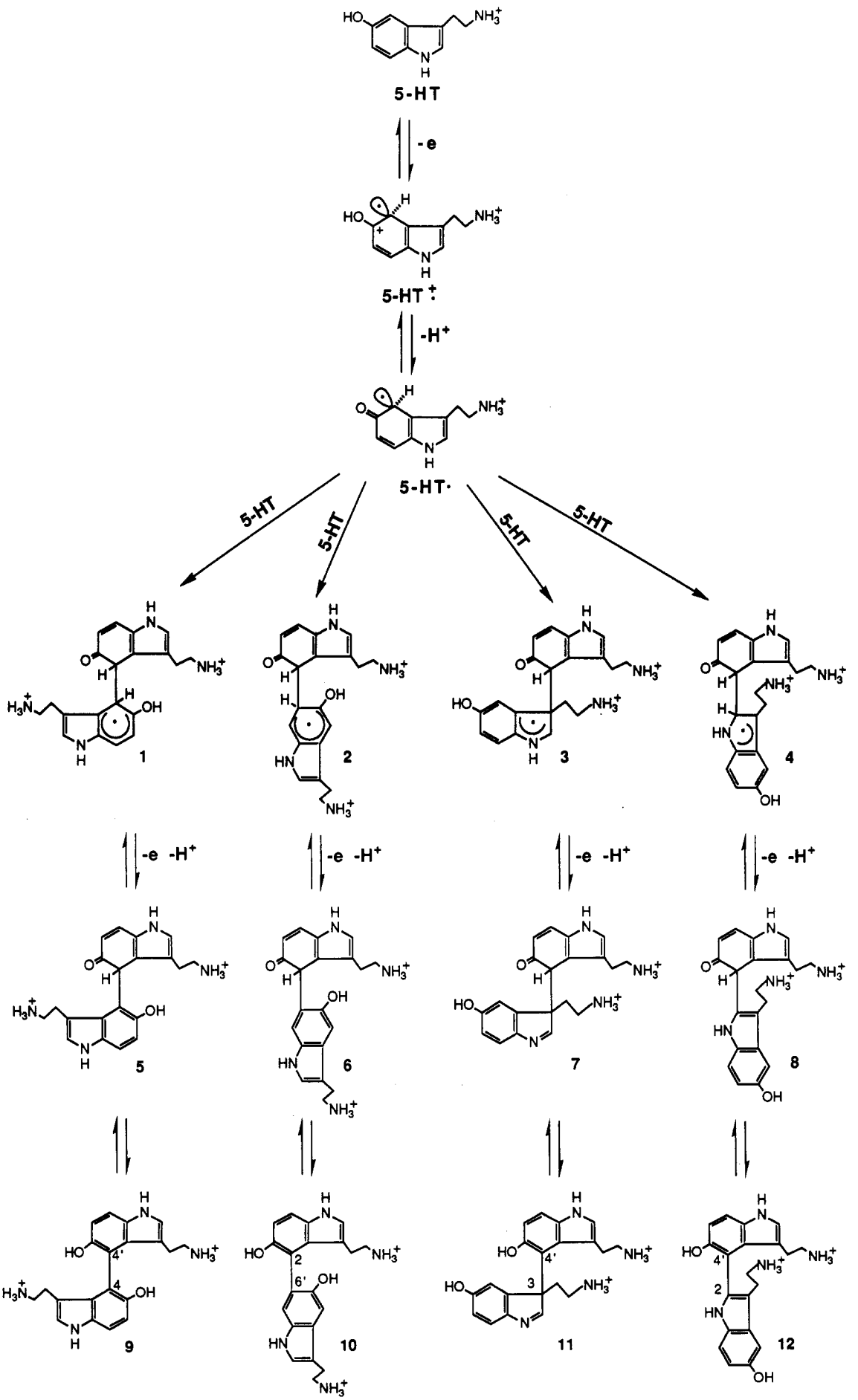
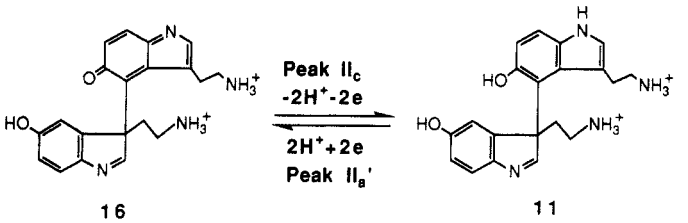
to the presence of chiral centers in the side chain of each 5-HTPP residue in these dimers and restricted rotation about the bond linking the two indolic residues, diastereomers of **14** and **15** are formed. In the case of 5-HTOL three simple dimers (4 → 4′, 2 → 4′, and 4,6′-linked) have been isolated as electrooxidation products.[64]

The reactions shown in Scheme I represent the major processes occurring upon electrochemical oxidation of 5-HT at peak I_a potentials. However, none of the isolated dimers (**9–12**) give reversible couples corre-

SCHEME I



SCHEME II

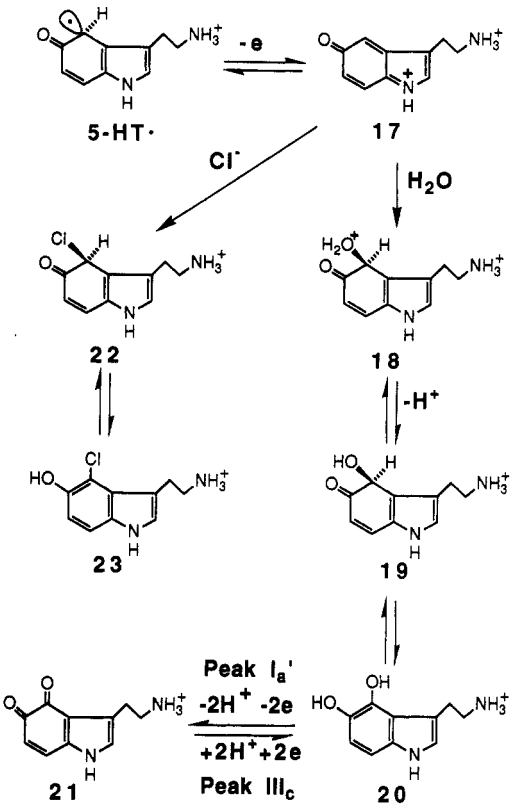


sponding to peaks III_c/I_a' and II_c/II_a' (Figures 2 and 3). This implies, therefore, that additional oxidation reactions occur to generate species responsible for these couples. At peak I_a potentials, the unusual asymmetric 3,4′-linked indolenine–indole **11** is further oxidized to the corresponding indolenine–quinone imine **16**,[56-58] and it is this species that is responsible for reduction peak II_c observed in CVs of 5-HT. The peak II_c reaction is a two-electron two-proton reduction of **16** to give **11**, which is reversibly oxidized in the peak II_a' reaction as illustrated in Scheme II.

Peak III_c observed in CVs of 5-HT is due to reduction of tryptamine-4,5-dione (**21**) to 4,5-dihydroxytryptamine (**20**). The yield of dione **21** increases as the applied potential used to electrooxidize 5-HT is made more positive and/or the concentration of 5-HT oxidized is decreased. Indeed, controlled-potential electrooxidation of ≤50 μM solutions of 5-HT at pH 2 at high applied potentials (e.g., 0.70 V vs SCE) results in almost quantitative formation of **21**. At lower applied potentials the 4,4′-linked dimer **9** increasingly replaces dione **21** as a product. Accordingly, radical 5-HT$^{\bullet}$ must be further oxidized to yield, ultimately, **21**. This occurs by a one-electron oxidation of 5-HT$^{\bullet}$ to the quinone iminium species **17**, which is rapidly attacked by water to yield 4,5-dihydroxytryptamine (**20**) by the reaction sequence shown in Scheme III. Compound **20** is easily oxidized ($E^{\circ\prime}_{20/21}$ = 0.105 V at pH 2) to dione **21**. In CVs of 5-HT, peak III_c is due to reduction of **21** to **20** and oxidation peak I_a' to the reverse reaction. Further evidence in support of the mechanism shown in Scheme III comes from the observation that electrooxidation of 5-HT in the presence of Cl^- gives 4-chloro-5-hydroxytryptamine (**23**) and that the yield of this compound increases at increasingly positive potentials as would be predicted if 5-HT$^{\bullet}$ is oxidized to quinone imine **17**. Electrochemical oxidations of 5-HTPP[62,63] and 5-HTOL[64] also give dione species analogous to **21** by identical reaction pathways.

The nature of the dimers formed upon peak I_a electrooxidation of 5-HT[56-58] (and 5-HTPP[62,63] and 5-HTOL[64]) in acidic aqueous solution clearly indicates that the C(4) position of these indoles is activated. However, in very acidic (1.05 M $HClO_4$) acetonitrile electrooxidation of 5-HT at a platinum electrode gives the 3,4′ indolenine–indole **11** in more than 80% yield.[66] On the basis of analysis of the characteristics of the voltammetric oxidation peak of 5-HT in acidic acetonitrile, it was concluded[66] that the initial heterogeneous one-electron abstraction generates a radical cation (**24**) in which the unpaired electron is located at the C(3) position. A radical–substrate coupling reaction was proposed to yield the dimer radical cation **25** (Scheme IV). A disproportionation-like second electron transfer in solution between **24** and **25** gives dication **26**, which deprotonates from position C(4′) to yield (protonated)

SCHEME III



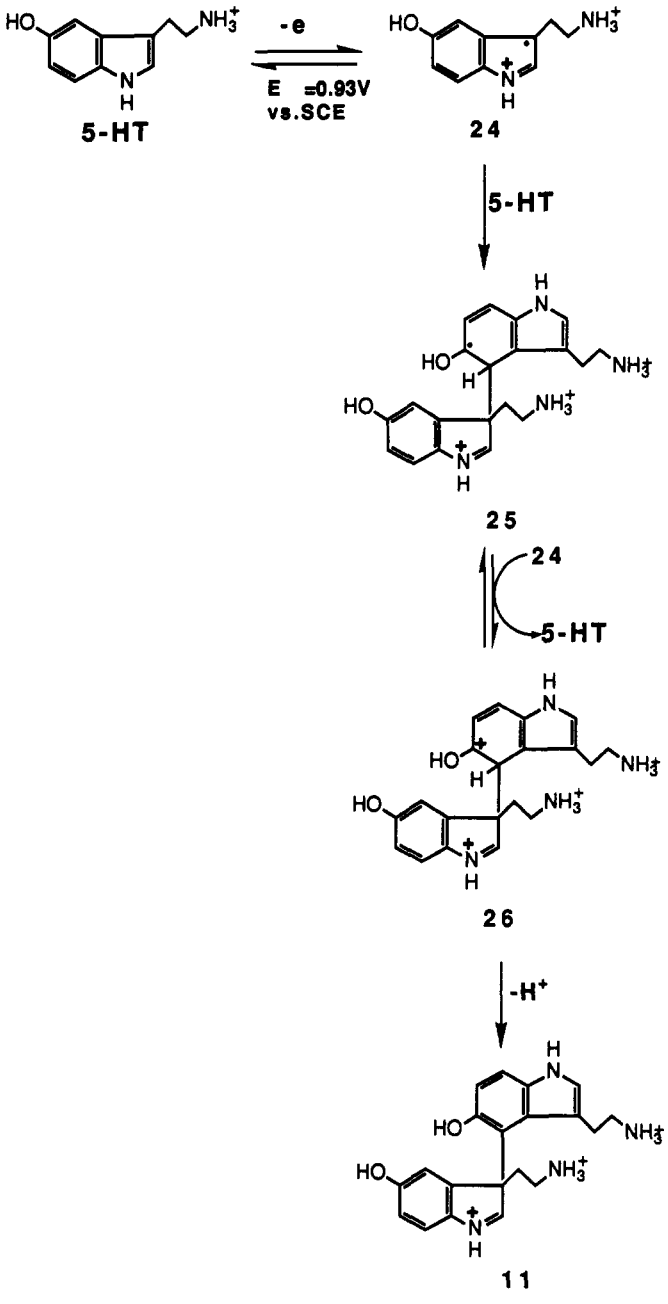
11. It is unfortunate that additional dimeric products were not isolated following electrooxidation of 5-HT in acidic acetonitrile in which linkages via C(3) are present in order to chemically confirm that it is the latter site that is activated. The mechanism outlined in Scheme IV, in fact, could equally well be imagined to proceed via a C(4) radical cation (i.e., 5-HT$^{\bullet+}$ in Scheme I).

Dimers **9**–**12** and **16** and monomers **21** and **23** are not the only products formed upon electrooxidation of 5-HT in aqueous solution. Many additional products appear as a result of further reactions of dione **21** and the oxidized dimer **16**.

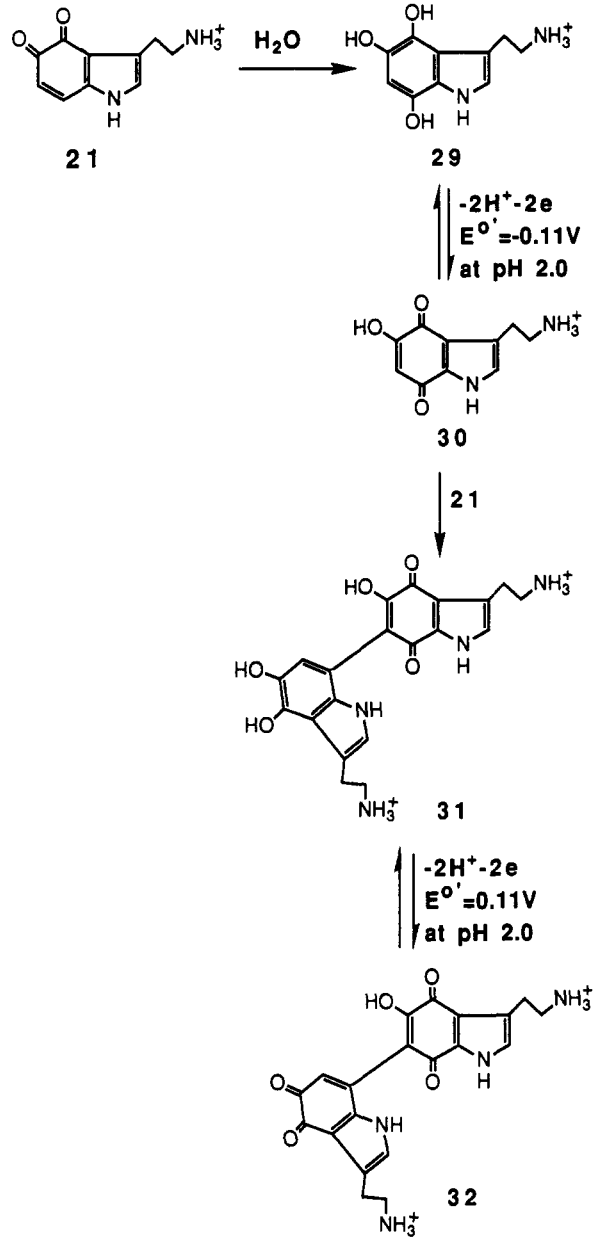
Tryptamine-4,5-dione (**21**) is a rather reactive compound. It cannot be isolated from solution, and hence, its structure was inferred by isolation of the product formed upon condensation with *o*-phenylenediamine.[56] In dilute aqueous solution, **21** slowly reacts with water to form 4,5,7-trihydroxytryptamine (**26**; Scheme V). The latter compound is very easily autoxidized to 5-hydroxytryptamine-4,7-dione (**30**), which reacts with **21** to generate dimer **32** as illustrated in Scheme V. The structure of **32** has not been unequivocally elucidated. However, mass spectra are in accord with the structure shown. In addition, CVs of **32** exhibit reversible couples characteristic of dione **21**[56] and *p*-quinone **30** residues.[67]

Attempts to concentrate even very dilute solutions of the purple dione **21** (e.g., by lyophilization) cause it to form the purple dimer 7,7′-bi(5-hydroxytryptamin-4-one) (**33**; Scheme VI).[68] This interesting compound can be reversibly reduced to 7,7′-bi(4,5-dihydroxytryptamine) (**34**) or reversibly oxidized to 7,7′-bi(tryptamine-4,5-dione) (**35**). Dione **21** also reacts directly with at least one simple dimer of 5-HT, i.e., 5,5′-dihydroxy-2,4′-bitryptamine (**12**), to give trimer **37** as shown in Scheme VII.[68] Electrooxidation of 5-HT also gives other oligomers and polymers that have not yet been characterized.[68] Such oligomers and polymers are

SCHEME IV



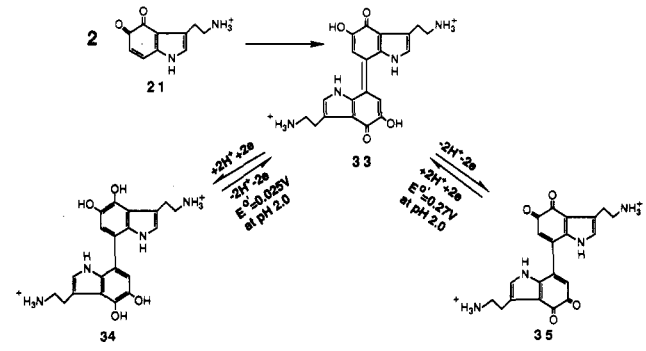
SCHEME V



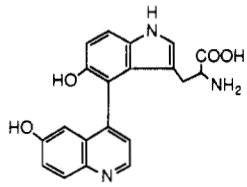
particularly prone to form when the electrooxidation product solution is concentrated by, for example, lyophilization. However, one such species decomposes to release 5-hydroxytryptamine-4,5-dione (**30**).[68] The latter compound is a major product of electrochemical oxidation and autoxidation of the indolic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT).[67] Accordingly, it was concluded that a minor oxidative pathway for 5-HT proceeded via intermediary formation of 5,7-DHT.[67] Subsequent studies, however, show that this conclusion is incorrect[68] (see also later discussion of 5,7-DHT).

Other ultimate products of electrochemical oxidation of 5-HT in acidic aqueous solution are derived from the 3,4′-linked indolenine–quinone imine **16**. During the course of a few hours **16** spontaneously reacts to give a complex mixture of products, which, rather interestingly, includes its reduced form **11**.[58] One major product of these reactions is the indole–quinoline dimer **45**. Formation of **45** from **16** must necessarily be a rather complex process, and since species intermediate

SCHEME VI



between the two compounds have not been isolated, the mechanistic pathway must remain speculative. Nevertheless, since diastereomers of an analogous indole–quinoline dimer (**46**) have been isolated from among the electrooxidation products of 5-HTPP[62] but not among the electrooxidation products of 5-HTOL,[64] it appears that the pyridine ring in **45** must be derived from the side chain of the indolenine residue of **16**. This implies



46

that the C(2)–C(3) bond of the latter residue must be opened. It has been speculated[58] that the reaction proceeds via addition of the elements of water across the N(1)=C(2) bond of **16** to give the indolines **38/39** (Scheme VIII).[69] Subsequent cleavage of the C(2)–C(3) bond of indoline **39** gives quinone *N*-formylimine **40**, which hydrolyzes to *p*-quinone **42**. Cyclization and oxidation then give **45** as conceptualized in Scheme XVIII. The ultimate oxidation step (i.e., **44** → **45**) must involve molecular oxygen or the relatively strong oxidant **16** (see later discussion), yielding **11**. Additional evidence for putative intermediate **39** has been provided by isolation of dimer **48** as another product formed spontaneously from **16**.[58] Nucleophilic attack by water on the quinone imine residue of **39** yields **47** (Scheme IX). The resultant 5,6-dihydroxyindoline residue in **47** would be expected to be easily oxidized to **48** by either molecular oxygen or **16**. Compound **16** decomposes to additional products including the partially characterized tetramer **51**, possibly by the reaction pathway shown in Scheme X.

Dimer **16** is a relatively strong oxidizing agent $E^{\circ\prime}_{16/11}$ = 0.30 V vs SCE at pH 2[58]), which accounts for the suggestion that it is responsible, at least in part, for the chemical oxidation of several putative intermediate species (e.g., **44** in Scheme VIII, **47** in Scheme IX, and **50** in Scheme IX) with concomitant formation of its reduced form **11**, which is therefore detected among the ultimate electrooxidation products of 5-HT even though it is much more easily oxidized than the latter compound.

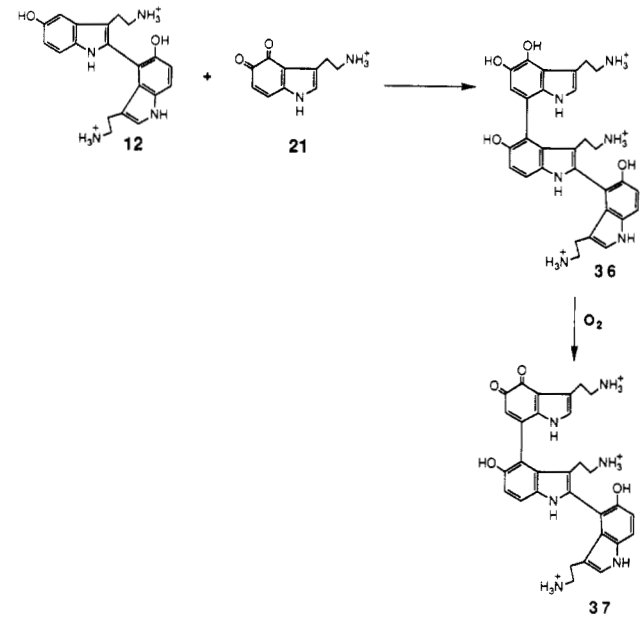
A 3,4′ indolenine–indole dimer analogous to **11** (or **16**) can be isolated as a product of electrooxidation of 5-HTOL[70] but not as a result of electrooxidation of 5-HTPP.[62,63] However, diastereomers of **46** are major oxidation products of 5-HTPP.[63] Since **46** must be formed by a reaction scheme analogous to that shown in Scheme VIII, it must be concluded that the carboxyl group associated with the indolenine residue side chain accelerates the ring-closure reactions that ultimately lead to this compound.[63]

Electrochemical oxidation of 5-HTOL in acidic solution also gives three dimeric products containing residues of tryptophol-4,5-dione and 5-HTOL (i.e., **56–58**; Scheme XI).[64] It has been proposed that these dimers are formed by reactions between two electrochemically generated intermediates, namely quinone imine **52** and 4,5-dihydroxytryptophol (**20**), as illustrated in Scheme XI.

IV. Oxidation Chemistry of Indolic Neurotoxins

5,6- and 5,7-Dihydroxytryptamine are widely used pharmacological agents for the selective chemical destruction of serotonergic neurons.[29,30,35,71–76] The selectivity of these dihydroxyindolamines is derived from their high affinity uptake by the membrane pump of serotonergic neurons. The molecular mechanisms by

SCHEME VII



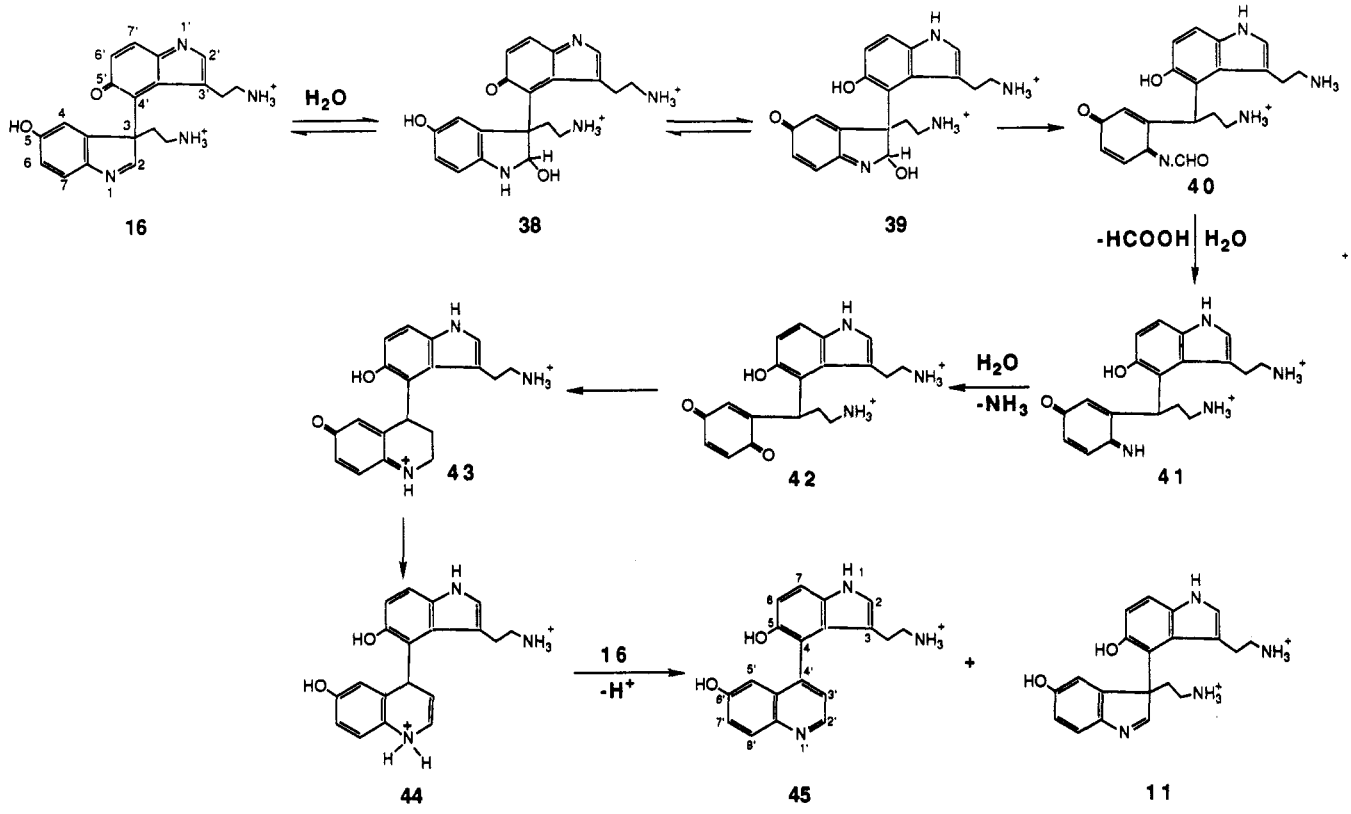
12 21 36 37

which 5,6- and 5,7-DHT express their neurodegenerative effect is widely believed to be related to an intrinsic chemical property, i.e., ease of oxidation. In vivo this oxidation is thought to be caused by dissolved oxygen without catalysis by an enzyme, a process generally known as autoxidation. Autoxidation of 5,7-DHT has been proposed to give the electrophilic quinone imines **59/60**, which can alkylate neuronal membrane proteins as a result of nucleophilic attack by their thiol residues as illustrated in Scheme XII.[77] Such a reaction would presumably modify the neuronal membrane to such an extent that the neuron is denervated.

Autoxidation of 5,6-DHT has been speculated to form *o*-quinone **62**, which alkylates and cross-links neuronal membrane proteins as conceptualized in Scheme XIII. Indeed, experiments with radiolabeled 5,6-DHT indicate that its autoxidation product(s) covalently binds with protein nucleophiles both in vitro[78] and in vivo.[79] It has also been suggested that byproducts of the autoxidation of 5,6- and 5,7-DHT are the cytotoxic reduced oxygen species $O_2^{\bullet-}$, $HO^{\bullet}$, and H_2O_2 (Schemes XII and XIII), which are the ultimate neurotoxins.[74,75,78–80]

Autoxidation of 5,7-DHT is first order in terms of both O_2 and the indolamine.[81] This observation is consistent with a mechanism in which O_2 is incorporated into the 5,7-DHT nucleus and is not consistent with the transformation of 5,7-DHT into quinone imines **59/60**. Sinhababu and Borchardt[81] have concluded that O_2 is initially incorporated into the C(4) position of 5,7-DHT, giving a free-radical superoxide complex. The key step in the autoxidation process was speculated to be formation of the C(4) radical although direct evidence in support of such an intermediate was not obtained.[81] Information bearing on such an intermediate has been obtained from an investigation of the electrochemical oxidation of 5,7-DHT.[82] A CV of 5,7-DHT is shown in Figure 4. Oxidation peak O_1 is an adsorption prepeak. In aqueous solution the primary step in the peaks O_1/O_2 process has been proposed to be a one-electron, one-proton abstraction from 5,7-DHT to give a radical intermediate represented as the resonance hybrid **66** in Scheme XIV. A number of simple dimers result from this putative radical, but the precise

SCHEME VIII



SCHEME IX

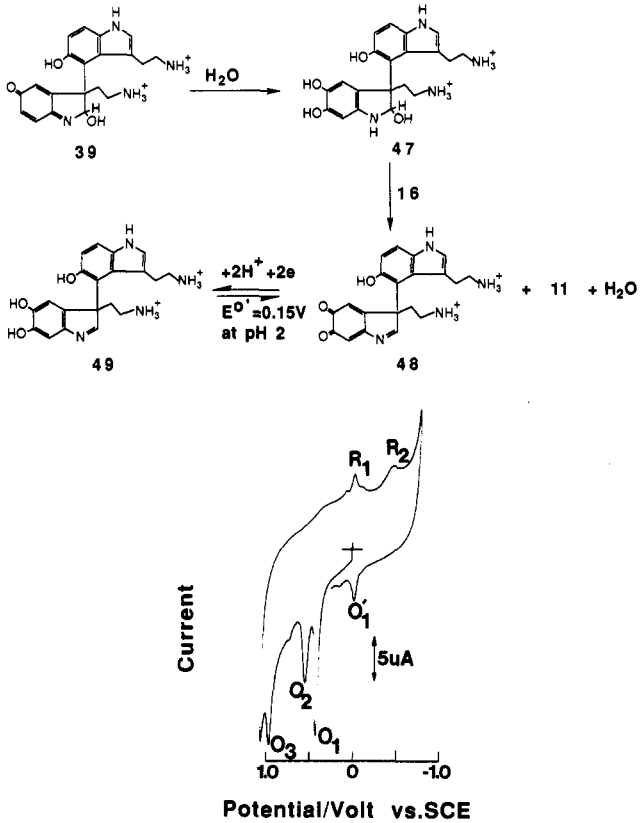
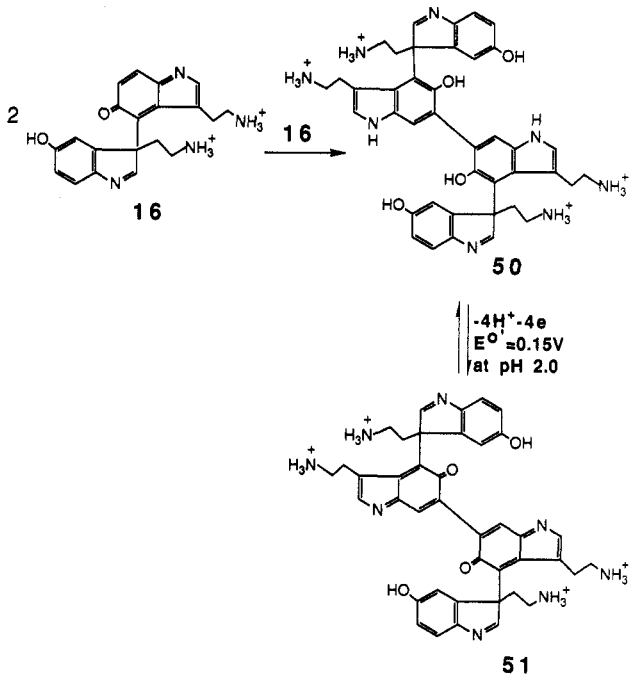


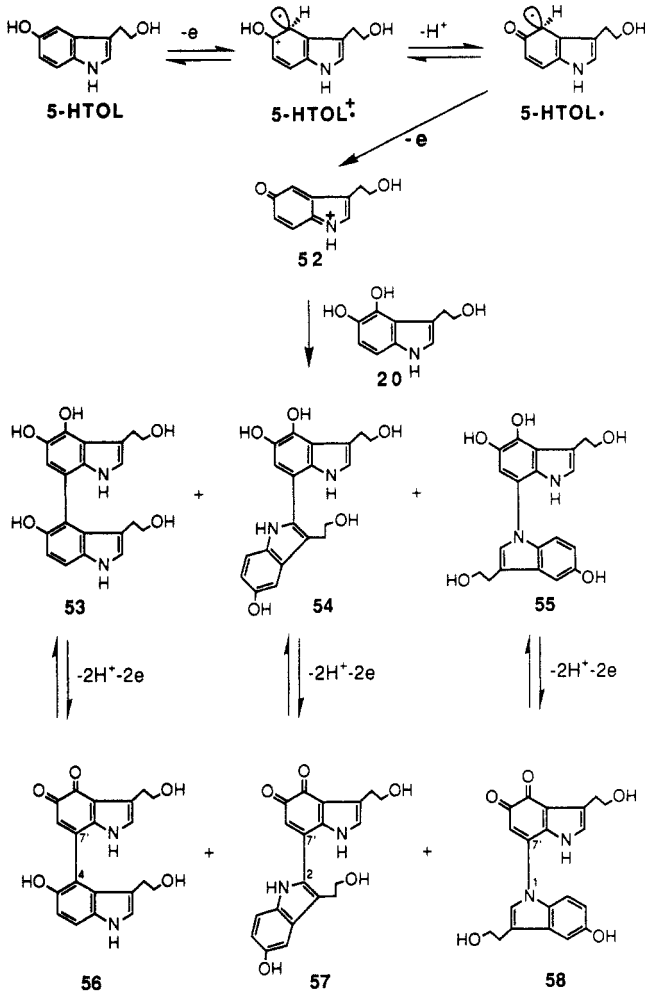
Figure 4. Cyclic voltammogram at the PGE of 0.1 mM 5,7-dihydroxytryptamine in aqueous solution at pH 1.5. Sweep rate 200 mV s^{-1} (reprinted from ref 82; copyright 1989 American Chemical Society).

SCHEME X

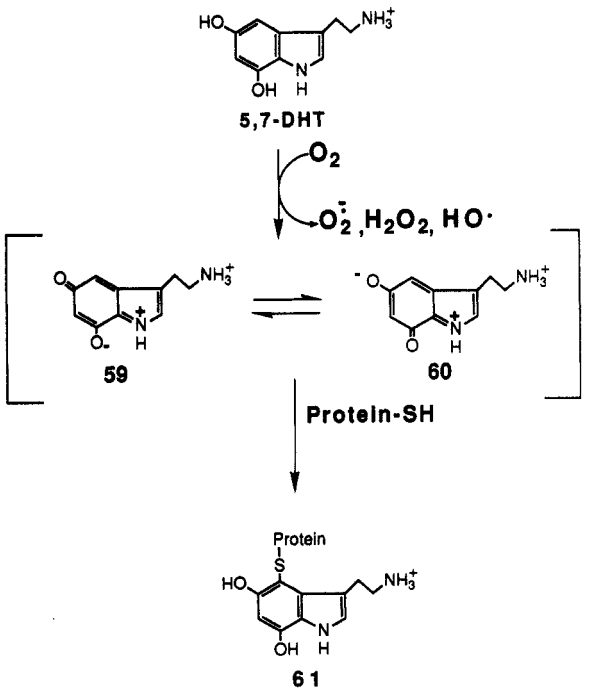


nature of the dimerization reaction remains to be elucidated. Nevertheless, the major dimeric product of the oxidation is the 4,4'-linked compounds **70–73** (Scheme XIV). Thus, the predominant form of the primary radical is **67** in which the unpaired electron is located at C(4). Dimerization of **67** gives 4,4'-bi(5,7-dihydroxytryptamine) (**69**), which at peak O_1/O_2 potentials is further oxidized to **70–73**. That some form of radical coupling reaction leads to **69** was inferred from the observation that the yield of dimeric products decreased as the applied potential for the electrolysis was made more positive and, correspondingly, the yield of 5-hydroxytryptamine-4,7-dione (**30**) increased. This observation indicates that radical **66** can be quite readily oxidized to quinone imine **74**, which is then

SCHEME XI

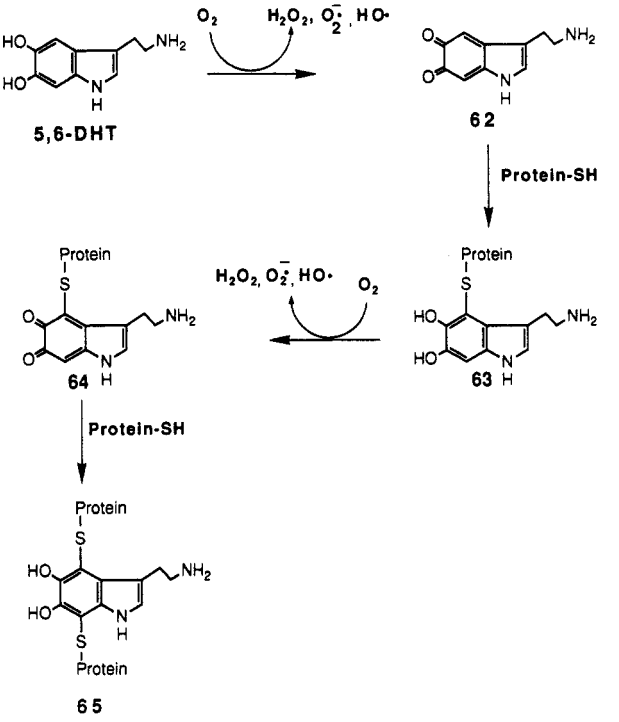


SCHEME XII



attacked by water to give trihydroxytryptamine **29**, which is immediately oxidized to **30** (Scheme XIV).[82] Peak R_1 observed in CVs of 5,7-DHT (Figure 4) is due to reduction of **30** to **29** and oxidation peak O_1' to the reverse reaction. Peak R_2 corresponds to reduction of

SCHEME XIII



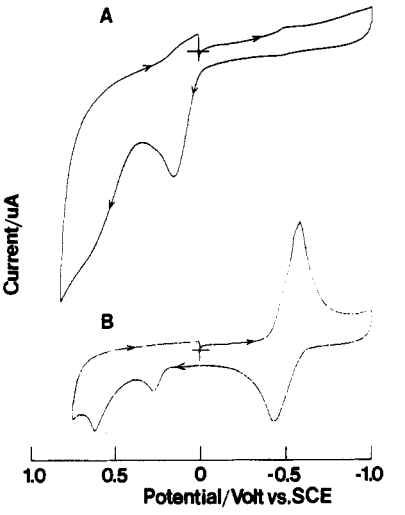
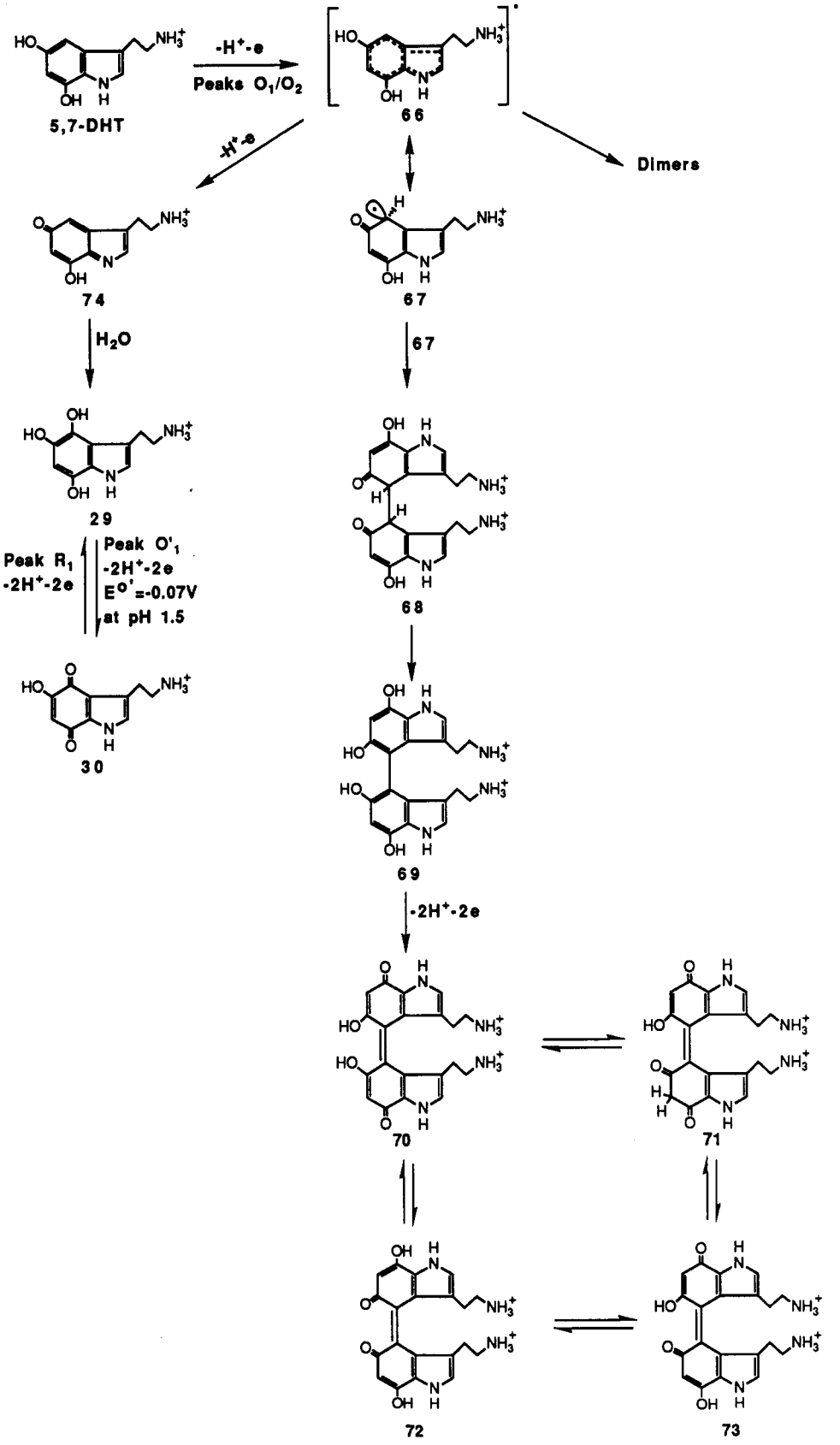


Figure 5. Cyclic voltammograms at the PGE of (A) 0.5 mM 5,7-dihydroxytryptamine in pH 7.4 phosphate buffer and (B) the same solution after stirring in air for several hours. Solutions were deaerated with N_2 before voltammograms were recorded. Sweep rate 200 mV s^{-1} (reprinted from ref 83; copyright 1990 American Chemical Society).

the oxidized dimer **71** to the cyclic ether **78** by the route shown in Scheme XV. Compound **78** is an easily oxidized compound ($E^{\circ\prime}$ = 0.08 V vs SCE at pH 1.5) and hence upon exposure to air is rapidly converted to quinonoid ether **79**. These electrochemical studies,[82] therefore, suggest that a C(4) radical species is a probable primary oxidation product of 5,7-DHT in agreement with the proposition of Sinhababu and Borchardt.[81]

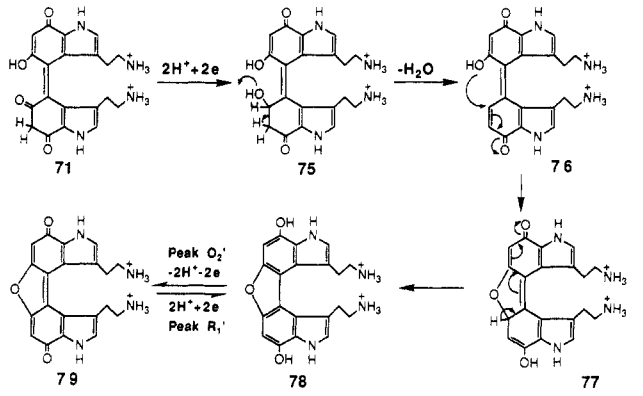
At physiological pH, 5,7-DHT exhibits a voltammetric oxidation peak at 0.19 V vs SCE (Figure 5A).[83] Such a low oxidation potential is in accord with the susceptibility of 5,7-DHT to autoxidation. Exposure of such a solution to air results in a systematic decrease of the voltammetric oxidation peak of 5,7-DHT. Figure 5B shows a CV of the final product solution. On the

SCHEME XIV

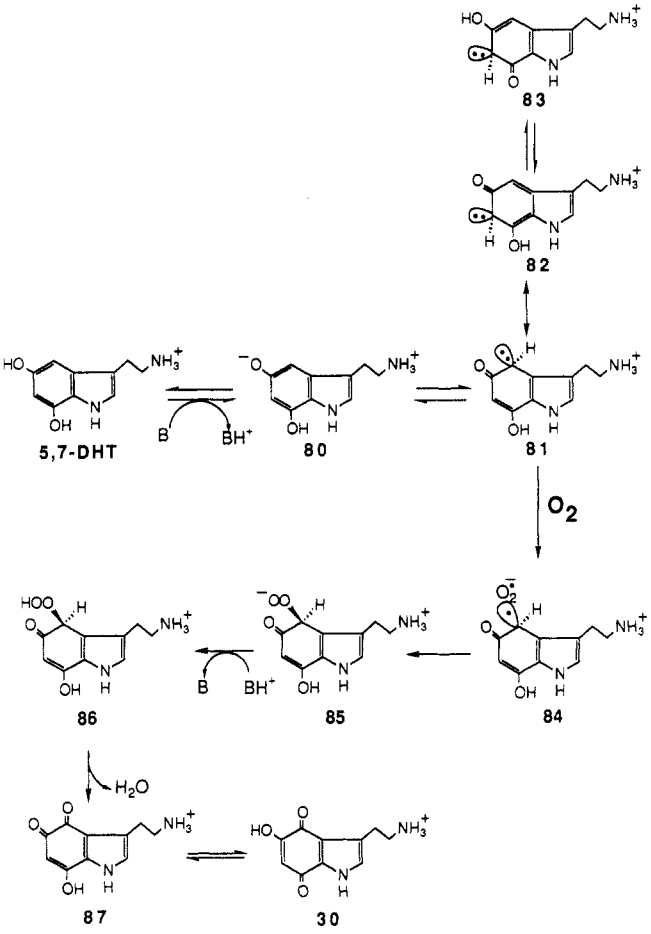


first cathodic sweep, a reduction peak appears at −0.59 V, and on the reverse sweep, a quasi-reversible oxidation peak appears at −0.43 V. On the first anodic sweep two, apparently irreversible, oxidation peaks appear at 0.275 and 0.62 V. The appearance of two oxidation peaks provided the first clue that autoxidation of 5,7-DHT gives more than one product.[83] Indeed, 5-hydroxytryptamine-4,7-dione (**30**) and 6,6′-bi(5-hydroxytryptamine-4,7-dione) (**97**) have been identified as the major autoxidation products.[83] Both **30** and **97** show quasi-reversible reduction peaks at ca. −0.59 V. However, **30** shows a voltammetric oxidation peak at 0.62 V whereas **97** shows an oxidation peak at 0.275 V. On the basis of NMR studies in D_2O,[81] it has been

SCHEME XV



SCHEME XVI



concluded[83] that at physiological pH the carbanions **81**–**83** are the primary electron donors to O_2 in the initial stage of autoxidation of 5,7-DHT. Formation of dimer **97** and earlier electrochemical studies[82] provide support for the conclusion that autoxidation of 5,7-DHT proceeds by a radical mechanism.[83] The mechanism leading to **30** has been proposed[81,83] to involve attack by O_2 on carbanion **81**, giving the radical superoxide complex **84** (Scheme XVI). Recombination of the superoxide of **84** with the incipient C(4) radical yields hydroperoxide anion **85**, which, upon proton abstraction, gives hydroperoxide **86**. Base-catalyzed decomposition of the secondary hydroperoxide **86** forms *o*-quinone **87** and hence the more stable *p*-quinone **30**. Attack by O_2 on carbanions **82** and **83** also leads to free-radical superoxide complexes **88** and **89**, respectively (Scheme XVII). However, since oxygen is not

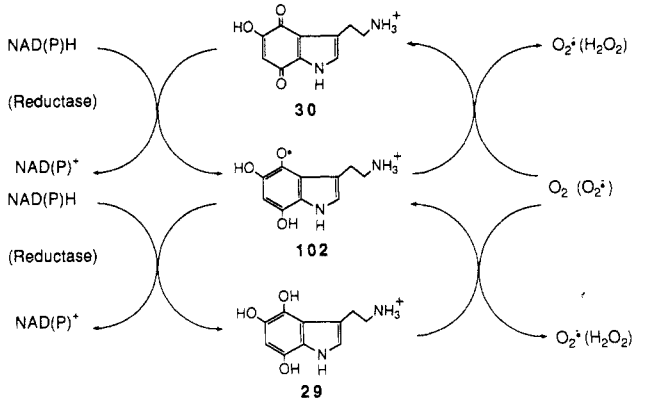


Figure 6. Mechanism proposed for redox cycling of 5-hydroxytryptamine-4,7-dione (**30**). Adapted from ref 84.

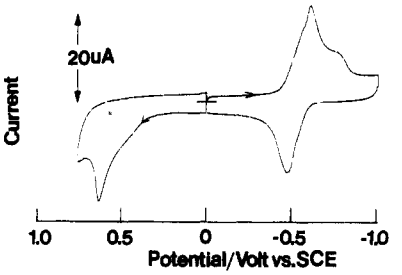


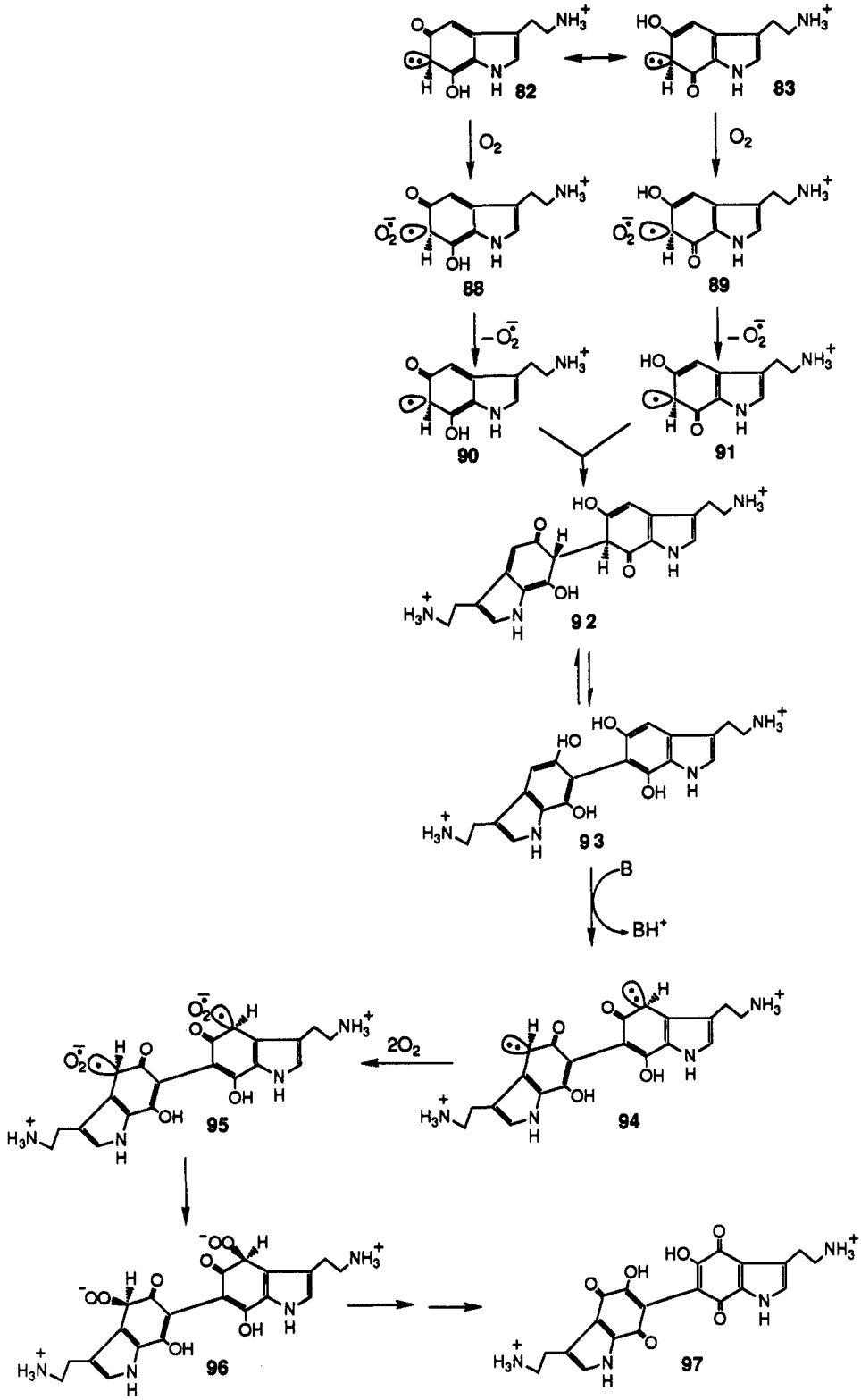
Figure 7. Cyclic voltammogram at the PGE of 0.5 mM 5-hydroxytryptamine-4,7-dione (**30**) in pH 7.4 phosphate buffer. Sweep rate 200 mV s^{-1} (reprinted from ref 83; copyright 1990 American Chemical Society).

ultimately incorporated into the C(6) position, these complexes must follow a different pathway to **84**. Accordingly, it has been proposed[83] that **88** and **89** dissociate to give radicals **90** and **91**, respectively, which react together to give dimer **93**. This dimer has the same structural functionalities as 5,7-DHT except that the C(6) position is blocked. Hence, a sequence of reactions similar to those proposed for 5,7-DHT in Scheme XVI leads to the ultimate dimer **97** (Scheme XVII).

It is of interest to note that 5-hydroxytryptamine-4,7-dione (**30**) accounts for one-third of 5,7-DHT that is autoxidized[83] but is 3 times more neurotoxic than 5,7-DHT in mouse brain.[67] This leads to the suggestion[83] that **30**, formed in vivo by autoxidation of 5,7-DHT, is the active neurodegenerative species. Sinhababu and Borchardt[84] have proposed that redox cycling of **30** might account for its neurotoxic effect. Redox cycling of many quinones has been shown to occur in several subcellar compartments including mitochondria and microsomes and is effected by many enzyme systems.[85–88] A plausible redox cycling process, outlined in Figure 6,[84] would generate cytotoxic reduced oxygen species that might be the ultimate neurodegenerative species. Such a redox cycling process, however, would also consume oxygen and, if sufficiently efficient, would have the potential to inflict hypoxia-induced damage on the target neuron.[84] Cyclic voltammograms of **30** (Figure 7) shows a quasi-reversible reduction peak at ca. −0.6 V at pH 7.4, indicating that it is relatively easily reducible.

The neurotoxic properties of dimer **97**, which is the major autoxidation product of 5,7-DHT, have yet to be determined. However, CV of **97** (Figure 8) reveals that it also shows a quasi-reversible reduction peak at ca. −0.6 V and hence, in principle, could participate in re-

SCHEME XVII



dox cycling reactions as effectively as **30**. In addition, **97** is significantly more easily oxidizable than **30**, suggesting, therefore, that further autoxidation of this compound in vivo might play some functional role in expressing the neurotoxic effects of 5,7-DHT. The oxidation chemistry of **97**, however, remains to be studied.

At physiological pH 5,6-DHT shows a voltammetric oxidation peak at 0.15 V (Figure 9), confirming its ease of oxidation. After scan reversal a reduction peak coupled to the oxidation peak is not observed, indicating that the initial oxidation product is very reactive.[89] As was noted earlier (Scheme XIII), autoxidation of 5,6-DHT appears to be a prerequisite step to initiate the neurodegenerative effects of this compound.[73-75,78-80,90,91] Klemm et al.[90] have demonstrated that H_2O_2 is formed during the autoxidation of 5,6-DHT and have concluded that the reaction is autocatalytically promoted by H_2O_2. The autoxidation reaction at physiological pH ultimately results in formation of a black, insoluble, melanin-like polymer of unknown structure.[90] The major initial autoxidation product of

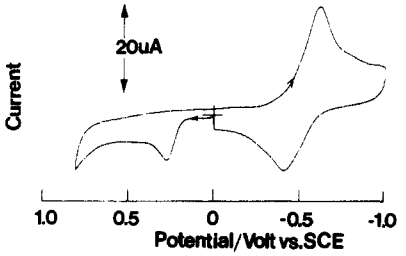


Figure 8. Cyclic voltammogram at the PGE of 0.5 mM 6,6′-bi(5-hydroxytryptamine-4,7-dione) (**97**) in pH 7.4 phosphate buffer. Sweep rate 200 mV s^{-1} (reprinted from ref 83; copyright 1990 American Chemical Society).

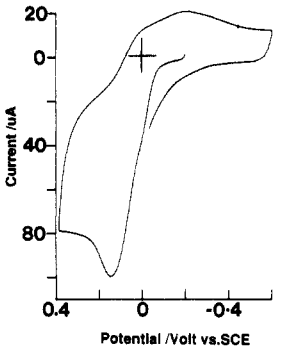


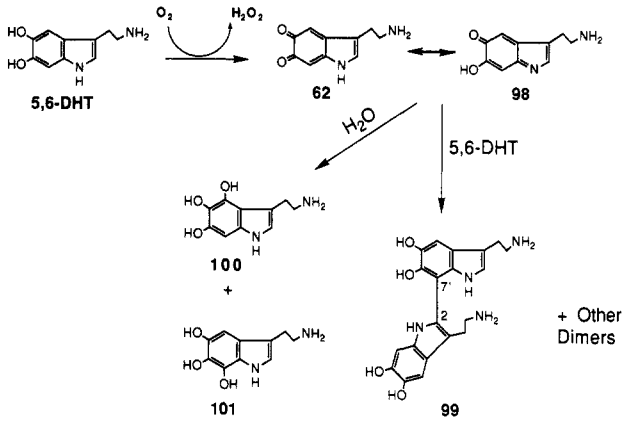
Figure 9. Cyclic voltammogram at the PGE of 2 mM 5,6-dihydroxytryptamine in pH 7.2 phosphate buffer. Sweep rate 200 mV s^{-1} (reprinted from ref 89; copyright 1990 American Chemical Society).

5,6-DHT is 2,7′-bi(5,7-dihydroxytryptamine) (**99**) although other dimers and some trihydroxytryptamines are also formed (Scheme XVIII).[89] Voltammetric measurements indicate that the oxidation potentials of all the initial monomeric and dimeric autoxidation products are equal to or more negative than that of 5,6-DHT.[89] In other words, the autoxidation products are as or more easily oxidized than 5,6-DHT. Thus, the apparent acceleration of the autoxidation reaction of 5,6-DHT during its latter stages might reflect the more rapid oxidation of products rather than the influence of H_2O_2 as proposed by Klemm et al.[90] Simultaneous autoxidation of **99** along with other dimers and trihydroxyindoles (Scheme XVIII) to quinoid intermediates no doubt explains the ultimate formation of highly cross-linked insoluble polymer.

V. Biological Implications

The metabolic transformations of 5-HT initiated by the enzyme monoamine oxidase (Figure 1) clearly play a vital role in the inactivation of the neurotransmitter in the CNS. However, several studies[10,13,14] both in vitro and in vivo suggest that the ultimate metabolite, 5-HIAA and its conjugates, represents only a fraction of the 5-HT metabolized. It has been suggested, for example, that further hydroxylation of 5-HT occurs,[44,92] but in fact there is little direct evidence to support this speculation. However, recently Commins et al.[93,94] have reported that administration of methamphetamine (MA) and *p*-chloroamphetamine (PCA), both of which deplete brain 5-HT, to rat results in formation of the neurotoxin 5,6-dihydroxytryptamine (5,6-DHT). The latter compound was identified on the basis of its retention time in high-performance liquid chromatography, and hence formation of 5,6-DHT must be viewed

SCHEME XVIII



with some skepticism. Nevertheless, the authors[93,94] hypothesize that the toxic effects of MA and PCA are mediated by formation of 5,6-DHT. There are also a number of reports on the formation of, but as yet unidentified, abnormal oxidation products of 5-HT and 5-HTPP in the CNS of individuals afflicted with Alzheimer's disease.[95,96] Serotonergic abnormalities have been extensively reported in dementia of the Alzheimer type.[97-99] Chen et al.[100] have suggested that an alteration of 5-HT metabolism in vivo might lead to the production of toxic indole derivatives, which, through long-term accumulation, could have profound effects on neuronal activity. Unknown indolic compounds have also been found in the urine of individuals in many other pathological states, e.g., carcinoidosis.[101] These and earlier reports appear to present an overwhelming case implicating chemical and/or biochemical oxidation reactions of endogenous indoles in disease states, particularly of the CNS. But, as noted previously, until very recently only the vaguest outline of this chemistry was known.

Electrochemical studies on 5-HT, 5-HTPP, and 5-HTOL, reported above, provide evidence that these endogenous indoles are easily oxidized species. Their electrooxidation appears to form very reactive radical intermediates that, under the conditions studied, react primarily with the parent molecules in a radical–substrate reaction to yield dimers. It is not difficult to imagine that such radicals, if generated in the CNS, could undergo reactions, possibly toxic in result, with many endogenous species. Electrochemical investigations also reveal that the primary radicals formed upon oxidation of endogenous indoles are also easily oxidized to an extremely reactive electrophilic quinone imine. The major solution reactions of these quinone imines in vitro are with water to yield, ultimately, 4,5-dihydroxyindoles and hence, the corresponding 4,5-diones (see Scheme III). In the complex environment of the CNS it is not difficult to imagine that quinone imine species (e.g., **17** Scheme III) would be extremely dangerous compounds subject to reaction with any nucleophiles present including, of course, water. It is known that the resultant product from quinone imine **17** is 4,5-dihydroxytryptamine (**20**) and, furthermore, that this is a potent CNS neurotoxin.[33,36] The mechanism(s) by which **20** expresses its neurodegenerative effect is not known. However, it is likely that in vivo it is autoxidized to dione **21** and that this might be a neurotoxin since it is known to react avidly with thiol residues of amino acids and peptides (e.g., cysteine and

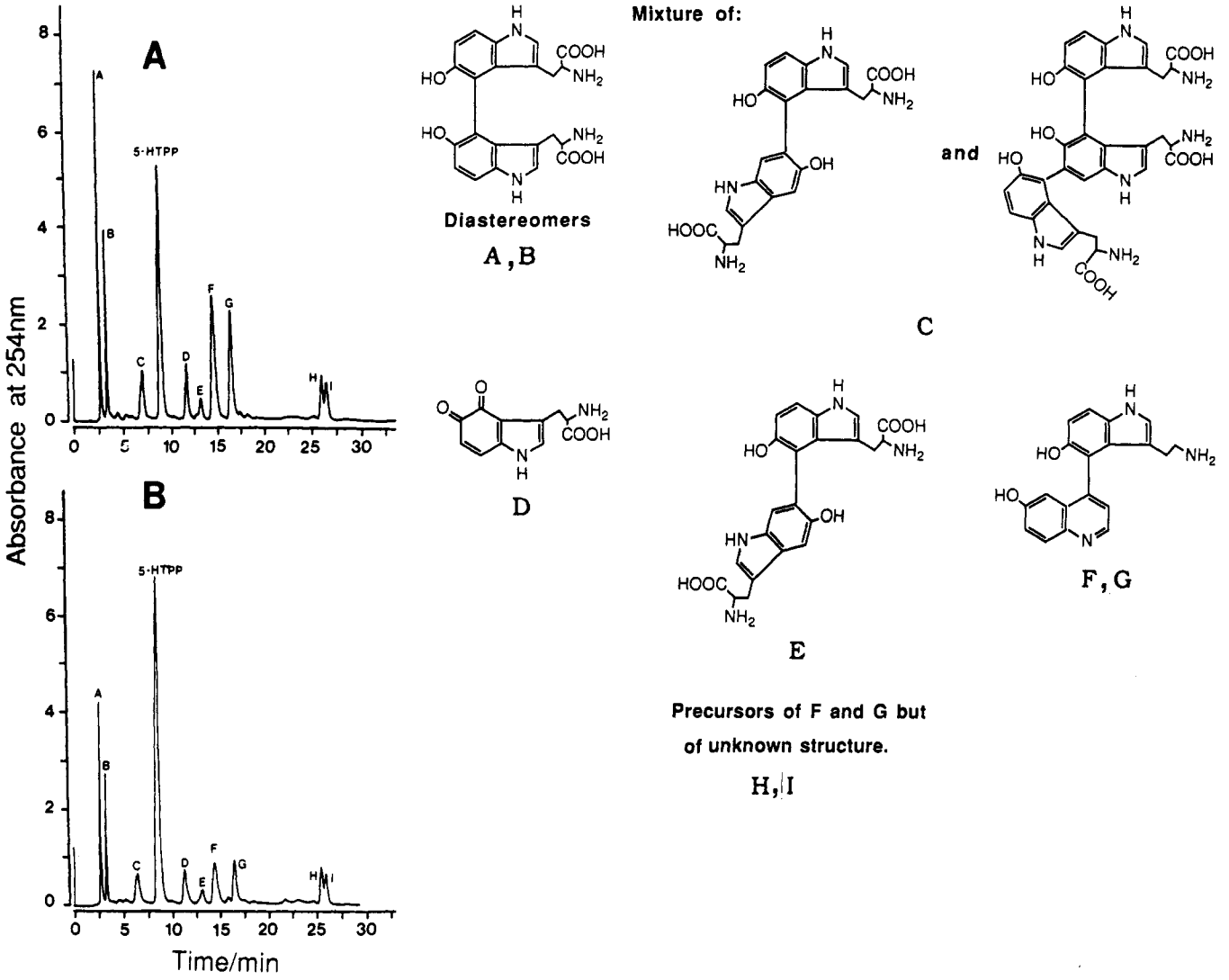


Figure 10. High-performance liquid chromatograms of the product solutions formed by (A) controlled-potential electrooxidation at 0.65 V and (B) Type VI peroxidase–H_2O_2 oxidation of 1.0 mM 5-hydroxytryptophan at pH 2.6 (reprinted from ref 63; copyright 1990 The Electrochemical Society).

glutathione).[96,102] As noted in Scheme V, dione **21** can be further attacked by water to yield 4,5,7-trihydroxytryptamine (**29**) and hence 5-hydroxytryptamine-4,7-dione (**30**). In vivo experiments in mouse[67] have demonstrated that **30** is approximately 3 times as toxic in the CNS as the widely used indolic neurotoxins 5,6- and 5,7-DHT.

Recently, Chen et al.[100] employed in vitro superfusion and incubation experiments to investigate the effects of an electrochemically oxidized solution of 5-HT on rat brain fragments. This solution was prepared by electrooxidation of 5-HT in 0.01 M HCl[56] and was thought to consist predominantly of tryptamine-4,5-dione (**21**) although independent analyses in this laboratory indicate a complex mixture of products are in fact present (see for example Schemes I, III, and V–X). Nevertheless, in vitro superfusion experiments revealed that the electrooxidation product solution containing **21** significantly increased 5-HT efflux from both rat hippocampal and striatal fragments. In vitro incubation experiments showed that electrooxidized 5-HT solutions evoked 5-HT efflux from rat hippocampus in a dose-dependent fashion. The indolic neurotoxin 5,6-DHT similarly induces 5-HT release from in vitro synaptosomal preparations by causing vesicularly stored 5-HT to enter the cytoplasm.[103] Thus, apparently **21** or perhaps other electrooxidation products of 5-HT express their neurotoxicity by a mechanism similar to that proposed for 5,6-DHT. Other studies have demonstrated that administration of the solution formed by electrooxidation of 5-HT into the lateral ventricles of rat causes cell death.[96] Thus, **20**[33,36] **21**,[100] **29**, **30**,[67] and possibly other products of electrochemical oxidation of 5-HT have powerful neurodegenerative properties.

Analysis of the cerebrospinal fluid (CSF) of individuals with Alzheimer's disease, using HPLC with an electrochemical detector, failed to detect **21** although very many peaks of unknown but easily electrooxidized compounds were detected.[96] However, if in fact dione **21** is a toxin of significance in the etiology of Alzheimer's disease or related dementias, it is very unlikely that it would exist in the free state in CSF because of its avid reaction with nucleophiles such as thiol groups.[96,102]

Electrochemical studies of the oxidation chemistry of biogenic indoles reported earlier have, for various practical reasons, been largely restricted to aqueous solution at relatively low pH. These studies, however, appear to provide much information about the fundamental oxidation chemistry of these compounds and clearly demonstrate that oxidation of biogenic indoles

are facile and produce very complex mixtures of products. Such studies have led to the discovery of at least three neurotoxins (**20, 21, 30**). Further work is currently under way to establish whether other oxidation products derived from 5-HT and other endogenous indoles possess neurodegenerative properties. Preliminary studies have been carried out on the electrochemical oxidation of the biogenic indoles at physiological pH.[104,105] These indicate many similarities to the results obtained at lower pH although more complex follow-up chemistry and electrochemistry appears to take place.

Peroxidase derived from horseradish and mammalian peroxidase–H_2O_2 systems has been reported to oxidize biogenic indoles although reaction products were not identified.[106] It has been speculated[106] that peroxidative 5-hydroxyindolamine catabolism, for example, might play a prominent role in situations where the enzyme monoamine oxidase is inhibited as well as in cancerous tissue, which often possesses high levels of peroxidase.[107] A comparison of the peroxidase–H_2O_2 and electrochemical oxidation of 5-HTPP has been carried out.[63,104] Chromatograms of the product solutions formed as a result of these oxidations are shown in Figure 10. Clearly, the product profiles resulting from both types of oxidation reactions are identical, suggesting that the chemical mechanisms leading to these products are the same. Peroxidase-catalyzed oxidations of organic substrates are widely believed to proceed via radical intermediates.[108,109] Hence, it seems likely that the general pathways outlined in Schemes I and III for the electrochemical oxidation of biogenic indoles also apply to the peroxidase-mediated oxidations. Comparisons of the electrochemical oxidations of 5-HT,[110] 5-HTPP,[104] and 5-HTOL[105] with those catalyzed by various peroxidases, tyrosinase, and ceruloplasmin have revealed that the product profiles and, by inference the chemical reaction pathways, are very similar.

VI. Conclusions

Electrochemical studies of biogenic indoles have provided some valuable insight into their fundamental oxidation chemistry. Such insight would be difficult to elucidate by direct investigations of enzyme-mediated oxidations. Very many structurally interesting products of the electrochemical oxidations have been isolated, and some of these have already been demonstrated to possess neurodegenerative properties. Indeed, the search is now under way to detect some of these products in the central nervous system of patients suffering from Alzheimer's disease and related dementias. Preliminary results have clearly established that the oxidative biochemistry of biogenic indoles, mediated by enzymes endogenous to the CNS, follows parallel pathways to the electrochemically driven reactions.

Several aspects of the still-unfolding story about the oxidative chemistry and biochemistry of indolic neurotoxins have been resolved by application of electroanalytical techniques. Ultimately, a combination of electrochemical, chemical, biochemical, and in vivo approaches will lead to a full understanding of the mechanisms by which the indolic neurotoxins express their neurodegenerative properties in the CNS.

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References

(1) Dryhurst, G. *Electrochemistry of Biological Molecules*; Academic Press: New York, 1977.
(2) Dryhurst, G.; Kadish, K. M.; Scheller, F.; Renneberg, R. *Biological Electrochemistry*; Academic Press: New York, 1982.
(3) Dryhurst, G., Niki, K., Eds. *Redox Chemistry and Interfacial Behavior of Biological Molecules*; Plenum Press: New York, 1988.
(4) Dryhurst, G. In *Comprehensive Treatise on Electrochemistry*; Srinivasan, S., Chizmadzhev, Yu, A., Bockris, J. O.'M., Conway, B. E., Yaeger, E., Eds.; Plenum Press: New York, 1985; Chapter 2.
(5) Kadish, K. M., Ed. *Electrochemical and Spectrochemical Studies of Biological Redox Components*; Advances in Chemistry Series 201; American Chemical Society: Washington, DC, 1982.
(6) Sawyer, D. T., Ed. *Electrochemical Studies of Biological Systems*; ACS Symposium Series 38; American Chemical Society: Washington, DC, 1977.
(7) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; Wiley: New York, 1980.
(8) Dryhurst, G.; Kadish, K. M.; Scheller, F.; Renneberg, R. *Biological Electrochemistry*; Academic Press: New York, 1982; Vol. I, Chapter 6.
(9) Wightman, R. M. *Anal. Chem.* **1981**, *53*, 1125A–1134A.
(10) Udenfriend, U.; Titus, E.; Weissbach, H.; Peterson, R. E. *J. Biol. Chem.* **1956**, *219*, 335–344.
(11) Sjoerdma, A.; Smith, T. E.; Stevenson, T. D.; Udenfriend, S. *Proc. Exp. Biol. Med.* **1955**, *89*, 36–37.
(12) Mitoma, C.; Posner, H. S.; Reitz, H. C.; Udenfriend, S. *Arch. Biochem. Biophys.* **1956**, *61*, 431–441.
(13) Erspamer, V. *J. Physiol. (London)* **1955**, *127*, 118–133.
(14) McIsaac, W. M.; Page, I. H. *J. Biol. Chem.* **1959**, *234*, 858–864.
(15) Kveder, S.; Iskric, S.; Keglivic, D. *Biochem. J.* **1962**, *85*, 447–449.
(16) Eccleston, D.; Moir, A. T. B.; Reading, H. W.; Ritchie, I. M. *Br. J. Pharmacol.* **1966**, *28*, 367–377.
(17) Amin, A. H.; Crawford, T. B. B.; Gaddum, J. H. *J. Physiol. (London)* **1954**, *126*, 596–618.
(18) Twarog, B. N.; Page, I. H. *Am. J. Physiol.* **1953**, *175*, 157–161.
(19) Page, I. H. *Physiol. Rev.* **1958**, *38*, 277–335.
(20) Novotna, R. *Physiol. Bohemslov.* **1980**, *29*, 243–253.
(21) Woolley, D. W.; Shaw, E. *Proc. Natl. Acad. Sci. U.S.A.* **1954**, *40*, 228–231.
(22) Gaddum, J. H. *J. Physiol. (London)* **1953**, *119*, 363–368.
(23) Himwich, H. E.; Kety, S. S.; Smythies, J. R. *Amines and Schizophrenia*; Pergamon Press: New York, 1967.
(24) Woolley, D. W. *The Biochemical Bases of Psychoses*; Wiley: New York, 1962.
(25) de la Torre, J. C. *Dynamics of Brain Monoamines*; Plenum Press: New York, 1972.
(26) Kaplan, R. D.; Mann, J. J. *Life Sci.* **1982**, *31*, 583–588.
(27) Dalgleisch, C. E. *Proceedings of the Symposium on 5-Hydroxytryamines*; Pergamon Press: New York, 1957; p 60.
(28) Eriksen, N.; Martin, G. M.; Benditt, E. P. *J. Biol. Chem.* **1960**, *235*, 1662–1667.
(29) Baumgarten, H. G.; Björklund, A.; Lachenmeyer, L.; Nobin, A.; Stenevi, U. *Acta Physiol. Scand. Suppl.* **1971**, *33*, 1–15.
(30) Baumgarten, H. G.; Evetts, K. D.; Holmes, R. B.; Iversen, L. L.; Wilson, G. *J. Neurochem.* **1972**, *19*, 1587–1597.
(31) Baumgarten, H. G.; Goethert, M.; Holstein, A. F.; Schlossberger, H. G. *Z. Zellforsch. Mikrosk. Anat.* **1972**, *128*, 115–134.
(32) Björklund, A.; Nobin, A.; Stenevi, U. *Brain Res.* **1973**, *53*, 117–129.
(33) Björklund, A.; Nobin, A.; Stenevi, U. *Z. Zellforsch. Mikrosk. Anat.* **1973**, *145*, 479–501.

(34) Saner, A.; Pieri, L.; Moran, J.; Da Prada, M.; Pletscher, A. *Brain Res.* **1974**, *76*, 109–117.
(35) Baumgarten, H. G.; Lachenmeyer, L. *Z. Zellforsch. Mikrosk. Anat.* **1972**, *135*, 399–414.
(36) Björklund, A.; Horn, A. S.; Baumgarten, H. G.; Nobin, A.; Schlossberger, H. G. *Acta Physiol. Scand. Suppl.* **1975**, *429*, 29–60.
(37) Porter, C. C.; Titus, D. C.; Sanders, B. E.; Smith, E. V. C. *Science* **1957**, *126*, 1014–1015.
(38) Sanker, D.; Siva, V. *Fed. Proc. Am. Soc. Exp. Biol.* **1959**, *18*, 441–442.
(39) Curzon, G.; Vallet, L. *Biochem. J.* **1960**, *74*, 279–287.
(40) Frieden, B.; Hsieh, H. S. *Exp. Biol. Med.* **1976**, *74*, 505.
(41) Barrass, B. C.; Coult, D. B.; Pindor, R. M.; Skeels, M. *Biochem. Pharmacol.* **1973**, *22*, 2891–2895.
(42) Barrass, B. C.; Coult, D. B. *Biochem. Pharmacol.* **1972**, *21*, 677–685.
(43) Martin, G.; Eriksen, N.; Benditt, E. P. *Fed. Proc. Am. Soc. Exp. Biol.* **1958**, *17*, 477–478.
(44) Blum, J. J.; Ling, J. S. *Biochem. J.* **1959**, *73*, 530–535.
(45) Martin, G. M.; Benditt, E. P.; Eriksen, N. *Arch. Biochem. Biophys.* **1960**, *90*, 208–217.
(46) Uemura, T.; Shimazu, T. *Biochem. Biophys. Res. Commun.* **1980**, *93*, 1074–1081.
(47) Alivisatos, S. G. A.; Williams-Ashman, H. G. *Biochem. Biophys. Acta* **1964**, *86*, 392–395.
(48) Borg, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1965**, *53*, 829–836.
(49) Perez-Reyes, E.; Mason, R. P. *J. Biol. Chem.* **1981**, *256*, 2427–2432.
(50) Walaas, E.; Wallaas, O. *Arch. Biochem. Biophys.* **1961**, *95*, 151–162.
(51) Uemura, T.; Matsushita, H.; Ozawa, M.; Fiori, A.; Chiesara, E.; *FEBS Lett.* **1979**, *101*, 59–62.
(52) Uemura, T.; Shimazu, T.; Miura, R.; Yamano, T. *Biochem. Biophys. Res. Commun.* **1980**, *93*, 1074–1081.
(53) Saavendra, J. M.; Brownstein, M.; Axelrod, J. *J. Pharmacol. Exp. Ther.* **1973**, *186*, 508–515.
(54) Brownstein, M. J. In *Basic Neurochemistry*; Siegel, G. J., Albers, B. W., Angraff, B. W., Katzman, R., Eds.; Little, Brown; Boston, 1981; p 220.
(55) Gershon, M. D.; Tamir, J. In *Serotonin, Current Aspects of Neuro-chemistry and Function*; Haber, B., Gabay, S., Issidorides, M. R., Alivisatos, S. G. A., Eds.; Plenum Press: New York, 1981; p 43.
(56) Wrona, M. Z.; Dryhurst, G. *J. Org. Chem.* **1987**, *52*, 2817–2825.
(57) Wrona, M. Z.; Humphries, K.; Dryhurst, G. In *Redox Chemistry and Interfacial Behavior of Biological Molecules*; Dryhurst, G., Niki, K., Eds.; Plenum Press: New York, 1988; pp 425–445.
(58) Wrona, M. Z.; Dryhurst, G. *J. Electroanal. Chem. Interfacial Electrochem.* **1990**, *278*, 249–267.
(59) Matsuda, H.; Ayabe, Y. *Z. Elektrochem.* **1955**, *59*, 494–503.
(60) Nadjo, L.; Savéant, J. M. *J. Electroanal. Chem. Interfacial Electrochem.* **1973**, *44*, 327–366.
(61) Wrona, M. Z.; Dryhurst, G. *J. Org. Chem.* **1989**, *54*, 2718–2721.
(62) Humphries, K.; Dryhurst, G. *J. Pharm. Sci.* **1987**, *76*, 839–847.
(63) Humphries, K.; Dryhurst, G. *J. Electrochem. Soc.* **1990**, *137*, 1144–1149.
(64) Cheng, F.-C.; Dryhurst, G. *J. Pharm. Sci.* **1990**, *79*, 266–272.
(65) Wrona, M. Z.; Westmark, P.; Dryhurst, G. Unpublished results.
(66) Anne, A.; Moiroux, J. *J. Org. Chem.* **1988**, *53*, 2816–2820.
(67) Wrona, M. Z.; Lemordant, D.; Lin, L.; Blank, C. L.; Dryhurst, G. *J. Med. Chem.* **1986**, *29*, 499–505.
(68) Wrona, M. Z.; Dryhurst, G. *J. Pharm. Sci.* **1988**, *77*, 911–917.
(69) Remers, W. A. In *Indoles*; Houlihan, W. T., Ed.; Wiley-Interscience: New York, 1972; Part I, p 191.
(70) It was originally reported[64] that such a 3,4′ indolenine–indole dimer is not formed upon electrooxidation of 5-HTOL. More recent work, however (Westmark, P.; Wrona, M. Z.; Dryhurst, G. Unpublished observations), indicates such a dimer is indeed formed in relatively high yield.
(71) Baumgarten, H. G.; Björklund, A.; Lachenmeyer, L.; Nobin, A. *Acta Physiol. Scand., Suppl.* **1973**, *391*, 1–19.
(72) Jacoby, J. L., Lytle, D., Eds. Serotonin Neurotoxins. *Ann. N.Y. Acad. Sci.* **1978**, *305*, 1–702.
(73) Jonsson, G. *Annu. Rev. Neurosci.* **1980**, *3*, 169–187.
(74) Baumgarten, H. G.; Klemm, H. P.; Sievers, J.; Schlossberger, H. G. *Brain Res. Bull.* **1982**, *9*, 131–150.
(75) Baumgarten, H. G.; Jenner, S.; Björklund, A.; Klemm, H. P.; Schlossberger, H. G. In *Biology of Serotonergic Transmission*; Osborne, N. N., Ed.; Wiley: New York, 1982; Chapter 10.
(76) Jonsson, G. In *Handbook of Chemical Neuroanatomy*; Björklund, A., Hokfelt, T., Eds.; Elsevier: Amsterdam, 1983; Vol. 1, Chapter XII and references cited therein.
(77) Rotman, A.; Daly, J. W.; Crevelling, R. C. *Mol. Pharmacol.* **1976**, *12*, 887–899.
(78) Creveling, C. R.; Rotman, A. *Ann. N.Y. Acad. Sci.* **1978**, *305*, 57–73.
(79) Baumgarten, H. G.; Klemm, H. P.; Lachenmeyer, L.; Björklund, A.; Lovenberg, W.; Schlossberger, H. G. *Ann. N.Y. Acad. Sci.* **1978**, *305*, 3–24.
(80) Cohen, G.; Heikkila, R. E. *Ann. N.Y. Acad. Sci.* **1978**, *305*, 74–84.
(81) Sinhababu, A. K.; Borchardt, R. J. *J. Am. Chem. Soc.* **1985**, *107*, 7618–7626.
(82) Dryhurst, G.; Anne, A.; Wrona, M. Z.; Lemordant, D. *J. Am. Chem. Soc.* **1989**, *111*, 719–726.
(83) Tabatabaie, T.; Wrona, M. Z.; Dryhurst, G. *J. Med. Chem.* **1990**, *33*, 667–672.
(84) Sinhababu, A. K.; Borchardt, R. J. *Neurochem. Int.* **1988**, *12*, 273–284.
(85) Kappus, H.; Sies, H. *Experientia* **1981**, *37*, 1233–1241.
(86) Borg, D. C.; Schaich, K. M. *Isr. J. Chem.* **1984**, *24*, 38–53.
(87) Smith, M. T.; Evans, C. G.; Thor, H.; Orrenius, S. In *Oxidative Stress*; Sies, H., Ed.; Academic Press: London, 1985; pp 91–113.
(88) Kappus, H. *Biochem. Pharmacol.* **1986**, *35*, 1–6.
(89) Singh, S.; Jen, J.-F.; Dryhurst, G. *J. Org. Chem.* **1990**, *55*, 1484–1489.
(90) Klemm, H. P.; Baumgarten, H. G.; Schlossberger, H. G. *J. Neurochem.* **1980**, *35*, 1400–1408.
(91) Klemm, H. P.; Baumgarten, H. G. *Ann. N.Y. Acad. Sci.* **1978**, *305*, 36–56.
(92) Lemberger, L.; Axelrod, J.; Kopin, I. J. *J. Pharmacol. Exp. Ther.* **1971**, *177*, 169–176.
(93) Commins, D. L.; Axt, K. J.; Vosmer, G.; Seiden, L. S. *Brain Res.* **1987**, *403*, 7–14.
(94) Commins, D. L.; Axt, K. J.; Vosmer, G.; Seiden, L. S. *Brain Res.* **1987**, *419*, 253–261.
(95) Volicer, L.; Langlais, P. J.; Matson, W. R.; Mark, K. A.; Gameche, P. H. *Arch. Neurol.* **1985**, *42*, 1158–1161.
(96) Volicer, L.; Chen, J.-C.; Crino, P. G.; Vogt, B. A.; Fishman, J.; Rubins, J.; Schnepper, P. W.; Wolfe, N. Proceedings, 1st International Conference on Alzheimer's Disease and Related Disorders.
(97) Perry, E. K.; Marshall, E. F.; Blessed, G. Br. *J. Psychiatry* **1983**, *142*, 188–192.
(98) Bowen, D. M.; Allen, S. J.; Benton, J. S.; Goodhart, M. J.; Haan, E. A.; Palmer, A. M.; Sims, N. R.; Smith, C. C. T.; Spillane, J. A.; Esiri, M. M.; Neary, D.; Snowdon, J. S.; Wilcock, G. K.; Davidson, A. N. *J. Neurochem.* **1983**, *41*, 266–272.
(99) Palmer, A. M.; Francis, P. T.; Benton, J. S.; Sims, N. R.; Mann, D. M. A.; Neary, D.; Snowden, J. S.; Bowen, D. M. *J. Neurochem.* **1987**, *48*, 8–15.
(100) Chen, J.-C.; Crino, P. B.; Schnepper, P. W.; To, A. C. S.; Volicer, L. *J. Pharmacol. Exp. Ther.* **1989**, *250*, 141–148.
(101) Dalgleish, C. E. *Biochem. J.* **1955**, *64*, 481–485.
(102) Wong, K. S.; Dryhurst, G. *Bioorg. Chem.* **1990**, in press.
(103) Wolf, W. A.; Bobik, A. *J. Neurochem.* **1988**, *50*, 534–542.
(104) Humphries, K. Ph.D. Dissertation, University of Oklahoma, 1989.
(105) Cheng, F.-C. Ph.D. Dissertation, University of Oklahoma, 1989.
(106) Nelson, D. R.; Huggins, A. K. *Biochem. Pharmacol.* **1985**, *24*, 181–192.
(107) Neufeld, H. A.; Lucas, F. V.; Martin, A. P.; Stotz, E. *Cancer Res.* **1955**, *15*, 550–555.
(108) Saunders, B. C.; Holmes-Siedle, A. G.; Stark, B. P. *Peroxidase*; Butterworths: Washington, DC, 1964.
(109) Walsh, C. *Enzymatic Reaction Mechanisms*; W. H. Freeman: New York, 1979; pp 490–494.
(110) Wrona, M. Z.; Dryhurst, G. Unpublished observations.