Phe 2.00, Arg 3.45; TLC R_f (BPAW) 0.63; FABMS $C_{102}H_{156}N_{32}O_{30}S$, found m/e 2342 $(M + H)^+$.

Deamino[dicarba^{105,121}]r-ANF(105–126) (16): AAA Asp/Asn 1.96, Ser 1.85, Gln 1.00, Gly 5.14, Ala 0.97, Ile 2.00, Leu 1.01, Tyr 0.94, Phe 1.98, Arg 3.14; TLC R_f (BPAW) 0.63; FABMS $C_{103}H_{158}N_{32}O_{30}$, found m/e 2325 $(M + H)^+$.

Deamino[γ,δ-dehydrodicarba^{105,121}]r-ANF(105–126) (17): AAA Asp/Asn 1.99, Ser 1.81, Gln 0.98, Gly 5.18, Ala 1.00, Ile 1.90, Leu 0.98, Tyr 0.83, Phe 2.01, Arg 3.31; TLC R_f (BPAW) 0.63; FABMS $C_{103}H_{156}N_{32}O_{30}$, found m/e 2323 $(M + H)^+$.

Superfused Rabbit Aorta Rings. The descending thoracic aorta from New Zealand albino rabbits weighing approximately 1.5 kg was excised and placed in Krebs solution and bubbled with 5% CO_2 in O_2 to maintain the pH at 7.4. The aorta was cleaned of extraneous tissue and cut transversally into six 4 mm wide ring segments. Each segment was mounted on a force transducer (Grass Instruments, Model FT.03) and connected to a polygraph for isometric recording according to the method described by Hooker et al.[24] The rings were placed under tension and readjusted until a stable 10-g resting tension was obtained. The tissues were superfused with Krebs solution at a rate of 15 mL/min with a multichannel perilstaltic pump (Piper, Model PL MT fitted with 3L-type pumpheads). After an equilibration period of 45 min, phenylephrine hydrochloride was added to the superfusate (1×10^{-7} M) causing a 40–60% increase in tension. This increase was maintained for the remainder of the experiment. A known concentration of the test compound (50 μL) was added to the superfusate 3–4 cm above the tissue. Once the tissue had recovered to the initial level of contractility a second dose of higher concentration was administered. A standard dose–response curve was constructed and the EC_{50} value was determined.

In Vivo Antihypertensive Studies. The antihypertensive effect of the ANF analogues was evaluated in DOCA–salt hypertensive rats[25] and in 5/6 nephrectomized rats.[26] Briefly, animals were anesthetized with halothane after infiltration through the skin with a 2% lidocaine solution, and the left femoral artery and vein were dissected and cannulated with PE50 and PE10 tubing, respectively. Both cannulas were exteriorized at a point near the tail and the skin wound was sutured. After a period of recovery, arterial blood pressure was measured with a Gould P50 transducer and a Gould Model 2800 S recorder. The test compounds were infused for 30-min intervals at increasing concentrations (0.1, 0.2, 0.5, and 1.0 μg/kg per min) at a rate of 70 μL/kg per min following a control period with saline. The animals were allowed to recover under saline infusion and a dose of hydralazine (100 μg/kg per min) was administered for standardization.

Diuresis and Natriuresis. Normotensive male Sprague–Dawley rats (300–325 g) were anesthetized with halothane. Following administration of a 2% lidocaine solution, the femoral artery was cannulated for measurement of blood pressure and the femoral vein was cannulated for administration of the test compound. The bladder was cannulated to measure urine flow. Following surgery the animals were allowed to recover for at least 1 h in plastic restraining cages, and administration of Ringer's solution 1.2 mL/h) was started. Urine samples were collected at 10-min intervals during a 30-min control period. The test compound was then infused at a rate of 0.5 μg/kg per min over 30 min, and urine samples were again collected at 10-min intervals. Ringer's solution was again infused during the next 30-min recovery period and urine samples were collected once again. Urine volume was determined and electrolyte concentrations were measured with a Nova Biomedical electrolyte analyzer. Systolic and diastolic blood pressures were recorded during the experiment to ascertain that an adequate perfusion pressure was maintained.

Acknowledgment. This work was supported in part by an operating grant from the Industrial Development Office of The National Research Council of Canada (project no. CA90-5-0876/876). We are indebted to Drs. M. Cantin and A. DeLéan of the multidisciplinary Hypertension Group of the Clinical Research Institute of Montreal. We wish to acknowledge the excellent technical assistance of F. Liard, N. Lapeyre, J. C. Vigeant, W. Paris, and V. M. Cruz.

Registry No. 1, 123963-64-4; 2, 123963-65-5; 3, 123963-66-6; 3 (BOC-deblocked), 123963-72-4; 4, 124095-60-9; 5, 123963-67-7; 6a, 123963-68-8; 6b, 123963-73-5; 8, 88898-17-3; 9, 90817-13-3; 10, 95079-20-2; 11, 107865-22-5; 12, 123992-46-1; 13, 123992-47-2; 14, 123963-69-9; 15, 123963-70-2; 16, 103340-30-3; 17, 124093-79-4; fragment A, 122294-89-7; fragment B, 103339-80-6; fragment C, 122313-02-4; Fm-OH, 24324-17-2; $BrCH_2CH_2COO$-Fm, 123963-71-3; H-Cys-OH, 52-90-4; H-Hcy-OH, 6027-13-0; H-Pen-OH, 113-41-3.

Autoxidation of the Serotonergic Neurotoxin 5,7-Dihydroxytryptamine

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The indolic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) has been widely speculated to express its neurodegenerative effects as a result of intraneuronol autoxidation. Until recently, it was believed that autoxidation led to reactive electrophilic quinone imine species which alkylated neuronal membrane proteins and that byproducts of the autoxidation reaction were cytotoxic reduced-oxygen species. This study reveals that at physiological pH carbanions of 5,7-DHT act as the primary electron-donor species to yield C(4)- and C(6)-centered free radical superoxide complexes in a 1:2 ratio. The C(4)-centered complex reacts to yield, ultimately, 5-hydroxytryptamine-4,7-dione which has been shown to be a significantly more powerful neurotoxin than 5,7-DHT. The C(6)-centered radical superoxide complexes react to give 6,6′-bis(5-hydroxytryptamine-4,7-dione). It is likely that the latter reaction yields $O_2^{\bullet-}$ as a cytotoxic byproduct.

5,7-Dihydroxytryptamine (5,7-DHT) is used extensively for the selective chemical denervation of central serotonergic neurons.[1-8] The selectivity of 5,7-DHT probably results from its high affinity uptake by the membrane pump of serotonergic neurons. The toxicity of 5,7-DHT is widely believed to be related to its rapid oxidation by

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dissolved oxygen at physiological pH without catalysis by an enzyme, a process generally known as autoxidation. Autoxidation of 5,7-DHT in vivo has been proposed to give reactive quinone imine intermediates which can alkylate nucleophiles such as thiol residues of neuronal membrane proteins.[9] Such a reaction would be expected to modify the neuronal membrane with resulting destruction of the function of the neuron. A second theory to explain the neurotoxicity of 5,7-DHT invokes formation of cytotoxic reduced-oxygen species ($O_2^{\bullet-}$, $HO^{\bullet}$, H_2O_2) as byproducts of the autoxidation reaction.[6,7,8-11] It has also been speculated that quinone imine intermediates express the neurotoxic effect of 5,7-DHT by interacting with the electron-transport chain.[12] There is little experimental evidence to support any of these theories. For example, it has not been established that autoxidation of 5,7-DHT leads to transient formation of electrophilic quinone imine intermediates and there is no direct experimental evidence for the chemical reaction of such intermediates with neuronal proteins or model compounds. Cohen and Heikkila[10] have reported that small amounts of H_2O_2 are generated during autoxidation of 5,7-DHT although this observation could not be confirmed by Klemm et al.[12] There is also no experimental evidence that cytotoxic oxygen radicals ($O_2^{\bullet-}$ and $HO^{\bullet}$) are formed as a result of autoxidation of 5,7-DHT. However, radical scavengers such as 1-phenyl-3-(2-thiazolyl)-2-thiourea, nialamide, thiourea, and ethanol appear to provide significant protection of peripheral nerves from damage by 5,7-DHT.[10-14] Unfortunately, it is not known whether such radical scavengers provide similar protection to serotonergic neurons.

Recently, it was reported that in pH 7.4 phosphate buffer autoxidation of 5,7-DHT was first order with respect to both O_2 and 5,7-DHT.[15] This observation is consistent with a mechanism involving the incorporation of molecular O_2 into the 5,7-DHT nucleus and is not consistent with the transformation of 5,7-DHT into quinone imine species. According to Sinhababu and Borchardt,[15] O_2 is initially incorporated into the C(4)-position of 5,7-DHT to give a free radical superoxide complex. The key step in this autoxidation mechanism was speculated to be incipient formation of a C(4)-centered radical. The latter workers did not isolate the ultimate autoxidation product of 5,7-DHT. However, based upon the reactions of several methyl derivatives, it was concluded that the autoxidation product of 5,7-DHT was the *p*-quinone of 4,5,7-trihydroxytryptamine (4,5,7-THT), which subsequently reacted further to give more complex tricyclic compounds which also were not isolated.

The first step toward elucidating the mechanisms by which 5,7-DHT expresses its neurotoxicity is to understand the autoxidation reactions and to identify the products of this compound. In this report it will be demonstrated that at physiological pH the autoxidation of 5,7-DHT is a relatively slow reaction, that radical intermediates are involved, and that two major products are formed.

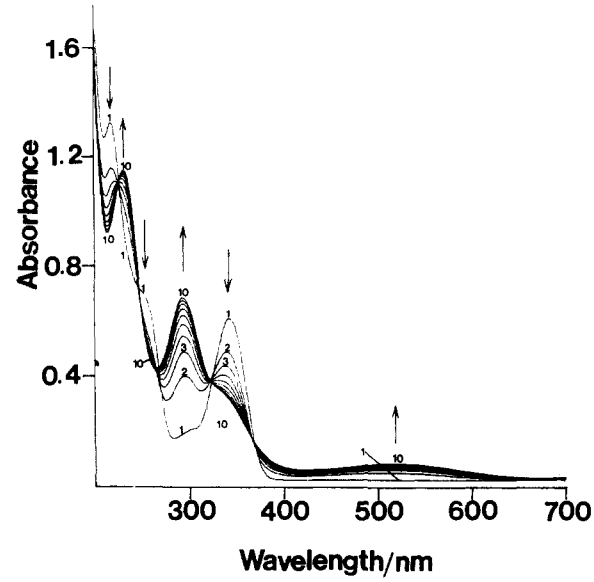

Figure 1. UV-visible spectra observed during the autoxidation of 0.12 mM 5,7-DHT in pH 7.4 phosphate buffer (μ = 0.1) at room temperature. Curve 1 was recorded immediately after dissolution of 5,7-DHT. Subsequent curves were recorded at 60-min intervals. Arrows indicate the direction of absorbance changes as the oxidation proceeds.

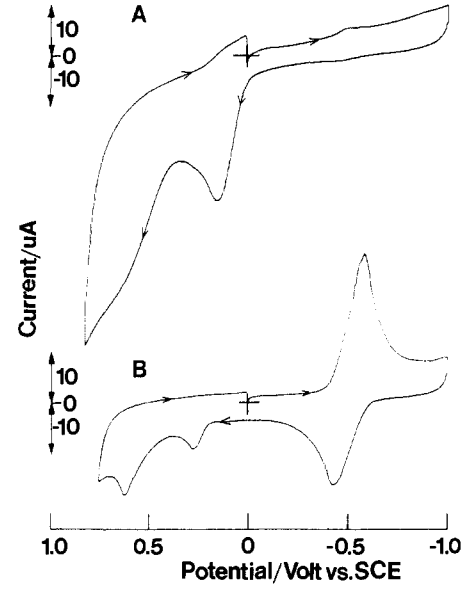

Figure 2. Cyclic voltammograms at the PGE of (A) a freshly prepared solution of 5,7-DHT (0.5 mM) in pH 7.4 phosphate buffer (μ = 0.1) and (B) the same solution after stirring in air for 24 h. The solutions were thoroughly deaerated with N_2 before recording the voltammograms. Sweep rate: 200 mV s^{-1}.

Results

Spectral changes which accompany the autoxidation of 5,7-DHT (0.12 mM) at pH 7.4 at 25 °C are shown in Figure 1. Curve 1 is the spectrum of 5,7-DHT (λ_{max} 344, 250 (sh), 214 nm). As the autoxidation reaction progresses, all of the latter bands systematically decrease. Correspondingly, new bands appear and grow at 525, 296, and 231 nm. Curve 10 in Figure 1 is the spectrum of the solution when all the 5,7-DHT has been oxidized. The appearance and growth of the low, broad band at 525 nm accounts for the initially colorless solution of 5,7-DHT turning pink-purple as the oxidation proceeds.

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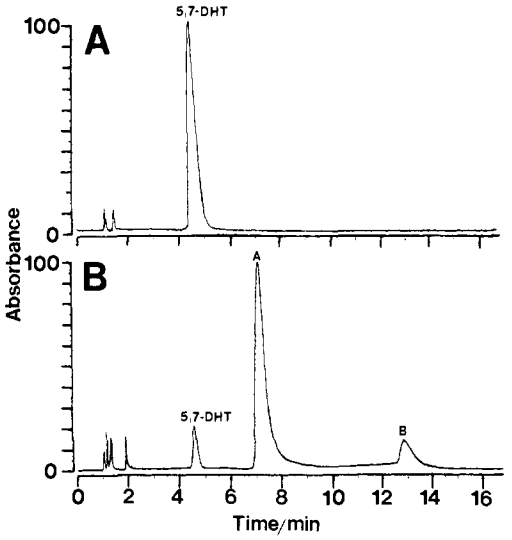


Figure 3. High-performance liquid chromatograms of (A) a freshly prepared solution of 5,7-DHT (1 mM) in pH 7.4 phosphate buffer (μ = 0.2) at 37 °C and (B) the same solution after autoxidation for 2.5 h. Chromatographic conditions are described in the Experimental Section. Injection volume: 200 μL. Absorbance is in arbitrary units.

Figure 2A shows a cyclic voltammogram of 5,7-DHT in a deaerated solution at pH 7.4. On the initial cathodic sweep no reduction peaks appear. On the first anodic sweep the primary oxidation peak of 5,7-DHT appears at a peak potential (E_p) of 0.19 V. After exposure of the solution to air for several hours the cyclic voltammogram shown in Figure 2B was recorded. On the first cathodic sweep a reduction peak appears at $E_p \approx -0.59$ V. Close inspection of this peak indicates that it represents two or more overlapping peaks. On the reverse sweep a quasi-reversible oxidation peak appears ($E_p \approx -0.43$ V). In addition, two apparently irreversible oxidation peaks appear at E_p = 0.275 and 0.62 V. The latter two peaks can be observed without initially scanning through the reduction peak at −0.59 V. Cyclic voltammograms recorded over the course of several hours of autoxidation show a systematic decrease of the 5,7-DHT oxidation peak at 0.19 V and a corresponding growth of all of the peaks shown in Figure 2B.

HPLC analysis of the product solution formed upon autoxidation of 5,7-DHT showed that as the peak for the latter compound (t_R = 4.7 min, Figure 3A) decreased two new major chromatographic peaks (A and B in Figure 3B) appeared. Peaks A and B appeared simultaneously. Chromatographic peak A is due to 6,6′-bis(5-hydroxytryptamine-4,7-dione) (**16**) and peak B to 5-hydroxytryptamine-4,7-dione (**8**). Quantitative analysis of the solution obtained following complete autoxidation of 0.54 mM 5,7-DHT showed that it contained 0.165 mM **16** and 0.20 mM **8**. Autoxidation of 1.07 mM 5,7-DHT gave 0.36 mM **16** and 0.39 mM **8**. Thus, autoxidation of millimolar concentrations of 5,7-DHT gives **8** and **16** in equimolar yields. This indicates that twice as much 5,7-DHT is converted to dimer **16** as is converted to monomer **8**. UV–visible spectra and cyclic voltammograms of monomer **8** and dimer **16** are presented in Figures 4 and 5, respectively. The spectra of **8** and **16** are quite similar and account for the spectral changes observed during autoxidation of 5,7-DHT and illustrated in Figure 1. The molar absorptivity of **16** is approximately 5 times that of **8** at 254 nm, the detector wavelength employed in HPLC analysis. This difference accounts for the fact that the peak height for **16** is significantly larger than that for **8** in HPLC traces (Figure 3B) although the concentrations of both compounds are approximately equal.

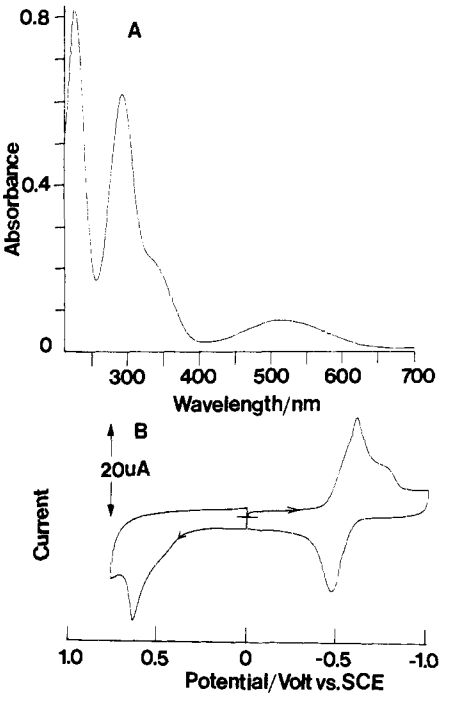


Figure 4. (A) UV–visible spectrum of 5-hydroxytryptamine-4,7-dione (**8**, 0.098 mM) in pH 7.4 phosphate buffer (μ = 0.1) and (B) cyclic voltammogram (0.49 mM **8**) at the PGE at a sweep rate of 200 mV s^{-1}. Spectra were recorded using 0.5-cm optical pathlength cells.

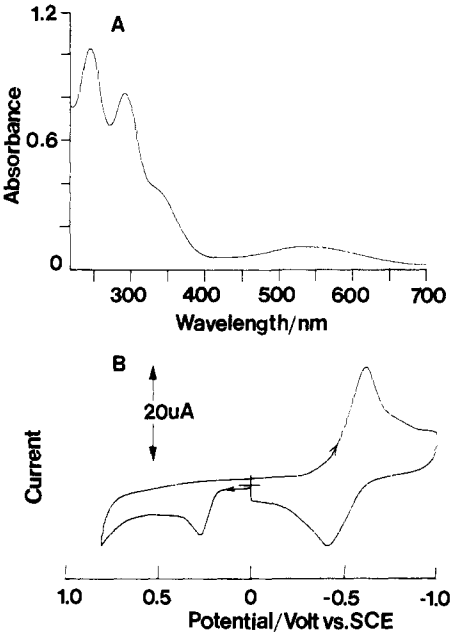


Figure 5. (A) UV–visible spectrum of 6,6′-bis(5-hydroxytryptamine-4,7-dione) (**16**, 0.1 mM) in pH 7.4 phosphate buffer (μ = 0.1) and (B) cyclic voltammogram (0.49 mM **16**) at the PGE at a sweep rate of 200 mV s^{-1}. Spectra were recorded using 0.5-cm optical pathlength cells.

Both **8** and **16** exhibit a quasi-reversible voltammetric reduction peak at ca. −0.6 V. However, **8** shows an apparently irreversible oxidation peak at 0.630 V while **16** exhibits an irreversible oxidation peak at 0.275 V. The quasi-reversible reduction peaks noted in cyclic voltammograms of both **8** and **16** can be observed without initially scanning their irreversible oxidation peaks. Similarly, the

latter peaks are observed without initially scanning the quasi-reversible reduction peaks. Cyclic voltammograms (CVs) of autoxidized solutions of 5,7-DHT (Figure 2B) clearly exhibit the presence of **8** and **16**. The processes responsible for the various peaks observed in CVs of **8** (Figure 4B) and **16** (Figure 5B) have yet to be determined.

Information bearing on the kinetics of the autoxidation reaction could not easily be obtained by monitoring one or more of the UV bands of 5,7-DHT because both **8** and **16** absorb in the same spectral regions. Accordingly, kinetic information was obtained by periodically sampling an autoxidizing solution followed by HPLC analysis. The pH of the mobile phase employed the HPLC (3.30) was such that it quenched the autoxidation reaction. As a further precaution the mobile phase was always thoroughly degassed before use. The peak area for the HPLC peak of 5,7-DHT was monitored throughout autoxidation reactions. Rate plots (ln peak area vs time) over the initial 2 h of autoxidation of 5,7-DHT (0.5–1.0 mM) at 37 °C in pH 7.4 phosphate buffer (μ = 0.2) through which air was vigorously bubbled were linear. Thus, under such conditions the autoxidation follows pseudo-first-order kinetics. The experimental pseudo-first-order rate constant under such conditions was 0.012 ± 0.001 min^{-1}. This corresponds to a half-life ($t_{1/2}$) for 5,7-DHT of approximately 58 min. When oxygen was bubbled through the solution under otherwise identical conditions the pseudo-first-order rate constant increased to 0.046 ± 0.001 min^{-1} ($t_{1/2} \approx 15$ min). The autoxidation of 5,7-DHT (0.5 mM) in air-saturated pH 7.4 phosphate buffer (μ = 0.2, 37 °C) in the presence of 0.1, 0.5, 50, and 70 mM EDTA also followed pseudo-first-order kinetics with experimental rate constants of 0.012, 0.017, 0.014, and 0.015 min^{-1}, respectively. It thus seems unlikely that trace metal ions play a significant catalytic role in the autoxidation reaction.

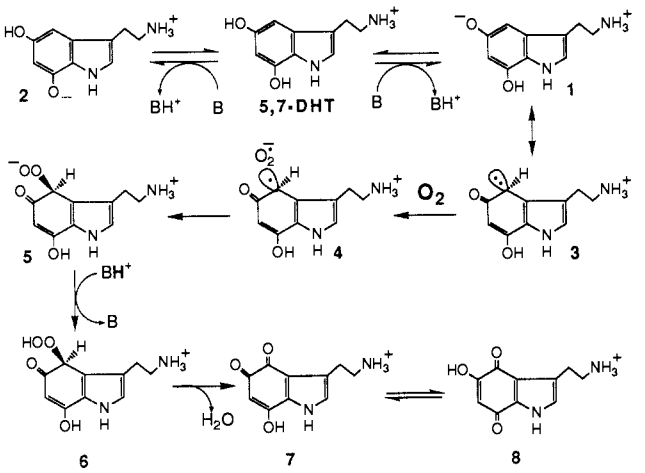
During preliminary experiments designed to isolate and purify **8** and **16** it was noted that there was a tendency for monomer **8** to form dimer **16**. In order to determine whether such a reaction was the source of **16** formed during autoxidation of 5,7-DHT, solutions of **8** in pH 7.4 phosphate buffer (μ = 0.2) were bubbled with air at 37 °C. After 3.5 h, HPLC analysis showed only a negligible conversion of **8** to **16**. Under the latter conditions approximately 20 days were required to cause 50% conversion of **8** to **16**.

Discussion

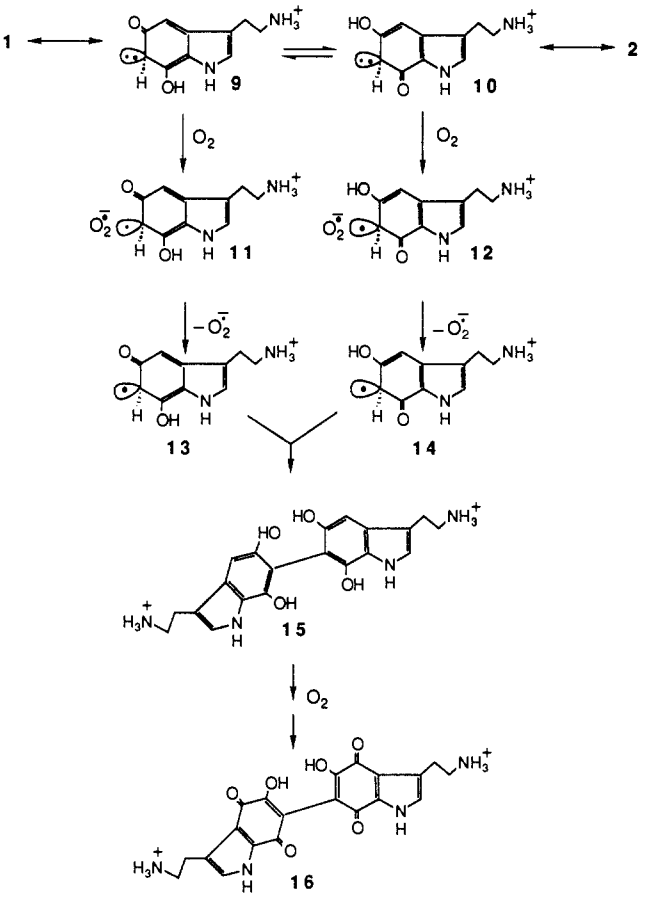
Schlossberger[16] first recognized that 5,7-DHT exhibits phenol–keto tautomerism characterized by a pH-dependent absorption maximum at 348 nm. Since the UV spectrum of 5,7-DHT exhibits several isosbestic points between pH 2–8, it is likely that free reversibility exists among the eight possible keto tautomers. Sinhababu and Borchardt[15] have compared the pH-dependent UV spectra of 5,7-DHT and various methylated analogues and concluded that the predominant keto tautomer involves ketonization of the 5-OH group with proton transfer to the C(4)-position. Since colored products were not formed upon autoxidation of 4-methyl-5,7-DHT, these workers concluded that the primary site of O_2 incorporation into 5,7-DHT is the C(4)-position. However, NMR studies of 5,7-DHT in D_2O indicate that the C(4)- and C(6)-protons are exchangeable and the latter proton exchanges twice as rapidly as the C(4)-proton.[15] This suggests that ketonization of the 5-OH and/or 7-OH groups might occur with proton transfer to the C(6)-position. The results from the present study indicate unequivocally that both the C(4)- and C(6)-positions are loci of the autoxidation reactions of 5,7-DHT. Failure to detect significant quantities of H_2O_2 as a by-product of autoxidation of 5,7-DHT[12,15] implies that quinone imine species are probably not the initial reactive intermediates formed. Electrochemical studies at low pH strongly suggest that 5,7-DHT is initially oxidized to radical intermediates.[17] Formation of dimer **16** and the latter facts support the conclusion that autoxidation of 5,7-DHT at physiological pH proceeds via a radical mechanism.

The pK_a for 5,7-DHT formally representing loss of a proton to form the 5-phenoxy species **1** and **2** (Scheme I) is 4.8. However, in view of the structures of the ultimate

Scheme I



Scheme II



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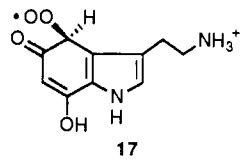
autoxidation products, it seems likely that carbanions **3**, (Scheme I) **9**, and **10** (Scheme II) are the primary electron donors to O_2. There are, in fact, several other ketonic species which could yield C(4)- or C(6)-centered carbanions, but with the present information it is not possible to specify which are the predominant species which react with O_2. The proposition that the initial step in the autoxidation reaction is formation of a free radical superoxide complex[15] is in accord with the various experimental results reported here and elsewhere. Attack of O_2 on carbanion **3** leads to the radical superoxide complex **4** (Scheme I). Recombination of the superoxide residue of **4** with the incipient C(4)-radical would produce hydroperoxide anion **5**[18,19] which upon proton abstraction generates hydroperoxide **6**. Base-catalyzed decomposition of the secondary hydroperoxide **6** generates *o*-quinone **7** and hence the more stable *p*-quinone **8**, which is an isolated product. The reaction pathway **3** → **8** is that proposed by Sinhababu and Borchardt[15] and elegantly rationalizes the observation that H_2O_2 is not formed as a byproduct of the autoxidation reaction. This pathway, however, only accounts for approximately one-third of the 5,7-DHT which is autoxidized.

Attack of O_2 on carbanions **9** and **10** would lead to the putative free radical superoxide complexes **11** and **12** (Scheme II). These complexes must follow a somewhat different reaction pathway to that of **4** because O_2 is not ultimately incorporated into the C(6)-position. We propose that **11** and **12** dissociate to give free radicals **13** and **14**, respectively. These radicals then react together to give dimer **15**. Since no evidence for a 4,6′-linked dimer product has been obtained, it appears that the radical superoxide complex **4** does not similarly dissociate to generate significant quantities of the C(4)-centered free radical. Dissociation of **11** and **12** would necessarily generate superoxide radical anion. Attempts have been made to detect $O_2^{\bullet-}$ by EPR spectroscopy by incorporating spin-traps such as 5,5-dimethyl-1-pyrroline 1-oxide (DMPO)[20] into the autoxidizing system. However, no signal could be detected for the DMPO–$O_2^{\bullet-}$ spin adduct. The slow rate of the autoxidation reaction ($t_{1/2} \approx 1$ h) and the known instability of the DMPO–$O_2^{\bullet-}$ spin adduct ($t_{1/2} \approx 35$ s at pH 8[21]) probably accounts for this fact. Dimer **15** has the same structural functionalities as 5,7-DHT except that the C(6)-position is blocked. Thus, dissociation of **15** leads to a 4 → 4′ dicarbanion which in a reaction sequence analogous to that of carbanion **3** reacts with oxygen to ultimately yield 6,6′-bis(5-hydroxytryptamine-4,7-dione) (**16**).

Sinhababu and Borchardt[15] noted that after the initial autoxidation of 5,7-DHT some O_2 was returned to the solution. This was speculated to be caused by dissociation of **4** to give the C(4)-centered free radical, which could react with O_2 to give peroxide radical **17**. Disproportionation



17

of **17** would produce O_2.[22,23] However, the failure to detect a 4,6′-linked dimeric product argues against transient formation of the C(4)-centered radical species. Dissociation of radical superoxide complexes **11** and **12** should yield $O_2^{\bullet-}$ and dismutation of this species or reaction of $O_2^{\bullet-}$ with hydroperoxide **6** or the equivalent dimeric hydroperoxide intermediate between **15** and **16** could be a source of the liberated O_2.[24-26] Previous workers have reported that little or no H_2O_2 is liberated during autoxidation of 5,7-DHT.[10,12] Furthermore, we have found that in anaerobic solution H_2O_2 (equimolar with 5,7-DHT) brings about little or no oxidation of 5,7-DHT. In addition, H_2O_2 added to an autoxidizing solution of 5,7-DHT causes no significant enhancement in the rate of the reaction or formation of additional products.

Conclusions

Available evidence supports the conclusion that, following uptake of 5,7-DHT into a target serotonergic neuron, the initial step in the reaction sequence by which this compound expresses its neurotoxicity is autoxidation. There is still, however, considerable uncertainty about the identity of the actual toxins and the ways in which they bring about neuronal damage. A recent report from this laboratory[27] demonstrated that **8** is a neurotoxin in mouse brain and, based on its LD_{50} value (~21 μg), it is approximately three times as toxic as 5,7-DHT. Perhaps fortuitously, one-third of 5,7-DHT is autoxidized to **8** in vitro, suggesting that the latter compound might be the ultimate neurotoxin or its immediate precursor. Sinhababu and Borchardt[28] have recently elaborated on this possibility and suggest that intraneuronally formed **8** might undergo redox cycling reactions which generate cytotoxic reduced-oxygen species. A natural consequence of such reactions would be oxygen depletion which might lead to hypoxia-induced damage to the neuron. While details of the redox chemistry of both **8** and its dimer **16** remain to be elucidated, it is clear from their cyclic voltammetric behaviors (Figures 4 and 5) that both **8** and **16** exhibit quasi-reversible redox behavior at ca. −0.5 V at pH 7.4. Thus, **16** should also be capable of redox cycling reactions of the type postulated for **8**. The latter workers have also suggested that a hydroperoxide intermediate such as **6** or the equivalent dimer might be converted to the reduced forms of **8** (4,5,7-trihydroxytryptamine) and **16** (6,6′-bis-(4,5,7-trihydroxytryptamine)), respectively, by enzymes such as glutathione peroxidase. Such reactions would lead to decreased levels of the intraneuronal antioxidant glutathione and yield reduced oxygen species.[28] It was noted previously that several radical scavengers protect at least peripheral nerves from damage by 5,7-DHT.[10-14] This observation suggests that short-lived radical intermediates such as **13** and **14** might play a functional role in the neurodegenerative action of 5,7-DHT.

Experimental Section

5,7-Dihydroxytryptamine (5,7-DHT, creatinine sulfate salt) was obtained from Sigma (St. Louis, MO) and was used without further purification. Cyclic voltammograms were obtained with

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a pyrolytic graphite electrode (PGE, Pfizer Minerals, Pigments and Metals Division, Easton, PA) having an approximate surface area of 4 mm^2. Voltammograms were obtained with a conventional operational amplifier-based potentiostat.[29] Voltammograms were corrected for iR drop. Potentials are referred to the saturated calomel electrode (SCE) at ambient temperature (22 ± 3 °C).

High-performance liquid chromatography (HPLC) employed a Gilson System 42 gradient system equipped with dual pumps, an Apple IIe controller, a Rheodyne Model 7125 loop injector, and a Gilson Holochrome UV detector (254 nm). HPLC separations were carried out with a reversed-phase semipreparative column (Brownlee Laboratories, RP-18, 5-μm particle size, 25 × 0.7 cm). A short guard column (Brownlee, RP-18, 5 μm, OD-GU, 5 × 0.5 cm) was always employed. The mobile phase was prepared as follows: 1.10 mL of triethylamine (Pierce Chemical Company, Sequanal grade) was added to 1 L of water and the pH of the resulting solution was adjusted to 3.30 by addition of glacial acetic acid. Then, 20 mL of HPLC-grade acetonitrile (Fisher) was added. All HPLC separations employed a mobile phase flow rate of 3.5 mL min^{-1}.

Low- and high-resolution mass spectrometry was carried out with a VG Instruments ZAB-E spectrometer. ^1H and ^{13}C NMR spectra (300 MHz) were recorded on a Varian Model 300 XL spectrometer. Infrared spectra were recorded on a Mattson Sirius 100 FT-IR spectrometer. UV–visible spectra were recorded on a Hitachi 100-80 spectrophotometer.

Isolation and Purification of Autoxidation Products. Autoxidations of 5,7-DHT (ca. 3 mM) were carried out in pH 7.4 phosphate buffer having an ionic strength (μ) of 0.20[30] at either 25 or 37 °C. In most experiments the solution containing 5,7-DHT was stirred with a Teflon-coated stirring bar and air was vigorously bubbled through the solution. In some experiments oxygen was bubbled through the solution. Upon completion of the autoxidation reaction, (6-7 h) as evidenced by HPLC analysis, cyclic voltammetry, and UV–visible spectrophotometry, the products were separated by HPLC with the conditions described previously. Two-milliliter injections of the product solutions were employed and the components which eluted under the peaks having retention times (t_R) of 7.15 and 12.9 min were collected separately. This procedure was repeated until the entire product solution had been separated. When the combined product solutions were freeze-dried in the presence of triethylammonium acetate, considerable decomposition of both products occurred. Accordingly, the collected products in the HPLC mobile phase were first desalted by passing the solutions through a column of Sephadex LH-20 (Pharmacia, Piscataway, NJ; 70 × 2.0 cm) using water adjusted to pH 2.0 with HCl as the mobile phase (60 mL h^{-1}). The triethylammonium acetate eluted rapidly from this column and hence the product could be readily desalted. The collected products were then freeze-dried. Each product was then dissolved in water and subjected to an identical HPLC/LC procedure for final purification.

5-Hydroxytryptamine-4,7-dione (8). The product which eluted with an HPLC t_R = 12.9 min was isolated as an orange solid (decomposition temperature = 175 °C). UV–visible spectra of this compound in phosphate buffers (μ = 0.1) at different pH values were as follows. λ_{max} (log ϵ_{max}): pH 2.15, 460 (2.94), 330 (3.49), 280 (4.12), 220 (4.10); pH 5.3, 510 (3.10), 330 (3.61), 290 (4.05), 230 (4.16); pH 7.4, 515 (3.17), 330 (3.67), 292 (4.11), 232 (4.22); pH 8.5, 515 (3.13), 330 (3.63), 293 (4.07), 232 (4.18). ^1H NMR (Me_2SO-d_6): δ 12.51 (s, 1 H, N(1)-H), 11.12 (b s, 1 H, OH), 8.07 (s, 3 H, NH_3^+), 7.04 (s, 1 H, C(2)-H), 5.73 (s, 1 H, C(6)-H), 3.02 (t, 2 H, CH_2), 2.96 (t, 2 H, CH_2). Assignments for these various resonances were based on homonuclear decoupling experiments and have been discussed elsewhere.[17,27] Addition of a few drops of D_2O to the above solution caused the resonances at δ 12.51, 11.2, 8.07, and 5.73 to disappear. Thus, the C(6)-H proton (δ 5.73) is exchangeable. The mass, IR, and ^1H NMR spectra and elemental analysis of **9** have been presented in detail in earlier reports.[17,27]

6,6′-Bis(5-hydroxytryptamine-4,7-dione) (16). This compound (HPLC t_R = 7.15 min) was isolated as a brown-orange solid (decomposition temperature = 230 °C). UV–visible spectra in phosphate buffers (μ = 0.1) at different pH values were as follows. λ_{max} (log ϵ_{max}): pH 2.15, 450 (3.02), 328 (3.74), 280 (4.24), 230 (4.31); pH 4.9, 510 (3.10), 340 (3.77), 285 (4.21), 240 (4.23); pH 7.4, 540 (3.26), 335 (3.88), 293 (4.23), 245 (4.33); pH 8.5, 545 (3.14), 335 (3.83), 293 (4.17), 245 (4.27). Fast atom bombardment mass spectra (FAB-MS, positive ion) in a 3-nitrobenzyl alcohol matrix, m/e (relative abundance): 411 (MH^+, 24), 329 (15), 307 (39), 289 (20), 176 (29), 155 (39), 139 (22), 138 (47), 137 (100). Accurate mass measurements on the pseudomolecular ion (MH^+) gave m/e = 411.1291 ($C_{20}H_{19}N_4O_6$, calculated m/e = 411.1305). ^1H NMR (Me_2SO-d_6): δ 12.58 (s, 2 H, N(1)-H, N(1′)-H), 10.70 (b s, ca. 2 H, 2 × OH), 8.16 (s, 6 H, 2 × NH_3^+), 7.08 (s, 2 H, C(2)-H, C(2′)-H), 3.08 (t, 4 H, 2 × CH_2), 3.01 (t, 4 H, 2 × CH_2). Addition of D_2O to the latter sample resulted in the disappearance of the resonances at δ 12.58, 10.70, and 8.16. ^{13}C NMR (Me_2SO-d_6): δ 178.38, 176.32, 156.06, 132.59, 123.99, 120.06, 118.97, 110.61, 38.16, 23.52. Compound **16** thus has a UV–visible spectrum which is quite similar to that of **8**. However, its molar mass (410 g) and molecular formula ($C_{20}H_{18}N_4O_6$) indicate that it must be a dimer of **8**. The simplicity of the ^1H NMR spectrum of **16** and the fact that only 10 resonances are observed in its ^{13}C NMR spectrum indicate that this compound must be a symmetrical dimer. The characteristic ^1H NMR resonance of the C(6)-H proton of **8** (δ 5.73) is absent in **16** but resonances due to two OH, two C(2)-H, and two N(1)-H protons can be clearly identified. Accordingly, **16** must consist of two residues of **8** linked together at their C(6)-positions. The ^{13}C NMR spectrum clearly indicates that in **16** two different carbonyl residues are present (δ 178.38, 176.32).

pK_a Determinations. Each compound was initially dissolved in phosphate buffer pH 2.2 (μ = 0.1; ca. 0.1 mM **8** or **16**). The UV–visibile spectrum of each compound was recorded and then the pH was adjusted in ca. 0.5 pH unit intervals to pH 10 by addition of a solution of NaOH. At each pH interval the spectrum was recorded. The shift in λ_{max} with pH for the two intense UV bands was used to calculate pK_a values. The experimental pK_a for **8** was 5.6 ± 0.2 and for **16** was 6.5 ± 0.5. The pK_a for 5,7-DHT could not be determined by this spectrophotometric method owing to the complicating effects of phenol–keto tautomerism (see earlier discussion). Potentiometric titration (under nitrogen) of 5,7-DHT (0.2 mM) with KOH gave a pK_a value of 4.8 ± 0.1.

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