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6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline formation by iron mediated dopamine oxidation: a novel route to endogenous neurotoxins under oxidative stress conditions.

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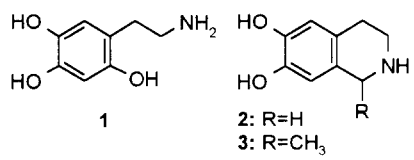
Abstract

Aerobic oxidation of dopamine mediated by iron ions gives 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**2**) and 3,4-dihydroxybenzaldehyde (**4**) in yields up to 10% and 15%, respectively. Based on ^{13}C labelling experiments, a reaction mechanism is proposed involving oxidative fission of the dopamine side chain to give **4** and formaldehyde, the latter giving **2** by Pictet-Spengler condensation with dopamine. This provides a novel route to endogenous generation of neurotoxic isoquinoline alkaloids under oxidative stress conditions.

Keywords: Alkaloids; Metabolites; Oxidation; Isoquinolines.

Oxidative stress is generally recognized as a critical condition underlying the loss of nigrostriatal dopaminergic neurons associated to senescence and neurodegenerative disorders such as Parkinson's disease [1,2]. A considerable body of evidence has been presented over the last decade suggesting that an increased lipid peroxidation [3], the release of iron ions from ferritin [4], and an enhanced production of H_2O_2 deriving from an upregulated monoamineoxidase activity [5] are typical markers of oxidative stress. Much credit has also been given to the hypothesis that a chronic diversion of dopamine metabolism occurring under such conditions [6] may give rise to endogenous neurotoxins capable of specifically impairing basic metabolic functions, leading eventually to neuron death. Extensive studies carried out since the late sixties have focused on 6-hydroxydopamine (**1**), whose ability of selectively destroying



1 **2**: R=H **3**: R=CH_3

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catecholaminergic nerve endings has been interpreted in terms of an oxidative conversion to toxic quinonoid species [7,8].

More recently, the occurrence of tetrahydroisoquinoline alkaloids, such as **2-3**, derived from dopamine in the brain of Parkinsonian patients on L-Dopa medication [9,10], and their potential of inducing Parkinsonism [11,12] by a mechanism similar to that ascribed to MPTP [13], has raised the possibility that these compounds may play a fundamental role in the ethiopathogenesis of the disease. However, how these neurotoxins may be generated intraneuronally has remained a matter of controversy.

A possible mechanism of endogenous generation of **1** was suggested by our recent studies showing that oxidation of dopamine in the presence of H_2O_2 [14] or polyunsaturated fatty acid hydroperoxides/ferrous ions [15] gives rise to the corresponding quinone. In this paper we report the formation of the 6,7-dihydroxytetrahydroisoquinoline **2** by aerobic oxidation of dopamine mediated by iron ions and provide evidence for a reaction mechanism which may become operative *in vivo* under conditions of oxidative stress.

Aerobic oxidation of 1.0 mM dopamine in phosphate buffer pH 7.0 is accelerated in the presence of ferrous ions [16] (e.g. 0.4 mM) and leads in the initial stages (6-8 h) to the formation of **1** as the major reaction product followed within 24-48 hrs by the appearance of a complex pattern of products, comprising two main components, A and B (Fig. 1).

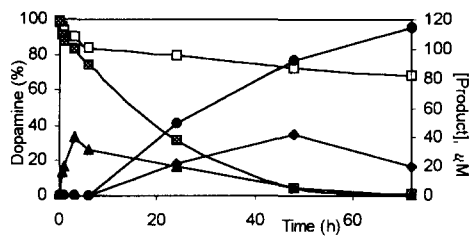
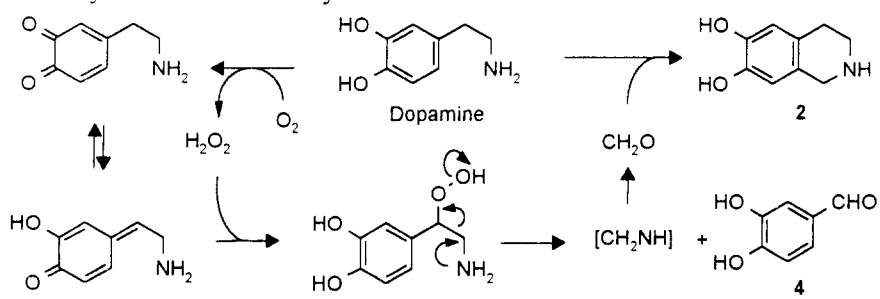


Figure 1. Time course of dopamine oxidation (left axis) and product concentration (right axis) by oxidation of 1.0 mM dopamine in 0.1 M phosphate buffer, pH 7.0. Dopamine decay: with (■), and without (□) 0.4 mM ferrous ions. Formation of **1** (△), product A (●), and product B (◆), (concentrations determined after identification, see text).

Isolation by preparative HPLC and spectral characterization allowed identification of compound A as the 3,4-dihydroxybenzaldehyde (**4**). The mass spectrum of compound B provided evidence for the presence of an additional methylene group with respect to dopamine. This feature was clearly apparent from analysis of proton and carbon spectra, which coupled with 2D experiments, led us to formulate the compound as the 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**2**) [17]. This structural assignment was corroborated by comparison with an authentic sample prepared by Pictet-Spengler reaction of dopamine with formaldehyde [18]. The dependence of formation yields of **2** on the reaction parameters was then investigated. Optimal yields up to 10% were obtained using the catecholamine at 10 mM concentration and a

1:1 Fe II/substrate molar ratio. Use of ferric in place of ferrous ions did not produce a marked increase of the yields, while addition of H_2O_2 over the first 2 hrs of the reaction led mostly to ill defined materials. Variation of the FeII/catecholamine ratio affected the extent of substrate depletion more than the product yields. Addition of hydroxyl radicals scavengers such as DMSO and mannitol did not affect thekineticsof the reaction and product distribution.

Formation of **2** and **4** by dopamine oxidation is at first sight not straightforward, although the significant yields of the aldehyde would point to a breakdown of the ethylamine side chain under the reaction conditions investigated. To gain an insight into the mechanism of formation of these species, the oxidation reaction was repeated using dopamine enriched with ^{13}C at the positions of the ethylamine side chain [19]. On ^{13}C NMR analysis isoquinoline **2** obtained from β-^{13}C dopamine [20] showed a single peak at δ 25.78 due to the CH_2 carbon at the 4-position. More interestingly, **2** from α-^{13}C dopamine [21] showed quantitative incorporation of the label, both at the 3-position as expected, and at the methylene group at position 1, demonstrating its origin from the α-carbon of the ethylamine chain of dopamine. On this basis, the mechanism outlined in Scheme 1 is proposed. In this, the key event is the nucleophilic attack to a transient quinonemethide generated by oxidation of dopamine, probably in the form of iron III chelate [15], by H_2O_2 smoothly released in the reaction medium by autooxidation of the catecholamine. The resulting β–aminohydroperoxide decomposes *via* carbonyl forming fragmentations [22] to give the benzaldehyde **4** and formaldehyde.



Pictet-Spengler reaction of the formaldehyde with dopamine at the 6-position activated by iron complexation leads eventually to the isoquinoline **2**. A different reaction course involving nucleophilic addition to quinonoid species arising from dopamine and subsequent muconic-type ring fission [23] may prevail when H_2O_2 is added to the mixture rather than slowly generated *in situ* accounting for the decrease of the yields of **2** observed. In line with the suggested mechanism, which rules out the intervention of hydroxyl radicals arising by Fe II reduction of H_2O_2 in a Fenton-type process, are the results of control scavenging experiments.

Apart from the chemical interest related to the peculiar reactivity exhibited by dopamine in oxidation processes promoted by iron ions, the results of this study provide a new mechanistic route to the tetrahydroisoquinoline **2** from dopamine under conditions of relevance to

neurodegenerative processes. Differently from the *R,S* enantiomers of salsolinol, whose formation has been largely documented and proved as a reaction of pyruvate with dopamine [24], the formation of **2** has so far remained speculative and interpreted in terms of the reactivity of dopamine with endogenous formaldehyde [25] in spite of very low basal levels. Although the relevance to the *in vivo* processes remains to be assessed, the finding that **2** may originate through a degradative fission of dopamine itself provides a new clue to definition of the metabolic pathway of this neurotoxic alkaloid.

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References and Notes

[1]Fhan, S.; Cohen, G. Ann. Neurol. 1992;32: 804-812.
[2]Spina, M.B.; Cohen. G. Proc. Natl. Acad. Sci. USA 1989;86:1398-1400.
[3]Dexter, D. T.; Carter. C. J.; Wells, F. R.; Javoy-Agid, F.; Agid, Y.; Lees, A. J.; Jenner, P.; Marsden, C. D. J. Neurochem. 1989;52:382-389.
[4]Ben-Shachar, D.; Riederer, P., Youdim, M. B. H. J. Neurochem 1991;57:1609-1614.
[5]Cohen, G. Advances in Neurology; M. D. Yahr, K. J. Bergman Eds.; Raven Press: New York, 1986;119-125
[6]Evans, J.M.; Cohen. G. Neurochem. Int. 1989;15:127-129.
[7]Ungerstedt, U. 6-Hydroxydopamine and Catecholamine Neurons; T. Malmfors, H. Thoenen Eds.; American Elsevier: New York, 1981;101-128.
[8]Slivka, A.; Cohen, G. J. Biol. Chem. 1985;29:15466-15472.
[9]Sandler, M.; Bonham-Carter, S.; Hunter, K. R.; Stern, G. M. Nature, 1973;241:439-443.
[10]Origitano, T.; Hannigan, J.; Collins, M. A. Brain Res. 1981;224:446-451.
[11]Yoshida, M.; Ogawa. M.; Suzuki, K.; Nagatsu, T. Adv. Neurol. 1993;60:207-211.
[12]Naoi, M.; Maruyama. W.; Dostert, P. Prog. Brain Res. 1995;106:227-239.
[13]Castagnoli, N. Jr; Chiba, K.; Trevor, A. J. Life Sci. 1985;36:225-230.
[14]Napolitano, A.; Crescenzi, O.; Pezzella, A.; Prota, G. J. Med. Chem. 1995;38:917-922.
[15]Pezzella, A.; d'Ischia, M.; Napolitano, A.; Misuraca, G.; Prota, G. J. Med. Chem. 1997;42:2011-2017.
[16]No precipitation of iron salts is observed under the reaction conditions due to the formation of a complex of dopamine with ferric ions [15] rapidly generated by aerobic oxidation of ferrous ions.
[17]LREIMS positive ions, m/z (relative intensity) 165 (M+, 19), 164 (45), 137 (15, M-C_2H_4), 136 (100); HREIMS 165.0792 exact mass calcd for $C_9H_{11}NO_2$ 165.0790; UV λ_{max} (H_2O) 280 nm (log ε 3.54); ^{1}H NMR (400 MHz, 0.1 M DCl) δ 2.96 (t, 2H, J=6.4 Hz, $CH_2$4), 3.45 (t, 2H, J=6.4 Hz, $CH_2$3), 4.21 (s, 2H, $CH_2$1), 6.70 (s, 1H, CH8), 6.75 (s, 1H, CH5); ^{13}C NMR (100 MHz, 0.1 M DCl) δ 25.78 (C-4). 43.69 (C-3), 45.97 (C-1), 115.49 (C-8), 117.60 (C-5), 121.41 (C-9), 125.46 (C-10), 144.83 (C-7), 145.69 (C-6).
[18]Whaley, W. M.; Govindachari, T. R. Organic Reactions; R. Adams, Ed., J. Wiley & Sons: New York, 1951;6:151-190.
[19] 2-(3,4-dihydroxyphenyl)[2-^{13}C] and [1-^{13}C]ethylamine were prepared as previously described: Crescenzi, O.; Kroesche, C.; Hoffbauer, W.; Jansen, N.; Napolitano, A.; Prota, G.; Peter, M.G. Liebigs. Ann. Chem. 1994;563-567.
[20]LREIMS positive ions, m/z 166 (M+); $^{12}C_8{}^{13}CH_{11}NO_2$ calc. 166.0823 found 166.0827.
[21]LREIMS positive ions, m/z 167 (M+); $^{12}C_7{}^{13}C_2H_{11}NO_2$ calc. 167.0857 found 166.0859.
[22]Becker, K.B.; Grob. C.A. The Chemistry of Functional Groups, Supplement A; S. Patai Ed.; J. Wiley and Sons: New York, 1977;653-723
[23]Pandell, A. J. J. Org. Chem. 1976;41:3992-3996.
[24]Dostert, P.; Strolin-Benedetti, M.; Bellotti, V.; Allievi, C.; Dordain, G. J. Neural Transm. 1990;81:215-223.
[25]Kajita, M.; Niwa. T.; Maruyama, W.; Nakahara, D.; Takeda, N.; Yoshizumi, H.; Tatematsu, A.; Watanabe, K.; Naoi, M.; Nagatsu, T. J. Chromatogr. 1994;654:263-269.