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Original Contribution

Oxidatively generated DNA damage after Cu(II) catalysis of dopamine and related catecholamine neurotransmitters and neurotoxins: Role of reactive oxygen species ☆

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ABSTRACT

There is increasing evidence supporting a causal role for oxidatively damaged DNA in neurodegeneration during the natural aging process and in neurodegenerative diseases such as Parkinson and Alzheimer. The presence of redox-active catecholamine neurotransmitters coupled with the localization of catalytic copper to DNA suggests a plausible role for these agents in the induction of oxidatively generated DNA damage. In this study we have investigated the role of Cu(II)-catalyzed oxidation of several catecholamine neurotransmitters and related neurotoxins in inducing oxidatively generated DNA damage. Autoxidation of all catechol neurotransmitters and related congeners tested resulted in the formation of nearly a dozen oxidation DNA products resulting in a decomposition pattern that was essentially identical for all agents tested. The presence of Cu(II), and to a lesser extent Fe(III), had no effect on the decomposition pattern but substantially enhanced the DNA product levels by up to 75-fold, with dopamine producing the highest levels of unidentified oxidation DNA products (383 ± 46 adducts/10^6 nucleotides), nearly 3-fold greater than 8-oxo-7,8-dihydro-2′-deoxyguanosine (122 ± 19 adducts/10^6 nucleotides) under the same conditions. The addition of sodium azide, 2,2,6,6-tetramethyl-4-piperidone, tiron, catalase, bathocuproine, or methional to the dopamine/Cu(II) reaction mixture resulted in a substantial decrease (>90%) in oxidation DNA product levels, indicating a role for singlet oxygen, superoxide, H_2O_2, Cu(I), and Cu(I)OOH in their formation. Whereas the addition of *N-tert*-butyl-α-phenylnitrone significantly decreased (67%) dopamine-mediated oxidatively damaged DNA, three other hydroxyl radical scavengers, ascorbic acid, sodium benzoate, and mannitol, had little to no effect on these oxidation DNA product levels, suggesting that free hydroxyl radicals may have limited involvement in this dopamine/Cu(II)-mediated oxidatively generated DNA damage. These studies suggest a possible contributory role of oxidatively generated DNA damage by dopamine and related catechol neurotransmitters/neurotoxins in neurodegeneration and cell death. We also found that a naturally occurring broad-spectrum antioxidant, ellagic acid, was substantially effective (nearly 50% inhibition) at low doses (1 μM) at preventing this dopamine/Cu(II)-mediated oxidatively generated DNA damage. Because dietary ellagic acid has been found to reduce oxidative stress in rat brains, a neuroprotective role of this polyphenol is plausible.

Significant evidence indicates that oxidative stress plays a central role in the aging brain [1–3] and in neurodegenerative diseases such as Alzheimer (AD)[2] [1,3,4] and Parkinson disease (PD) [5–7]. Postmortem studies of patients with neurologic degeneration have demonstrated increased levels of oxidatively damaged DNA in AD [4] and dementia with Lewy body brains [8] and increased indices of oxidative stress in PD brains, including increased levels of lipid peroxidation and iron and decreased mitochondrial complex I activity and levels of glutathione [9], compared to age-matched control subjects. A common trait shared between AD and PD is the degradation of neurons in specific regions of the brain, which is believed to be mediated, at least in part, by reactive oxygen species (ROS) [10]. Postmitotic cells, such as neurons, are believed to be particularly sensitive to the deleterious effects of ROS because damaged cells cannot be replaced. Moreover, the high oxygen metabolism taking place in the brain also increases neuronal vulnerability to oxidatively generated damage [11]. ROS have been shown to cause, in addition to neuronal cell death [12,13], damage to a wide range of neuronal cellular components, including membrane lipids [14–16], proteins [15,17], and DNA [5,7].

Both PD and AD are considered diseases of the aging brain because the most important risk factor for these diseases is advancing age

Abbreviations: AD, Alzheimer disease; PD, Parkinson disease; ROS, reactive oxygen species; nDNA, nuclear DNA; mtDNA, mitochondrial DNA; ST-DNA, salmon testis DNA; SOD, superoxide dismutase; TBP, *N-tert*-butyl-α-phenylnitrone; TMP, 2,2,6,6-tetramethyl-4-piperidone; PEI, polyethyleneimine; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; dSP, spiroiminodihydantoin nucleosides.

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[11,18]. Not only have increased levels of oxidatively damaged DNA in brain tissue been shown to be inversely proportional to life span [2,3] and correlate with increasing age of the human brain [3], but they have also been shown to play a key role in selective neuronal loss in both PD [6] and AD [3]. Nuclear DNA (nDNA) and mitochondrial DNA (mtDNA), in particular, seem to be important targets for the progression of neurodegeneration in the natural aging process as well as in several diseases including AD and PD [11,18]. Mitochondria provide the primary source of energy in the cell. mtDNA is exceptionally susceptible to oxidatively generated damage as it lacks protective histones and is located in close proximity to the respiratory chain, the cell's main source of ROS [11,18]. One line of evidence supporting mtDNA's increased vulnerability to oxidative stress is that elevated levels of oxidatively damaged DNA in both nDNA and mtDNA have been found in AD brains [19,20]; however, 3- to 10-fold higher levels of oxidized bases were identified in mtDNA [4,21]. Also noteworthy is that mtDNA has no noncoding sequences [22], thus potentially resulting in impaired functional consequences [4]. Impairment in mitochondrial function resulting from mutations in nDNA and mtDNA may result in neuronal cell death via defects in oxidative phosphorylation [4]. It should also be recognized that damage to either nDNA or mtDNA can indirectly affect the other. Damage to mtDNA can lead to impaired energy generation and potential leaks of ROS during respiration, which can in turn result in oxidatively generated damage to nDNA. Conversely, nDNA encodes the majority of mitochondrial proteins and thus its integrity has a direct impact on mitochondrial respiration and function [18].

A growing body of evidence indicates that dopamine [23–26], an essential neurotransmitter, and possibly other catecholamine neurotransmitters such as norepinephrine [27] contribute to neuronal cell death and the development of neurodegenerative diseases such as PD [28] and AD [29]. One common trait and plausible mechanism shared by these catechol neurotransmitters is their ability to redox cycle, producing cellular and DNA-damaging ROS [23,26,27,30]. The oxidation of these catecholamines to DNA-reactive species has been shown to occur nonenzymatically, in the presence of transition metals such as copper and iron, and has been proposed to proceed via quinone and semiquinone intermediates [30,31]. Both iron and copper are prevalent in human tissues, including the brain, and altered levels of these essential metals have been found in brain tissues of patients with various neurodegenerative diseases [31–33]. Because approximately 20% of the total copper is stored in the nucleus [34], DNA is a major target for copper-catalyzed oxidations. In this study we investigated oxidatively generated DNA damage formed by copper-mediated oxidation of dopamine and its catecholinergic analogs by employing a new ^{32}P-postlabeling thin-layer chromatography (TLC) system for the detection of oxidative DNA adducts. The specific ROS involved in this DNA adduction were also studied by employing a variety of known free radical scavengers.

Experimental procedures

Chemicals

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) or Aldrich (Milwaukee, WI, USA) unless stated otherwise. Sources of chemicals used for ^{32}P-postlabeling analysis were as described previously [35].

Oxidation DNA products

Salmon testis (ST) DNA (300 μg/ml), freed from RNA by treatment with RNases and solvent extractions [35], was incubated (37 °C, 4 h) with vehicle (1% dimethyl sulfoxide (DMSO)), catecholamine neurotransmitters, or dopamine analogs (30 μM) in the presence or absence of a metal catalyst (30 μM), $CuCl_2$, CuCl, $FeCl_3$, or $FeSO_4$, in 10 mM Tris–HCl, pH 7.4. Intervention with ROS scavengers was carried out as described above using dopamine (30 μM) as the substrate in the presence of $CuCl_2$ (30 μM) and 200 U/ml superoxide dismutase (SOD), 200 U/ml catalase, 200 U/ml tyrosinase, 10 mM tiron, 10 or 100 mM sodium azide, 10 mM 2,2,6,6-tetramethyl-4-piperidone (TMP), 150 μM bathocuproine, 10 mM 3-(methylthio)propionaldehyde (methional), 10 mM sodium benzoate, 10 mM mannitol, 10 mM *N-tert*-butyl-α-phenylnitrone (TBP), 150 μM ascorbic acid, or 1, 6, 30, or 150 μM ellagic acid. All ROS modifiers were dissolved in HPLC-grade water except bathocuproine, TBP, methional, and ellagic acid, which were dissolved in DMSO. Modified DNA was purified by solvent extraction and ethanol precipitation of DNA as previously described [35].

To generate readily detectable oxidation DNA products and to obtain reliable quantitative data in the presence of potent ROS scavengers, significantly higher levels of copper compared to known biological levels were used in these studies.

^{32}P-postlabeling analysis

Analyses of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and the unidentified oxidation DNA products were performed separately as described elsewhere [36,37]. Briefly, DNA (6 μg) was hydrolyzed to nucleoside 3′-monophosphates with micrococcal nuclease and spleen phosphodiesterase and adducts were enriched by treatment with nuclease P1 (unidentified DNA products) or one-directional polyethyleneimine (PEI)-cellulose TLC. The DNA products were 5′-^{32}P-labeled in the presence of molar excess of [γ-^{32}P]ATP (<3 μM; 550 Ci/mmol) and T4 polynucleotide kinase. The labeled nucleotides were treated with a mixture of nuclease P1 and potato apyrase to convert labeled bisphosphates of the residual normal nucleotides to monophosphates and unused [γ-^{32}P]ATP to $^{32}P_i$, respectively. The labeled 3′,5′-bisphosphates of 8-oxodG were converted to 5′-monophosphates by treatment with nuclease P1. The unidentified oxidation DNA products were resolved by two-directional PEI-cellulose TLC (D1, 45 mM sodium phosphate, pH 5.8/1 M formic acid onto a 6-cm Whatman No. 17 paper wick; D2, 100 mM sodium phosphate, pH 6.0/10% acetonitrile (v/v)). The 8-oxodG's were separated by two-directional PEI-cellulose TLC (D1, 0.6 M formic acid onto a 5-cm Whatman No. 17 paper wick; and D2, 3 M sodium phosphate, pH 6.0).

Total nucleotides were analyzed by labeling a dilute DNA digest (2 ng) in parallel with adducts followed by one-directional PEI-cellulose TLC in 0.5 M acetic acid/2 M formic acid [36]. The decomposition pattern was visualized and quantified by a Packard InstantImager. Oxidation DNA product levels were determined by relative adduct labeling (RAL), i.e., RAL = (cpm of the oxidative products/cpm of normal nucleotides) × 1/dilution factor. Levels are expressed as oxidation DNA products/10^6 nucleotides.

Cochromatography of oxidation DNA products of Cu(II)-catalyzed reaction of dopamine and H_2O_2 was performed by applying similar amounts of radioactivity of the DNA products in the two reaction mixtures, individually and in combination, onto PEI-cellulose thin layers and resolving the DNA products in two different solvents as indicated in the figure legend (see Fig. 3).

Statistical analysis

Statistical comparisons were made using the two-way ANOVA followed by Dunnett's multiple comparison test. Values were considered significantly different when $P \leq 0.05$.

Results

Detection of oxidatively damaged DNA

All catecholamine neurotransmitters and their congeners (30 μM) tested (Fig. 1), in the presence of Cu(II) (30 μM $CuCl_2$) and ST-DNA