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Original Article

The aminoethyl group is a crucial structural moiety in metal-mediated oxidative DNA damage by catecholamines

Koji Ueda, Yoshihiko Nishino, Yoshinori Okamoto, Nakao Kojima and Hideto Jinno

Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya 468-8503, Japan

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ABSTRACT — Oxidative stress is involved in the development of many neurological diseases. The interactions between catecholamines and copper or iron generate reactive oxygen species that lead to oxidative DNA damage *in vitro*. Furthermore, catechol structure is essential for DNA damage. Here, we clarified the effect of aminoethyl side chains on DNA damage. Endogenous catecholamines (dopamine, noradrenaline, and adrenaline) were more effective than other catechols (catechol, 4-ethylcatechol, and 3,4-dihydroxybenzylamine) in strand break and base oxidation of calf thymus DNA. The presence of copper caused more DNA damage than iron. Furthermore, adrenaline oxidized to adrenochrome more rapidly by copper than iron. Leukoadrenochrome, an oxidation intermediate formed by the intramolecular cyclization of aminoethyl side chains, rapidly increased the formation of 8-hydroxy-2′-deoxyguanosine compared with adrenalin. These results show the effect of aminoethyl side chains in catecholamine-induced oxidative DNA damage. This mechanism may partly show the vulnerability of catecholaminergic neurons against oxidative stress.

Key words: Catecholamine, Oxidative damage, Metal, Neurodegeneration, Neurological disorder

INTRODUCTION

Reactive oxygen species (ROS) are involved in many physiological and pathological processes. Furthermore, oxidative damage to nerve cells is involved in neurological disorders (Subba Rao, 2007). Catecholamines, a group of endogenous catechols that are neurotransmitters, generate ROS in the presence of transition metals, such as copper and iron, *in vitro* (Ando *et al*., 2009; Nishino *et al*., 2011; Oikawa *et al*., 2006). Iron and copper accumulate in lesions of the brain in patients with neurodegenerative diseases such as Parkinson's and Alzheimer's diseases (Dexter *et al*., 1989; Rajendran *et al*., 2009). These findings suggest that the pathology of neurodegeneration involves oxidative stress induced by excess ROS from the reaction between endogenous catechols and metals. Mechanisms for metal-mediated oxidative DNA damage by catechol structure are reduction-oxidation reactions among catechol, metals, and oxygen; however, the effect of side chains has not been clarified. The chemical composition of catechol side chains affects their ROS-generating ability. For example, we found that α-carbonyl groups suppress Cu-dependent oxidative DNA-damaging activity of catechols by chelate formation (Ando *et al*., 2010). Furthermore, we showed that endogenous catecholamines exert relatively strong DNA-damaging activity compared with catechols, 4-ethylcatechol, and a non-endogenous catecholamine, 3,4-dihydroxybenzylamine (Nishino *et al*., 2011). Here, we propose a mechanism that clarifies the strong DNA-damaging activity induced by endogenous catecholamines.

Correspondence: Koji Ueda (E-mail: cozy@meijo-u.ac.jp)

MATERIALS AND METHODS

Materials

Calf thymus DNA, 8-hydroxy-2′-deoxyguanosine (8-oxodG), 2′-deoxyguanosine, dopamine hydrochloride (DA), adrenaline (Ad), adrenochrome (AdC), and 3,4-dihydroxybenzylamine (3,4-DB) were purchased from Sigma (St. Louis, MO, USA). L-noradrenaline (NAd) and 4-ethylcatechol (4-EC) were purchased from Alfa Aesar (Ward Hill, MA, USA). Ethidium bromide (EtBr), 6 × loading dye, and agarose S were obtained from Bio-Rad Laboratories (Hercules, CA, USA), Takara Bio Inc. (Shiga, Japan), and Nippon Gene (Tokyo, Japan), respectively. Nuclease P1 was provided by Yamasa Shoyu (Chiba, Japan). Calf intestine alkaline phosphatase (CIP) was purchased from Roche Diagnostics (Mannheim, Germany). Copper(II) chloride dehydrate [Cu(II)], iron(III) nitrate nonahydrate [Fe(III)], and sodium periodate ($NaIO_4$) of the highest grade were purchased from Wako Pure Chemical Industries (Osaka, Japan). Nitrilotriacetic acid trisodium salt (NTA) and catechol (C) were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Fe(III)-NTA complex was prepared by mixing equimolar Fe(III) and NTA in aqueous solution. Leukoadrenochrome (LAdC) was prepared from AdC by reduction with ascorbic acid (Mattok, 1965).

Measurement of DNA strand breaks

A mixture containing a catechol compound (20 μM), Cu(II) or Fe(III)-NTA (20 μM), and calf thymus DNA (100 μM base) in 4 mM sodium phosphate buffer (pH 7.8) was incubated for 2 hr at 37°C, and 10 μL of the mixture was mixed with loading dye and subjected to agarose gel electrophoresis. The gel contained 1% agarose and 0.5 μg/mL EtBr in Tris-acetate EDTA buffer (pH 8.0). The EtBr-bound DNA was detected by UV irradiation (312 nm).

Measurement of 8-oxodG formation

A reaction mixture consisting of a catechol compound (20 μM), Cu(II) or Fe(III)-NTA (20 μM), and calf thymus DNA (100 μM base) in 400 μL of 4 mM sodium phosphate buffer (pH 7.8) was incubated at 37°C for 1, 2, 4, 6, 9, or 12 hr. After ethanol precipitation, DNA was dissolved in 20 mM sodium acetate buffer (pH 5.0) and digested to obtain nucleosides by incubating first with nuclease P1 (3.4 units) at 37°C for 30 min followed by CIP (1.3 units) at 37°C for 1 hr in 0.1 M Tris-HCl (pH 7.5). Mixtures were analyzed by HPLC (LC-10 series, Shimadzu, Kyoto, Japan) equipped with an electrochemical detector (ECD; Coulochem II, ESA, Chelmsford, MA, USA). HPLC conditions were as follows: column, ODS-80Ts (Tsk-gel, 150 × 4.6 mm i.d.; Tosoh, Tokyo, Japan); column temperature, 25°C; flow rate, 1 mL/min; and detection wavelength, 254 nm (for 2′-deoxyguanosine). The ECD conditions were as follows: guard cell, 400 mV; E, 150 mV; R, 100 μA; filter, 2; output, 1 V (channel 1); E, 300 mV; R, 200 nA; filter, 10; output, 1 V (channel 2).

Measurement of AdC formation

AdC formation was measured based on the method by Remiao *et al*. (2003). Reaction mixtures consisting of 1 mM Ad and 0.5 mM Cu(II) or Fe(III)-NTA in 400 μL of 4 mM sodium phosphate buffer (pH 7.8) were incubated at 37°C. At the indicated time points, the concentration of AdC was assessed by HPLC equipped with a photodiode-array detector (490 nm). Ad was considered to have been converted completely to AdC by 2 mM $NaIO_4$.

RESULTS AND DISCUSSION

We previously reported different mechanisms for copper and iron in oxidative DNA damage induced by catecholamines (Nishino *et al*., 2011). Catechol structure plays an essential role in the production of ROS (Oikawa *et al*., 2001). However, the role of aminoethyl side chains remains unclear. In this study, we clarified the effects of side chain variation on catecholamine-induced DNA damage. The side chain derivatives shown in Fig. 1 exert different activities on DNA cleavage, which was assessed using an assay by electrophoresis, as shown in Fig. 2. In the presence of Cu(II), there were differences in the overall size of cleaved DNA fragments between those mixed with endogenous catecholamines (DA, NAd, and Ad) and catechols with shorter side chain derivatives (C, 4-EC, and 3,4-DB). In the presence of Fe(III), the damage was moderate; however, there was a large difference in DNA fragment size since the DNA remained nearly intact in samples containing catechols with shorter side chain derivatives. In order to quantify oxidative DNA damage, we measured 8-oxodG formation. The time course curves were divided into two groups consisting of endogenous catecholamines and other catechols (Fig. 3). The results show that the aminoethyl side chain of endogenous catecholamines is important for strong DNA-damaging activity. This may be due to the formation of leukoaminochromes, which are formed from catecholamine quinones by the irreversible cyclization of the aminoethyl side chain (Bindoli *et al*., 1992). Leukoaminochromes may damage DNA through their oxidation process into aminochromes. The repetitive ROS-generating reactions may

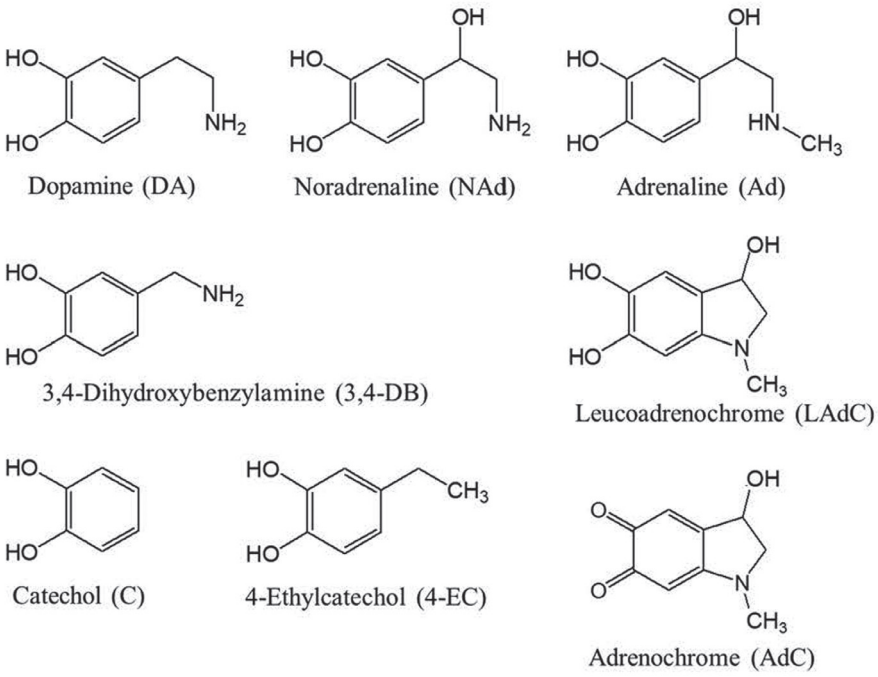

Fig. 1. Chemical structures of the catechol compounds used in this study.

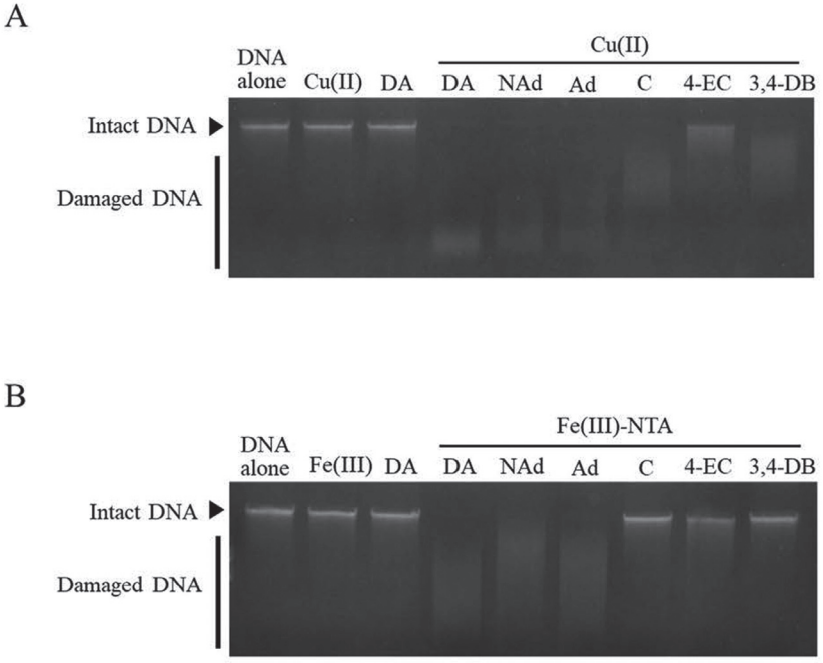

Fig. 2. DNA strand breaks by catechols in the presence of Cu(II) [A] and Fe(III)-NTA [B]. Calf thymus DNA was incubated with catechols and Cu(II) or Fe(III)-NTA for 2 hr. The treated DNA was analyzed by agarose electrophoresis. DNA strand breaks were detected as DNA fragments. Data are from at least three separate experiments.

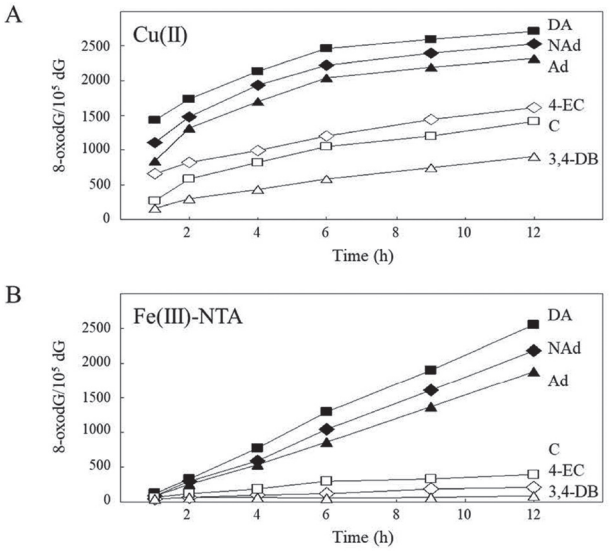

Fig. 3. DNA oxidation by catechols in the presence of Cu(II) [A] and Fe(III)-NTA [B]. The time course of 8-oxodG formation was measured by HPLC-ECD. Calf thymus DNA was incubated with the indicated catechols in the presence of Cu(II) or Fe(III)-NTA. Data are from at least three separate experiments.

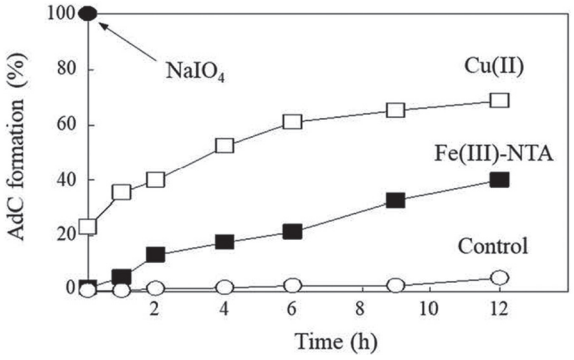

Fig. 4. Ad oxidation by Cu(II) and Fe(III)-NTA. The time course of AdC formation from Ad was determined by measuring the maximum absorption of AdC by HPLC. The amount of AdC is indicated as % of the amount in the reaction with $NaIO_4$. Data are from at least three separate experiments.

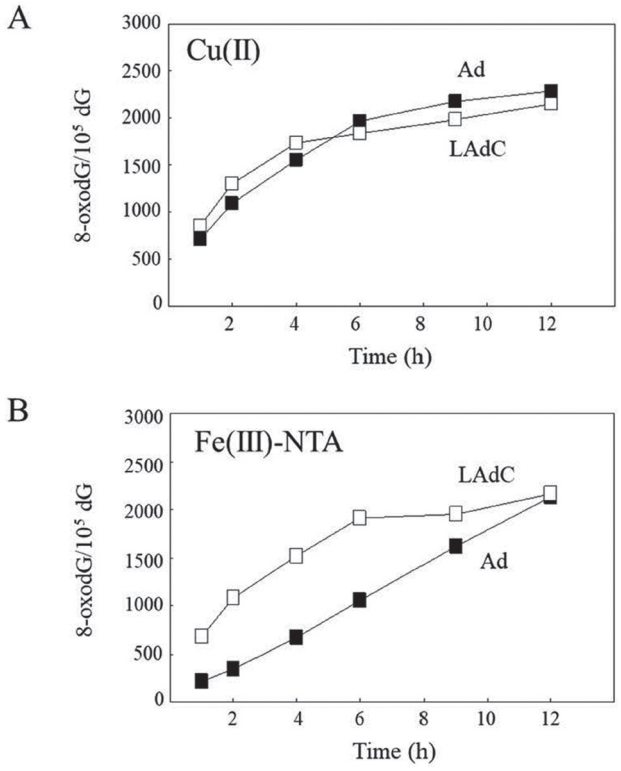

Fig. 5. DNA oxidation by Ad and LAdC in the presence of Cu(II) [A] and Fe(III)-NTA [B]. The time course of 8-oxodG formation was measured by HPLC-ECD. Calf thymus DNA was incubated with Ad or LAdC in the presence of Cu(II) or Fe(III)-NTA. Data are from at least three separate experiments.

enable catecholamines to exert increased DNA damage. In contrast, catechols without aminoethyl side chain are unable to perform this side chain cyclization. As shown in Fig. 4, catecholamines are able to form aminochromes in the presence of Cu(II) or Fe(III)-NTA. Ad was used since its aminochrome, AdC, is relatively stable and commercially available. DA and NAd have also been reported to produce aminochromes (Remiao *et al*., 2003). Cu(II) was more effective than Fe(III)-NTA in Ad oxidation. This is consistent with the results of DNA damage shown in Fig. 2 and 3. However, the additional reactions of leukoaminochromes are insufficient to clarify the large difference in DNA damage between endogenous and other catechols, especially those observed in Fe(III)-NTA. To determine whether leukoaminochromes are more effective in damaging DNA than catecholamines, we prepared LAdC from Ad using ascorbic acid and compared their 8-oxodG-forming ability (Fig. 5). LAdC increased the concentration of 8-oxodG more rapidly than Ad in the presence of Fe(III)-NTA. The small difference in Cu(II) results from the rapid oxidation of Ad into AdC as shown in Fig. 4. A possible mechanism is summarized in Fig. 6. The oxidation of catecholamines to quinones is mediated by metals and oxygen, and the mediation by Fe(III) is slower than Cu(II) during the initial oxidation. Catacholamine

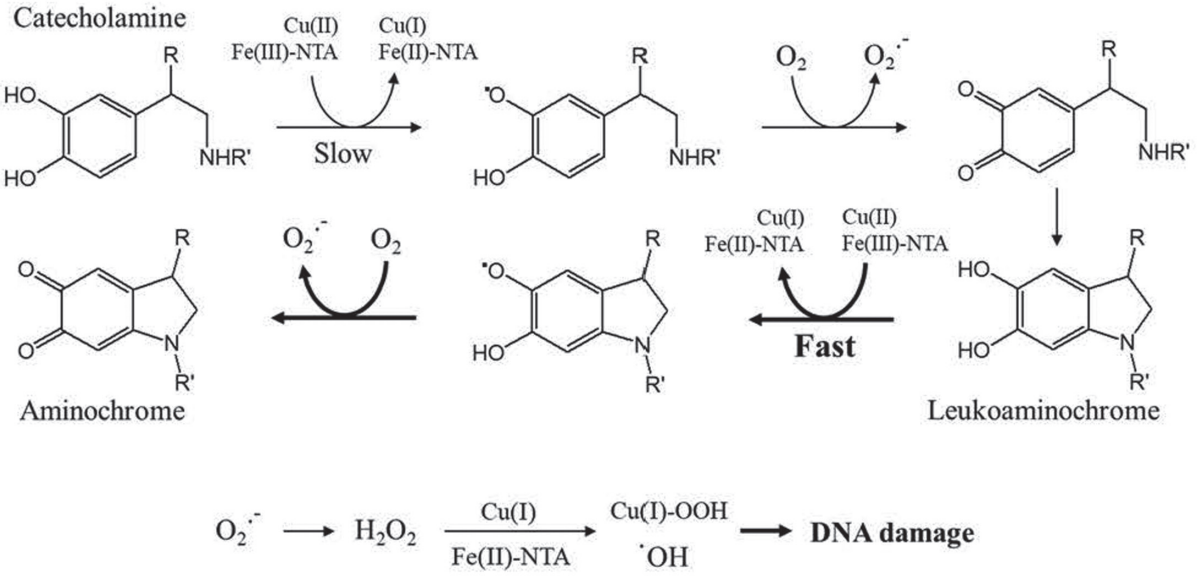

Fig. 6. Metal-mediated catecholamine oxidation via aminoethyl cyclization leading to DNA damage. The oxidation processes of catecholamines produced reduced forms of metals and superoxide anion radical ($O_2^{\cdot-}$). Hydrogen peroxide (H_2O_2) was formed by dismutation of $O_2^{\cdot-}$. Cu(I) and Fe(II) formed Cu(I)-OOH and hydroxyl radicals ($^{\cdot}OH$), respectively and had different processes in mediating oxidative DNA damage.

quinones return to their reduction potential by intramolecular rearrangements into leukoaminochromes and are followed by further oxidation reactions to form aminochromes. The oxidation reaction of leukoaminochromes is faster than that of catecholamines. Therefore, in addition to catechol structure, the presence of aminoethyl side chains is crucial for metal-mediated DNA damage by catecholamines. ROS and reduced forms of metals are generated in the oxidation process of catecholamines, and their interactions induce oxidative DNA damage (Kawanishi *et al*., 2001). This mechanism may partly show the vulnerability of catecholaminergic neurons against oxidative stress.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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