Radical Scavenging Reactivity of Catecholamine Neurotransmitters and the Inhibition Effect for DNA Cleavage

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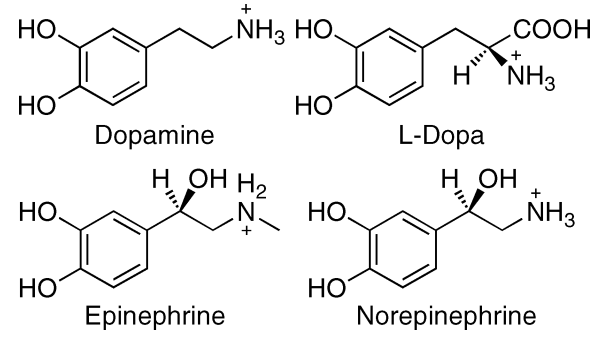
Neurotransmitters such as catecholamines (dopamine, L-dopa, epinephrine, norepinephrine) have phenol structure and scavenge reactive oxygen species (ROS) by hydrogen atom transfer (HAT) to ROS. Radical scavenging reactivity of neurotransmitters with galvinoxyl radical ($GO^{\bullet}$) and cumyloxyl radical ($RO^{\bullet}$) in acetonitrile at 298 K was determined by the UV−vis spectral change. The UV−vis spectral change for HAT from catecholamine neurotransmitters to $GO^{\bullet}$ was measured by a photodiode array spectrophotometer, whereas HAT to much more reactive cumylperoxyl radical, which was produced by photoirradiation of dicumyl peroxide, was measured by laser flash photolysis. The second-order rate constants (k_{GO}) were determined from the slopes of linear plots of the pseudo-first-order rate constants vs concentrations of neurotransmitters. The k_{GO} value of hydrogen transfer from dopamine to $GO^{\bullet}$ was determined to be 23 M^{-1} s^{-1}, which is the largest among examined catecholamine neurotransmitters. This value is comparable to the value of a well-known antioxidant: (+)-catechine (27 M^{-1} s^{-1}). The k_{GO} value of hydrogen transfer from dopamine to $GO^{\bullet}$ increased in the presence of Mg^{2+} with increasing concentration of Mg^{2+}. Such enhancement of the radical scavenging reactivity may result from the metal ion-promoted electron transfer from dopamine to the galvinoxyl radical. Inhibition of DNA cleavage with neurotransmitters was also examined using agarose gel electrophoresis of an aqueous solution containing pBR322 DNA, NADH, and catecholamine neurotransmitters under photoirradiation. DNA cleavage was significantly inhibited by the presence of catecholamine neurotransmitters that can scavenge hydroperoxyl radicals produced under photoirradiation of an aerated aqueous solution of NADH. The inhibition effect of dopamine on DNA cleavage was enhanced by the presence of Mg^{2+} because of the enhancement of the radical scavenging reactivity.

Introduction

Catecholamine neurotransmitters (epinephrine, norepinephrine, dopamine, and L-dopa, Chart 1) play an important role in a signal transduction system.[1] Since human beings consume more than 20% of oxygen in the brain, neurotransmitters are exposed to considerable amounts of reactive oxygen species (ROS).[2,3] ROS such as superoxide or peroxyl radicals and their metabolic products may play a key role in the etiology of certain disorders of the central nervous system.[3,4] The patients of schizophrenia are also known to be quite sensitive to the mental and physiological stress that usually increases the concentration of ROS.[5] Under such conditions, the catecholamine neurotransmitters may be easily oxidized to the corresponding quinone, which might disturb neurotransmission by sending abnormal signals to the receptors or by behaving as an agonist like methamphetamine.[6] Thus, relevance of active oxygen to schizophrenia has been predicted; however, there have been few reports on the reactivity of catecholamine neurotransmitter with ROS.[7,8]

It is well accepted that hydrogen atom transfer (HAT) from a phenolic hydroxyl group of antioxidants to ROS is responsible for the antioxidant activity.[9−12] There are three possible pathways for radical scavenging reactions by phenolic compounds: a one-step HAT, electron transfer (ET) followed by proton transfer (PT), and sequential proton loss electron transfer.[13−17] We have reported that the scavenging of the cumylperoxyl radical ($PhCMe_2OO^{\bullet}$) by the neurotransmitter proceeds via the one-step HAT.[7] In contrast to the case of $PhCMe_2OO^{\bullet}$, $GO^{\bullet}$ has been reported to act as a good electron acceptor in HAT reactions.[18,19] Thus, it is interesting to investigate the mechanism of HAT from the catecholamine neurotransmitter to $GO^{\bullet}$ and whether it occurs through a one-step HAT or ET/PT.

CHART 1



On the other hand, ROS causes a genetic abnormality via the DNA cleavage by ROS in the biological cell.[20] DNA cleavage induced by ROS is initiated by 5′-hydrogen atom abstraction from DNA, resulting in the site-selective nicking.[21] Hydrogen atom abstraction at other sites of the deoxyribose ring

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followed by strand scission likely is responsible for the nonspecific nicking.[21] Catecholamine neurotransmitters may protect from DNA damage with ROS by scavenging ROS. However, such inhibition of DNA cleavage with ROS by catecholamine neurotransmitters has yet to be examined.

We report herein the kinetics and mechanism of HAT from catecholamine neurotransmitters to GO• as a stable ROS model and also to the much more reactive cumyloxyl radical. The rates were determined by following the UV−vis spectral change in the HAT reactions in deaerated acetonitrile (MeCN) at 298 K. The reactivity of catecholamine neurotransmitters (Chart 1) against the highly reactive cumyloxyl radical was examined by laser flash photolysis measurements in which cumyloxyl radicals were produced by laser irradiation of dicumyl peroxide. The efficiency for the inhibition of DNA cleavage with ROS by catecholamine neurotransmitters was also examined using agarose gel electrophoresis.

Experimental Section

Materials. Dopamine hydrochloride, L-dopa, norepinephrine, and acetonitrile (MeCN; spectral grade) were purchased from Nacalai Tesque, Inc., Japan and used as received. Epinephrine, galvinoxyl radical (GO•), and dicumyl peroxide were commercially obtained from Aldrich Co. (+)-Catechin and dihydronicotinamide adenine dinucleotide (NADH) were purchased from Sigma Chemical Co. All catecholamines are used as ammonium salts. The ammonium salts of L-dopa, norepinephrine, and epinephrine were prepared by addition of 1 equiv of $HClO_4$. Magnesium perchlorate [$Mg(ClO_4)_2$] and DNA pBR322 were obtained from Wako Pure Chemical Ind. Ltd., Japan. Tetra-*n*-butylammonium perchlorate (Bu_4NClO_4) used as a supporting electrolyte in electrochemical measurements was purchased from Tokyo Chemical Industry Co. Ltd., Japan, and it was recrystallized from ethanol and dried under vacuum at 313 K prior to use.

Kinetic Analysis. A continuous flow of Ar gas was bubbled through an MeCN solution (2.0 mL) containing GO• (9.0×10^{-6} M) in a square quartz cuvette (10 mm i.d.) with a glass tube neck for 7 min. Air was prevented from leaking from the neck of the cuvette with a rubber septum. Typically, an aliquot of a neurotransmitter, which was also in deaerated MeCN, was added to a cuvette with a microsyringe to start the reaction. The UV−vis spectral change in the reaction was monitored using a Hewlett-Packard HP8453 photodiode array spectrophotometer. The rates of the GO•-scavenging reactions with neurotransmitters were determined by monitoring the absorbance change at 428 nm due to GO• (9.0×10^{-6} M). The pseudo-first-order rate constants ($k_{obs(GO)}$) were determined by a least-squares curve fit using a personal computer. The first-order plots of $\ln(A - A_\infty)$ vs time (A and A_∞ are denoted as the absorbance at the reaction time and the final absorbance when the reaction was completed, respectively) were linear until three or more half-lives with the correlation coefficient >0.99.

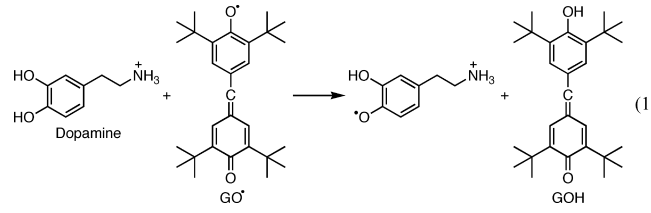
Laser Flash Photolysis. For nanosecond laser flash photolysis experiments, deaerated MeCN solutions of catecholamine neurotransmitters and dicumyl peroxide were excited by a Panther OPO pumped by a Nd:YAG laser (Continuum, SLII-10, 4−6 ns fwhm) at $\lambda = 355$ nm. The cumyloxyl radical was generated by 355 nm laser flash photolysis of a solution of dicumyl peroxide (0.45 M) in the presence of a neurotransmitter, where the peroxide concentration was chosen to give an O.D. ~ 0.3 at 355 nm. The photodynamics was monitored by continuous exposure to a xenon lamp (150 W) as a probe light and a photomultiplier tube (Hamamatsu 2949) as a detector. The rates of the cumyloxyl radial (COR)-scavenging reactions of neurotransmitters were determined by monitoring the absorbance change at 480 nm due to the cumyloxyl radical. The pseudo-first-order rate constants ($k_{obs(COR)}$) were determined by a least-squares curve fit using a personal computer. The first-order plots of $\ln(A - A_\infty)$ vs time (A and A_∞ are denoted as the absorbance at the reaction time and the final absorbance when the reaction was completed, respectively) were linear until three or more half-lives with the correlation coefficient >0.999.

Electrochemical Measurements. Cyclic voltammetry (CV) and second-harmonic alternating current voltammetry (SHACV)[22] measurements of catecholamine neurotransmitters were performed on an ALS-630B electrochemical analyzer in deaerated MeCN containing 0.10 M Bu_4NClO_4 (TBAP) as a supporting electrolyte. A Pt working electrode (BAS) was polished with BAS polishing alumina suspension and rinsed with MeCN before use. The counter electrode was a platinum wire. Measured potentials were recorded with respect to the Ag/$AgNO_3$ (0.01 M) reference electrode. The one-electron oxidation potentials (E_{ox} vs Ag/$AgNO_3$) of catecholamine neurotransmitters were converted to those vs SCE by adding 0.29 V.[23] All electrochemical measurements were carried out at 298 K under 1 atm of Ar.

DNA Cleavage. Typically, an aqueous buffer solution of NADH (2.1×10^{-2} M, 20 μL) and 1 μL of an aqueous solution of DNA pBR322 (0.32 g L^{-1}) were mixed in a micro test tube in the dark. Samples were incubated under photoirradiation with monochromatized light ($\lambda = 340$ nm) from a Shimadzu RF-5300PC spectrophotometer at 298 K. An amount of 2 μL of an aqueous solution of DNA pBR322 was diluted by adding 18 μL of water and then mixed with 2 μL of a loading buffer solution (0.1% bromophenol blue and 3.75% Ficol in TAE buffer) and loaded onto 1.4% agarose gel. The gel was run at a constant voltage of 130 V for 50 min in TAE buffer using a Nihon Eido electrophoresis kit, then washed with distilled water, soaked into 0.1% ethidium bromide aqueous solution, visualized under a UV transilluminator, and photographed using a digital camera. The evaluation of the ratio of DNA cleavage has been carried out by free graphic software (ImageJ, ver. 1.37, National Institute of Health, USA).[24]

Results and Discussion

Radical-Scavenging Reactivity by Catecholamine Neurotransmitters. When dopamine was added to a deaerated acetonitrile (MeCN) solution of GO•, the visible absorption band at 428 nm due to GO• decreased, accompanied by an increase in the absorption band at 392 nm due to GOH with a clear isosbestic point at 412 nm as shown in Figure 1. A decrease in the absorption band at 428 nm due to GO• indicates that HAT occurs from one of the OH groups of dopamine to GO• (eq 1).[25] Dopamine has both catechol and primary amine moieties. HAT by ROS has been reported to occur from attacking an OH group of the catechol moiety, not a CH_2 chain of the primary amine moiety.[26]



(1)

The decay of the absorbance at 428 nm due to GO• obeyed pseudo-first-order kinetics when concentration of dopamine was

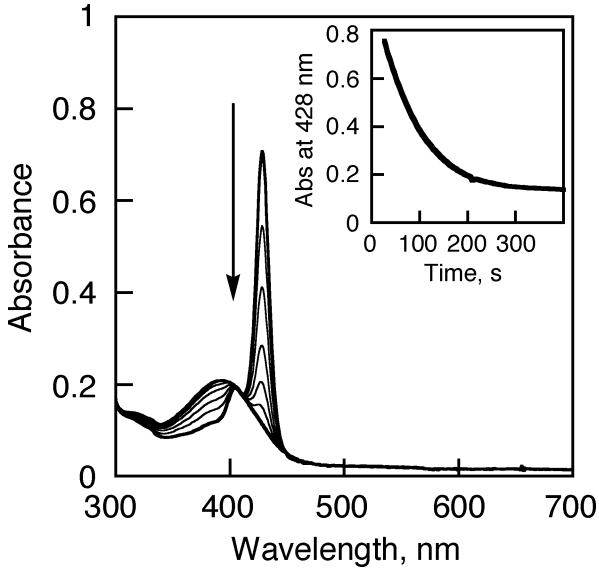


Figure 1. Spectral change observed upon addition of dopamine (6.1×10^{-4} M) to a deaerated MeCN solution of GO$^{\bullet}$ (9.0×10^{-6} M) at 298 K. Inset: Time profile of absorption change at 428 nm.

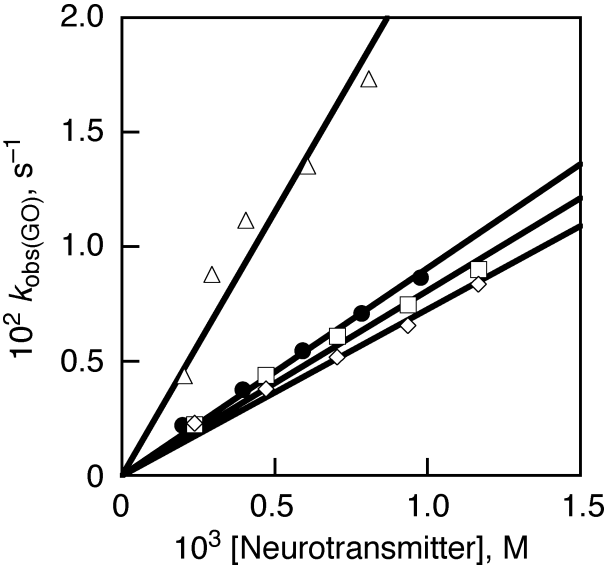


Figure 2. Plots of the pseudo-first-order rate constants ($k_{obs(GO)}$) vs concentration of neurotransmitter (dopamine (Δ), epinephrine (●), norepinephrine (□), and L-dopa (◇)) for the reaction of neurotransmitter with GO$^{\bullet}$ in deaerated MeCN at 298 K.

maintained at more than 10-fold excess of GO$^{\bullet}$ concentration. The observed pseudo-first-order rate constant ($k_{obs(GO)}$) increases linearly with an increase in concentration of dopamine (Figure 2). No natural decay of GO$^{\bullet}$ was observed during the measurements. Thus, there is no intercept as shown in Figure 2. The second-order rate constant (k_{GO}) of HAT from dopamine to GO$^{\bullet}$ in MeCN at 298 K was determined from the slope of the linear plot of $k_{obs(GO)}$ vs [dopamine] to be 23 M^{-1} s^{-1}. The semiquinone radical of dopamine thus formed is known to be unstable to disproportionate to produce the parent dopamine and an *o*-quinone-type oxidized product.[6,27] The k_{GO} values of HAT from other catecholamine neurotransmitters and (+)-catechin to GO$^{\bullet}$ were determined in the same way as shown in Figure 2, and the resulting k_{GO} values are listed in Table 1.

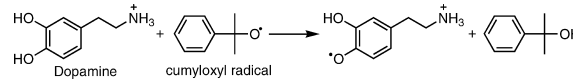
The radical-scavenging reactivity of catecholamine neurotransmitters was also examined using a much more reactive

TABLE 1: Rate Constants for Scavenging of the Galvinoxyl Radical (k_{GO}) and Cumyloxyl Radical (k_{COR}) by Neurotransmitters in Deaerated MeCN at 298 K, One-Electron Oxidation Potentials (E_{ox} vs SCE) in MeCN (0.1 M Bu_4NClO_4) at 298 K, and the HOMO Level

neurotransmitter	k_{GO},[a] M^{-1} s^{-1}	k_{COR},[b] M^{-1} s^{-1}	E_{ox} (vs SCE), V	HOMO,[c] eV
epinephrine	9.1	8.0×10^7	1.26	−8.68[d]
norepinephrine	8.1	7.6×10^7	1.28	−8.76[d]
L-dopa	7.3	5.3×10^7	1.28	−8.61[d]
dopamine	23	8.6×10^7	1.05	−8.53[d]
(+)-catechine	27	1.0×10^8	1.18	−5.73

[a] Experimental error: ± 5%. [b] Experimental error: ± 10%. [c] Taken from ref 7. The values calculated by DFT at the B3LYP/6-31G(d) level. [d] Calculated as an ammonium ion, charge = 1.

radical species than GO$^{\bullet}$, which is the cumyloxyl radical, by the nanosecond laser flash photolysis technique (see Experimental Section). The cumyloxyl radical (COR) was generated by 355 nm nanosecond laser flash illumination of dicumyl peroxide in MeCN at 298 K (see Experimental Section). The transient absorption spectrum of an intermediate produced upon laser photoexcitation is shown in Figure 3a. The spectrum of the intermediate agrees well with that of COR reported in the literature.[28,29] This band completely disappeared at 10 μs after laser excitation. The absorption bands of the phenoxyl radical and 2-phenylpropan-2-ol, which have absorption below 400 nm, were not observed under the present reaction conditions because of the significant overlap with the absorption due to dicumyl peroxide.[30] The rate of HAT from catecholamine neurotransmitters to the cumyloxyl radical was determined by following the decay of absorbance at 480 nm due to COR. The decay time profiles obey pseudo-first-order kinetics (Figure 3b). The pseudo-first-order rate constant ($k_{obs(COR)}$) was determined from the slope of the first-order plot (inset of Figure 3b), which increases linearly with increasing concentration of dopamine as shown in Figure 3c. The second-order rate constant of HAT (k_{COR}) from dopamine to the cumyloxyl radical was determined from the slope of the linear plot of $k_{obs(COR)}$ vs [dopamine] to be 8.6×10^7 M^{-1} s^{-1}. The k_{COR} values of HAT from other catecholamine neurotransmitters and (+)-catechin to the cumyloxyl radical (eq 2) were determined in the same way, and the resulting k_{COR} values are listed in Table 1.



(2)

The k_{COR} value of dopamine is the largest among the examined catecholamine neurotransmitters, being comparable to the value of (+)-catechine, which is known to be a strong antioxidant.[31,32]

Effects of Magnesium Ion on Galvinoxyl Radical Scavenging Reactions by Catecholamine Neurotransmitters. When $Mg(ClO_4)_2$ is added to the dopamine−GO$^{\bullet}$ system in deaerated MeCN, the rate of GO$^{\bullet}$-scavenging reaction by dopamine was significantly accelerated. The rate constants (k_{GO}) of GO$^{\bullet}$-scavenging reaction by dopamine increase linearly with increasing Mg^{2+} concentration ([Mg^{2+}]) as shown in Figure 4. The magnesium ion has been reported to act as an effective promoter in metal ion-coupled electron-transfer reactions by binding of Mg^{2+} to the one-electron reduced species produced in the electron-transfer reactions.[33−39] Thus, the GO$^{\bullet}$-scavenging reaction by dopamine may proceed via metal ion-coupled electron

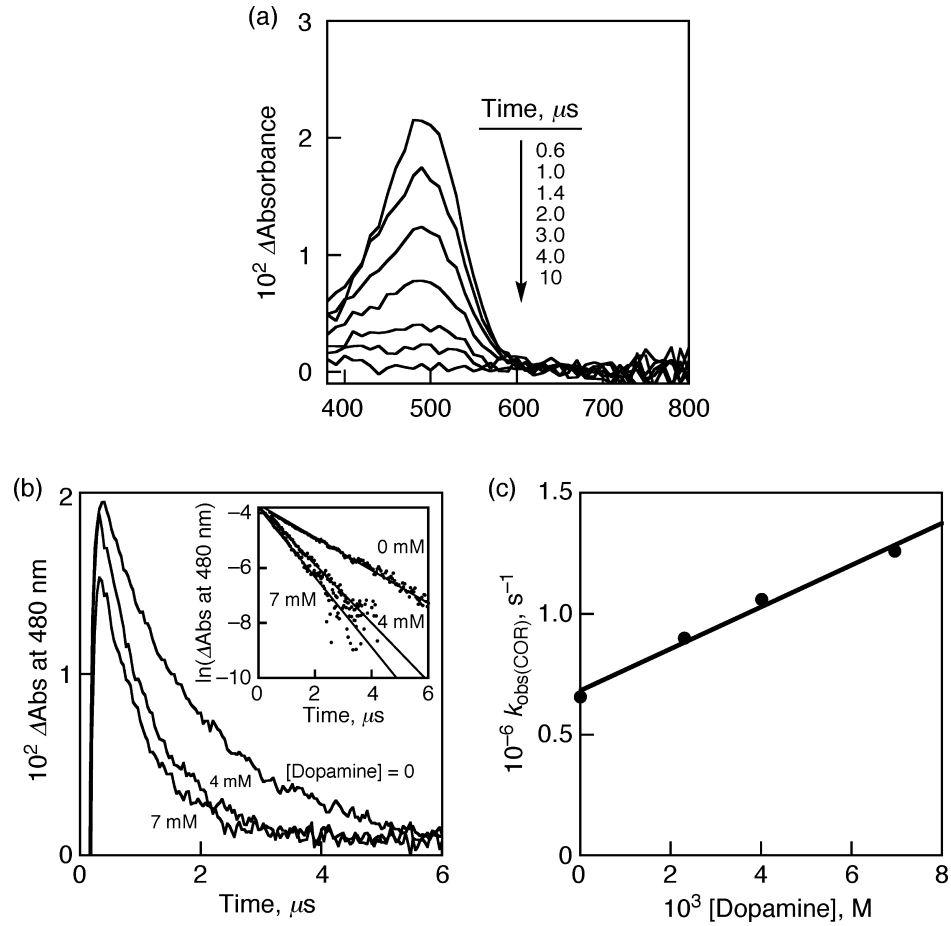


Figure 3. (a) Transient absorption spectra of the cumyloxyl radical generated by laser flash irradiation ($\lambda = 355$ nm) of an MeCN solution containing dicumyl peroxide (0.45 M). (b) Decay time profiles of the cumyloxy radical monitored by absorption change at 480 nm in the absence and presence of dopamine in deaerated MeCN at 298 K. Inset: First-order plots. (c) Plot of $k_{obs(COR)}$ vs [dopamine] for HAT from dopamine to the cumyloxy radical.

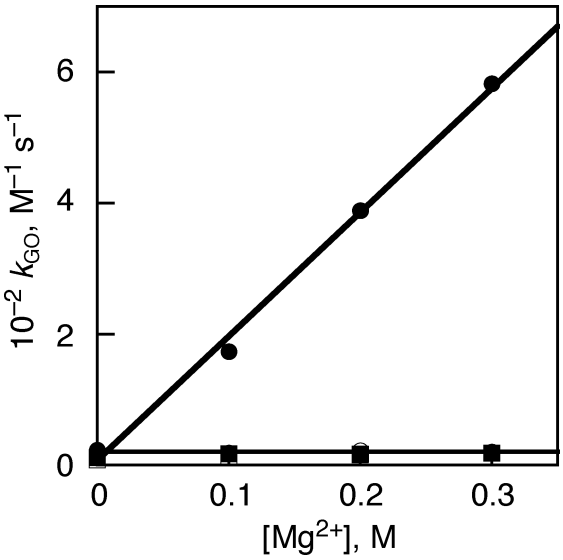
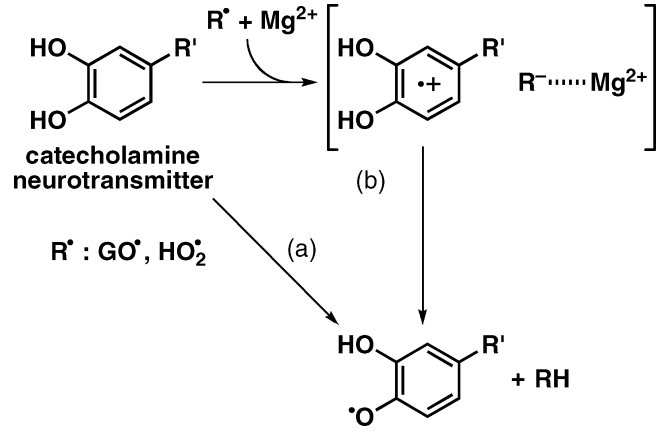


Figure 4. Plots of rate constants (k_{GO}) vs concentration of magnesium ion ([Mg^{2+}]) for HAT from catecholamine neurotransmitters [epinephrine (○), norepinephrine (■), dopamine (●), and L-dopa (□)] to GO• in deaerated MeCN at 298 K.

SCHEME 1: Two Mechanistic Pathways for the Free Radical-Scavenging Reaction by a Neurotransmitter: (a) One-Step HAT and (b) MCET/PT



transfer (MCET) from dopamine to GO•, followed by proton transfer (PT) from the radical cation of dopamine to $GO^- - Mg^{2+}$ as shown in Scheme 1. In such a case, the binding of Mg^{2+} to GO^- results in the acceleration of the electron transfer.

In contrast to the case of dopamine, the addition of Mg^{2+} caused virtually no change in the k_{GO} values of epinephrine, norepinephrine, and L-dopa as shown in Figure 4. These results suggest that the GO•-scavenging reaction by epinephrine, norepinephrine, and L-dopa proceeds via a one-step HAT pathway without contribution of MCET. The MCET pathway of dopamine rather than a one-step HAT pathway may result from a lower oxidation potential of dopamine ($E_{ox} = 1.05$ V) as compared with the E_{ox} values of other catecholamine neurotransmitters (Table 1). In addition, the HOMO energy of dopamine obtained by DFT calculations is the highest among catecholamine neurotransmitters used in this study (Table 1).[40] This may be the reason why only dopamine reacts by ET.

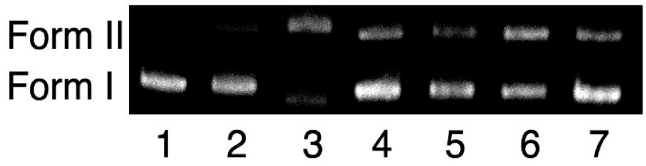


Figure 5. Agarose gel electrophoresis of cleaved supercoiled pBR322 DNA in the photoirradiated NADH (1.9×10^{-2} M) with O_2 in air-saturated aqueous buffer solution (pH 5.0) at 298 K. Cleavage of DNA after 20 min photoirradiation of monochromatized light ($\lambda = 340$ nm). Lane 1, control; lane 2, no photoirradiation within NADH; lane 3, photoirradiation within NADH; lane 4, +4 mM epinephrine; lane 5, +4 mM norepinephrine; lane 6, +4 mM L-dopa; lane 7, +4 mM dopamine.

Inhibition of DNA Cleavage with ROS by Catecholamine Neurotransmitters. The radical-scavenging reactivity of catecholamine neurotransmitters results in inhibition of DNA cleavage with ROS (vide infra). DNA has been reported to be cleaved efficiently under photoirradiation of an air-saturated aqueous solution of DNA with NADH.[41] The photoexcitation of NADH results in the formation of ^{1}NADH*, followed by intersystem crossing to produce ^{3}NADH*. Then, electron transfer from ^{3}NAHD* to O_2 occurs to form $NADH^{\bullet+}$ and $O_2^{\bullet-}$. The produced $O_2^{\bullet-}$ is in protonation equilibrium with $HO_2^{\bullet}$,[42] which acts as a chain carrier for the radical chain oxidation of NADH with O_2 to produce NAD^+ and H_2O_2,[43] leading to the DNA cleavage.[41] The supercoiled DNA (form I) is efficiently cleaved into form II (nicked DNA) by 25 min UVA-light irradiation of an air-saturated CH_3COOH/KOH buffer solution (1.0×10^{-2} M, pH 5.0) of NADH (1.9×10^{-2} M) with the use of a monochromatized light ($\lambda = 340$ nm) from a xenon lamp as shown in Figure 5 (lane 3). Under dark conditions, no DNA cleavage occurs in the presence of all the components, i.e., NADH and O_2. When the agarose gel electrophoresis of the NADH−DNA system was performed in the presence of dopamine to study the photoinduced DNA cleavage, the DNA cleavage by $HO_2^{\bullet}$ is significantly prohibited by dopamine (lane 7 in Figure 5). Addition of the other catecholamine neurotransmitters (epinephrine, lane 4; norepinephrine, lane 5; and L-dopa, lane 6) also inhibits DNA cleavage. Thus, the neurotransmitters can scavenge $HO_2^{\bullet}$, resulting in protection of DNA damage by ROS. In particular, dopamine acts as the strongest inhibitor for DNA damage by ROS. This is consistent with the fact that radical-scavenging reactivity of dopamine is the largest among examined catecholamine neurotransmitters.

When $Mg(ClO_4)_2$ is added into an air-saturated buffer solution (pH 5.0) of NADH (1.9×10^{-2} M) and dopamine, the ratio of uncleaved DNA after photoirradiation increased with increasing Mg^{2+} concentration (Figure 6). Thus, the $HO_2^{\bullet}$-scavenging reactivity of dopamine was enhanced by Mg^{2+} by the same token as the enhanced $GO^{\bullet}$-scavenging reactivity of dopamine by Mg^{2+} (vide supra). In contrast to the case of dopamine, little change was observed in the ratio of form II to form I in the case of epinephrine, norepinephrine, and L-dopa by the presence of Mg^{2+}. Again, this is consistent with little change in the $GO^{\bullet}$-scavenging reactivity of these catecholamine neurotransmitters by the presence of Mg^{2+} in Figure 4.

Summary

In summary, the galvinoxyl radical- and cumyloxyl radical-scavenging reactivities of dopamine at ambient temperature are the largest among examined catecholamine neurotransmitters, being comparable to the radical scavenging reactivity of (+)-catechine that is known as a strong antioxidant. Hydrogen atom transfer from dopamine to the galvinoxyl radical in the presence of Mg^{2+} proceeds via an MCET/PT pathway, whereas other catecholamine neurotransmitters (epinephrine, norepinephrine, and L-dopa) undergo a one-step HAT pathway, although further studies are required to clarify the possible effects of solvents on the two reaction pathways.[13,44] The largest radical-scavenging reactivity of dopamine is also demonstrated in protection of DNA damage by ROS, which is further enhanced by the presence of Mg^{2+}. Consequently, dopamine with Mg^{2+} acts as a very efficient scavenger for ROS that triggers serious diseases. However, the toxicity of the oxidized dopamine has yet to be clarified.

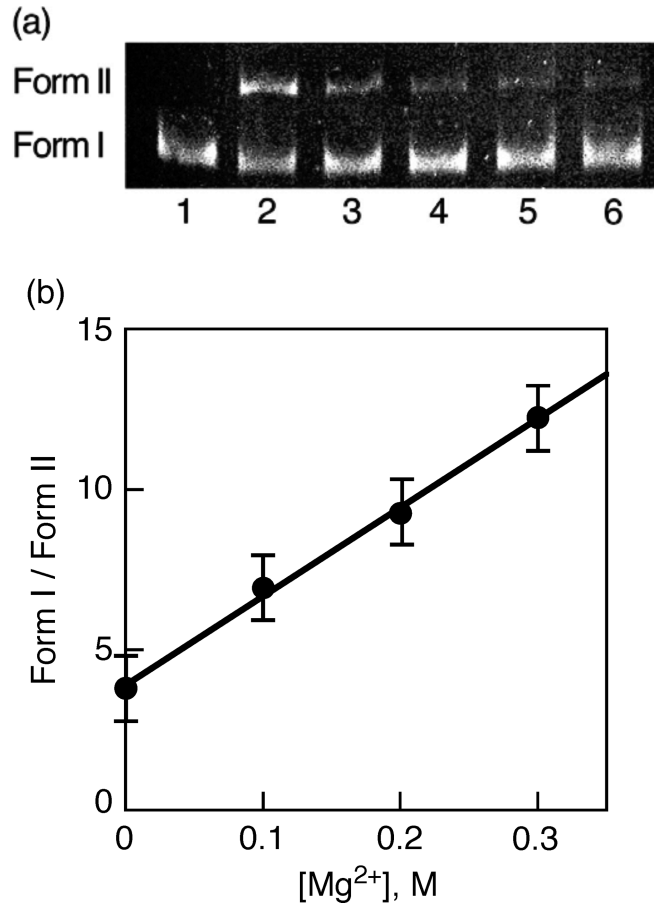


Figure 6. (a) Agarose gel electrophoresis of cleaved supercoiled pBR322 DNA after 20 min photoirradiation of an air-saturated aqueous buffer solution (pH 5.0) of NADH (1.9×10^{-2} M) and pBR322 DNA at 298 K with monochromatized light ($\lambda = 340$ nm). Lane 1, control; lane 2, photoirradiation with NADH; lane 3, +4 mM dopamine; lane 4, +4 mM dopamine and 0.1 M Mg^{2+}; lane 5, +4 mM dopamine and 0.2 M Mg^{2+}; lane 6, +4 mM dopamine and 0.3 M Mg^{2+}. (b) Plot of ratio of form I/form II vs $[Mg^{2+}]$.

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