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Effect of desferrioxamine, a strong iron (III) chelator, on 1-methyl-4-phenylpyridinium ion (MPP^+)-induced hydroxyl radical generation in the rat striatum

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Abstract

The present study was examined that the desferrioxamine, a strong iron (III) chelator, enhanced 1 methyl-4-phenylpyridinium ion (MPP^+)-induced hydroxyl radical (•OH) generation in the extracellular fluid of caudate nucleus anesthetized rats. Rats were anesthetized, and sodium salicylate in Ringer's solution (0.5 nmol/μl/min) was infused through a microdialysis probe to detect the generation of •OH as reflected by the non-enzymatic formation of 2,3-dihydroxybenzoic acid (DHBA) in the striatum. Induction of desferrioxamine (50 μM) drastically increased the formation of •OH trapped as 2,3-DHBA by the action of MPP^+, as compared with MPP^+-only-treated animals. Although desferrioxamine did not change the levels of MPP^+-induced dopamine, a marked elevation of •OH formation trapped as 2,3-DHBA was observed. When corresponding experiments were performed with reserpinized animals, the level of dopamine and 2,3-DHBA drastically decreased. However, the level of dopamine did not change, but desferrioxamine significantly increased the level of 2,3-DHBA in reserpinized animals. Iron (III) decreased MPP^+-induced 2,3-DHBA formations in the presence of dopamine (10 μM). Moreover, when iron (II) was administered to desferrioxamine-treated animals, a marked elevation of 2,3-DHBA was observed, compared with MPP^+-only-treated animals, that showed a positive linear correlation between iron (II) and •OH formation trapped as 2,3-DHBA ($R^2=0.981$) in the dialysate. The present study indicates that the suppression of MPP^+-induced •OH formation by iron (III) may play a key role in protective effect of iron (III) on the rat brain.

Keywords: Desferrioxamine; Iron (III); 1-Methyl-4-phenylpyridinium ion (MPP^+); Hydroxyl radical; Parkinson's disease

1. Introduction

Various free radicals were generated from biomedical reaction using known generating system. There is considerable evidence that intracellular iron mediates the toxicity of excess of reactive oxygen species, such as superoxide anion (O_2^-), hydrogen peroxide and hydroxyl free radical (•OH) to the cells. Although free radical reactions are a part of normal metabolism, the overproduction of reactive oxygen species may contribute to their cellular injury (McCord, 1985). Disorders of iron metabolism may cause high levels of free iron in various tissues. Iron has been implicated in cell degradation more than any other metal (Halliwell, 1989; Obata, 2002a). Reactive oxygen species has been implicated in dopaminergic toxicity caused by 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) and iron. MPTP producing a parkinsonian syndrome after its conversion to 1-methyl-4-phenylpyridine (MPP^+) by type B monoamine oxidase (EC 1.4.3.4) in the brain (Markey et al., 1984; Hirsch et al., 1988; Obata, in press), the etiology of this disease remains obscure. Although there are many papers showing that dopamine autoxidation and oxidative stress may be involved in Parkinson's disease (Gerlach et al., 1994; Maruyama et al., 1995; Obata, 2002a; Henze et al., 2005), the exact mechanism underlying MPP^+-enhanced depolarization evoked dopamine release from the nigrostriatal terminals is not known. MPP^+ is one of the most potent dopamine releasing agents (Obata, 2002a). Free radical formation has been linked to

Abbreviations: MPP^+, 1-methyl-4-phenylpyridinium ion; •OH, hydroxyl radical; 2,3-DHBA, 2,3-dihydroxybenzoic acid.

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the selective increase in content of iron in the substantia nigra (SN) (Dexter et al., 1989; Obata, in press). These reactions proceed via the well-established interaction between iron (II) and iron (III) with H_2O_2 (Minotti and Aust, 1987) and melanin (Pilas et al., 1988), respectively, to drive the Fenton-type reaction (Obata, 2002a). Although the function of iron content in brain is not known, its homeostasis is important for a normal brain function. There is substantial evidence for an increase in content of especially iron (III) (Malisza and Hasinoff, 1995, Obata, 2002a). A highly selective increase of iron (II) or iron (III) has been observed in parkinsonian substantia nigra (SN) (Gerlach et al., 1994). The major objective of this study was to investigate the mechanism of protective effect by iron (III) on the formation of •OH in the extracellular space of the striatum during dopamine release by MPP^+.

2. Materials and methods

2.1. Materials

MPP^+ was purchased from Research Biochemicals Inc. (USA). Desferrioxamine, ferrous ammonium sulfate, sodium salicylate, and its hydroxylate metabolites were purchased from Sigma Chemical Co. (St. Louis, MO, USA). In the case of reserpinized rats, reserpine (5 mg/kg; Daiichi Pharmaceutical, Japan) was injected intravenously into the rats 24 h before the experiments.

2.2. Animal preparation

Adult male Wistar rats (300–400 g) were housed in an environmentally controlled room (20–25 °C, 50–60% humidity) with available food and water ad libitum for 4 days prior to our experiments. The rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and prepared for intracranial microdialysis brain perfusion by a method previously used (Chiueh et al., 1992; Obata, in press). The Ethical Committee for Animal Experiments, Oita Medical University, Japan, approved this study.

2.3. Experimental protocol

In the preliminary experiments, the recovery rate of 10^{-7} M dopamine was 20.8±0.9% at a flow rate of 1 μl/min. The drugs were dissolved in Ringer's solution containing 147 mM NaCl, 2.3 mM $CaCl_2$ and 4 mM KCl, pH 7.4 for perfusion (1 μl/min) through a microdialysis probe into the striatum. The microdialysis probe was pre-washed with Ringer's solution for at least 30 min prior to stereotaxical implantation in the striatum (stereotaxic coordinates: AP: 1.0, R/L: 2.5, H: −7 mm from dura matter) (Paxinos and Watson, 1982). Thereafter for trapping •OH radicals (Chiueh et al., 1994; Obata, in press) in the striatum, sodium salicylate in Ringer's solution (0.5 nmol/μl/min) was perfused by a microinjection pump (Carnegie Medicine CMA/100 Stockholm, Sweden) and basal levels of 2,3-DHBA during a definite period time were determined. Brain dialysate (1 μl/min) was collected every 15 min into small collecting tubes containing 15 μl of 0.1 N $HClO_4$ to prevent amine oxidation and assayed immediately for 2,3-DHBA by high-performance liquid chromatographic-electrochemical (HPLC-EC) procedure (Smith and Bennett, 1997; Obata, in press). In a dose–response experiment, three different concentrations of ferrous ammonium sulfate, 2, 5 and 10 μM were administered directly through the dialysis probe in the rat brain for 15 min each. Desferrioxamine or iron (II) treatment alone had no effect on the formation of 2,3-DHBA.

2.4. Analytical procedures

The dialysate samples were immediately injected for analysis into an HPLC-EC equipped with a glassy carbon working electrode (EICOM Corp., Kyoto, Japan) and an analytic reverse-phase column on an Eicompak MA-5ODS column (5 μm 4.6×150 mm; EICOM). The working electrode was set at a detector potential of 0.75 V. Each liter in the mobile phase contained 1.5 g heptane sulfonic acid sodium salt (Sigma), 0.1 g Na_2EDTA, 3 ml triethylamine (Wako) and 125 ml acetonitrile (Wako) dissolved in H_2O. The pH of the solution was adjusted to 2.8 with 3 ml phosphoric acid (Wako).

2.5. Statistical analysis

All values are indicated as means±S.E.M. ANOVA combined with a Fisher's post hoc test or Student's *t*-test was used to determine the significance. A *P* value of less than 0.05 was regarded as statistically significant.

3. Results

The present results confirm that MPP^+ causes a sustained dopamine release (Miyake and Chiueh, 1989). The present microdialysis results demonstrated that MPP^+ evoked dopamine overflow lasting for more than 2 h. After a 60-min washout with pH 7.4 Ringer's solution, MPP^+ (5 mM or 5 nmol/μl/min) was infused into the striatum for 15 min (total dose; 75 nmol). Time-dependent changes in the level of dopamine and the formation of 2,3-DHBA from •OH were monitored in the dialysates from rat brain after MPP^+ treatment. The effect of desferrioxamine, a strong iron (III) chelator, on the sequential changes of the level of dopamine and •OH formation trapped as 2,3-DHBA in the dialysate obtained from six rats is shown in Fig. 1. When desferrioxamine (50 μM or 5 pmol/μl/min) was infused to MPP^+ pretreated animals, the level of dopamine did not change. However, a marked elevation of •OH formation was observed. When corresponding experiments were performed with reserpinized animals, the level of dopamine and 2,3-DHBA in rat brain dialysate was drastically decreased. However, desferrioxamine significantly increased the level of 2,3-DHBA formations in reserpinized animals (Fig. 2). When dopamine (10 μM or 10 pmol/μl/min) was administered to MPP^+-treated animals, dopamine significantly increased the formation of 2,3-DHBA, as compared to MPP^+-only-treated animals in the dialysate (data not shown). However, iron (III) applied at a variety of concentration (5, 10, 50 and 100 μM) decreased MPP^+-induced

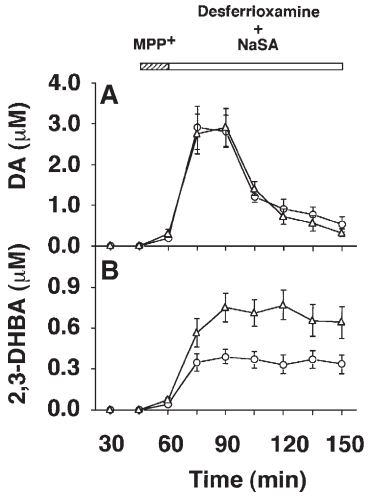

Fig. 1. Effect of histidine MPP^+-induced dopamine and the formation of •OH. Striatum was infused with MPP^+ (5 mM or 5 nmol/μl/min) for 15 min (shaded bar; total dose, 75 mM) to evoke the release of dopamine (A). At 60 min after probe implantation, Desferrioxamine (50 μM or 50 pmol/μl/min) including sodium salicylate (shaded bar, 5 mM or 0.5 nmol/μl/min) was infused through microdialysis probe for 90 min to trap •OH (B). MPP^+ (open circle) were compared with MPP^+ then Desferrioxamine (triangle). Values are mean ± S.E.M. for six animals.

2,3-DHBA formations in the presence of dopamine in a concentration-dependent manner (Fig. 3). To confirm the •OH generation by Fenton-type reaction, iron (II) (0, 2, 5 and 10 μM) was administered to MPP^+-pretreated animals, iron (II) clearly produced a dose-dependent increase in the levels of 2,3-DHBA,

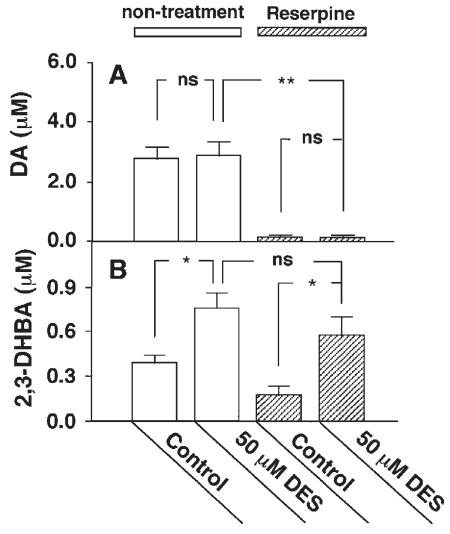

Fig. 2. Relationship between dopamine and •OH generation in the reserpinized rat. Dialysate dopamine (A) and in vivo trapping of highly reactive •OH trapped as 2,3-DHBA (B) in extracellular fluid of caudate nucleus were investigated by infusing sodium salicylate in Ringer solution (0.5 mM or 0.5 nmol/μl/min) through an intracranial microdialysis probe placed in rat striatum. When desferrioxamine (50 μM or 50 pmol/μl/min) was infused to reserpinized rat for 60 min, the level of 2,3-DHBA was examined. When MPP^+ (5 mM or 0.5 nmol/μl/min) was administered to reserpinized rats (shaded bar), dopamine and 2,3-DHBA were assayed (shaded column). Dialysate samples were assayed immediately by an HPLC-EC procedure. Values are means ± S.E.M. for six animals; $*P<0.05$, $**P<0.01$ versus each control (ANOVA and Fisher's rest). ns, nonsignificant.

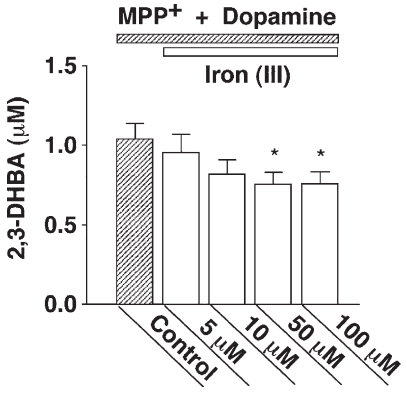

Fig. 3. Inhibitory effect of iron (III) on MPP^+-induced •OH formation. When iron (III) (open bar; 5, 10, 50 and 100 μM; open column) was administered to MPP^+ (0.5 mM or 0.5 nmol/μl/min) then dopamine (10 μM or 10 pmol/μl/min)-pretreated animals (control, shaded bar), dialysate 2,3-DHBA levels were compared (shaded column). Values are means ± S.E.M. for six animals; $*P<0.05$ versus control group (ANOVA and Fisher's test).

as compared with iron (II)-only treated tats, showing a positive linear correlation between iron (II) and •OH trapped as 2,3-DHBA ($R^2=0.981$) in the dialysate. However, when the corresponding experiments were performed with desferrioxamine (50 μM)-treated animals, a marked elevation in the level of 2,3-DHBA products was observed (Fig. 4).

4. Discussion

The present results of our study demonstrated that iron (III) is associated with protective effect by suppress of the •OH generation. The administration of drugs was carried out through the microdialysis probe. Oxygen free radicals are very reactive, and the nonenzymatic •OH adduct of salicylate, 2,3-DHBA provides an assay of •OH formation both in vitro and in vivo (Halliwell et al., 1991; Chiueh et al., 1994). This sensitive salicylate hydroxylation procedure can detect •OH during Fe^{2+}-catalyzed autoxidation of dopamine in vitro. 2,3-DHBA

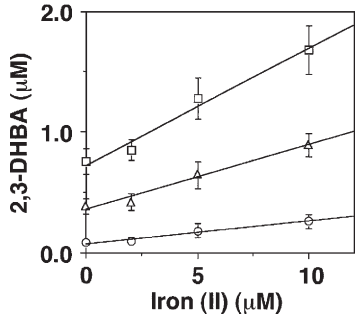

Fig. 4. Cumulative dose–response relationship between iron (II) and the formation of •OH products of salicylate in MPP^+ (5 mM or 5 nmol/μl/min)-treated rats. Iron (II) and sodium salicylate (0.5 mM or 0.5 nmol/μl/min) were infused through the dialysis probe. Three different concentrations of iron (II) (0, 2, 5 and 10 μM) were infused through the dialysis probe in the MPP^+-treated rats, and the level of 2,3-DHBA produced. MPP^+ alone (triangle), MPP^+, then desferrioxamine (square) and no MPP^+ (open circle) were compared. Dialysate samples were assayed immediately by an HPLC-EC procedure. Values are means ± S.E.M. for six animals. The ordinate shows the cumulative level of 2,3-DHBA out put over 90 min.

formations in brain dialysate from control rats were about 10% higher than in the in vitro perfusion reagent background. Accordingly, the concentration profile of the administered compounds in the surrounding interstitial space is unknown; in general, the extracellular concentration of a compound given through the probe would never attain the concentration levels seen in the dialysis probe (Benveniste, 1989). This is an unavoidable limitation of the microdialysis technique that should be kept in mind when interpreting the experimental data.

Although there are numerous reports about MPP^+ (Chiueh et al., 1992; Imperato et al., 1994; Obata, in press), the mechanism of •OH generation by MPP^+ is not clear. The present study indicates that some extracellular autoxidation of dopamine, occurring in the presence of oxygen and some transition metal, could lead to the formation of •OH radicals. As suggested by data obtained during the investigation of manganese-induced Parkinsonism (Higashi et al., 2004), sustained autoxidation of dopamine could lead to excessive accumulation of toxic quinones and potentially cytotoxic oxygen free radicals. Dopamine enhanced the formation of 2,3-DHBA (Chiueh et al., 1994), the nonenzymatic •OH adduct of salicylate. MPP^+ is one of the most potent dopamine releasing agents (Obata, 2002b). When the level of dopamine in the dialysate increased dramatically following administration of MPP^+, the elevation of 2,3-DHBA was observed in the brain dialysate (Fig. 1). Dopamine is known to be autoxidized in the presence of oxygen and transition metals (Riederer et al., 1989; Obata, 2002b).

Theoretically, •OH may be formed in vivo during nonenzymatic oxidation (Graham, 1984) and/or enzymatic oxidation of dopamine, especially in the brain regions (putamen, caudate nucleus, and substantia nigra, zona compacta) where there are high levels of dopamine, oxygen and iron. Free radical reactions are a part of normal metabolism. The sequence of reactions involving O_2^- and •OH with iron is known. I suggested that iron (II), by reacting with O_2^- is converted to iron (III) and thereby suppress formation of •OH. My data indicate that the elevation in dopamine release may cause •OH generation, as reflected by 2,3-DHBA levels in the brain dialysate. Dopamine is known to be autoxidized in the presence of oxygen and transition metals Graham, 1984; Obata, 2002b). Reserpine is known to release stored vesicular dopamine and block amine uptake by vesicles. Therefore, reserpine induces depletion of dopamine (Imperato et al., 1994). To confirm the effect of MPP^+-induced •OH formation, the levels of dopamine and 2,3-DHBA formations were measured in reserpinized animals. Reserpine-induced dopamine depletion may reduce MPP^+-induced •OH formation. The level of dopamine and 2,3-DHBA with reserpinized group was drastically reduced, as compared with that of MPP^+-only treated group. When desferrioxamine, a strong iron (III) chelator, was administered to MPP^+-treated animals, a marked elevation in the levels of 2,3-DHBA was observed, as compared to MPP^+-only-treated animals in the dialysate. Although desferrioxamine did not change the levels of MPP^+-induced dopamine, a marked elevation of •OH formation trapped as 2,3-DHBA was observed (Fig. 2). Fig. 2 demonstrated that desferrioxamine significantly increased •OH formation in the striatum of reserpinized rats, but the levels of radicals were not significantly changed as compared to nonreserpinized rats. I cannot completely rule out other possibilities concerning the mechanism of suppression of •OH formation because desferrioxamine has a strong ion (III) chelator. Although MPP^+ is known to induce •OH generation, the mechanism of •OH generation induced by MPP^+ is not fully understood. I cannot explain about this point in the present. However, further experiment is necessary to confirm the relationship between •OH and desferrioxamine. The inhibition of iron (III) enhanced •OH formation, which leads (possibly by an indirect mechanism) to the formation of cytotoxic •OH formation. Moreover, iron (III) decreased MPP^+-induced 2,3-DHBA in the presence of dopamine (10 μM) (Fig. 3). These results indicate that dopamine increasing MPP^+-induced •OH formation may attenuate by iron (III). To determine whether iron (III) has a radical scavenging or antioxidant effect, I examined the effect of desferrioxamine on the iron (II)-induced •OH generation. Induction of desferrioxamine (50 μM) drastically increased MPP^+-induced •OH formation trapped as 2,3-DHBA in the brain dialysate, compared with MPP^+-only-treated animals, that showed a positive linear correlation between iron (II) and •OH formation trapped as 2,3-DHBA ($R^2 = 0.981$) in the dialysate (Fig. 4). Therefore, iron (III) could participate in protective effect.

Although free radical reactions are a part of normal metabolism, the enzyme xanthine oxidase is also thought to be a source of O_2^- (Fig. 5). The O_2^- itself is somewhat poorly reactive in aqueous solution, but does participate in the reaction in which the iron ions are involved, leading to the generation of more damaging •OH species. O_2^- has an extremely short half-life (Halliwell et al., 1991) and rapidly undergoes dismutation yielding to hydrogen peroxide. H_2O_2 undergoes a Fenton-type reaction in the presence of iron yielding to cytotoxic •OH. In addition, •OH can also arise from an interaction between hydrogen peroxide and O_2^- (Haber–Weiss reaction) (Liu et al., 2004). However, iron (III) can be reduced further to iron (II). Desferrioxamine can be reduced to iron (III). The level of 2,3-DHBA markedly increases due to desferrioxamine treatment. Therefore, iron (II) in the presence of hydrogen peroxide results

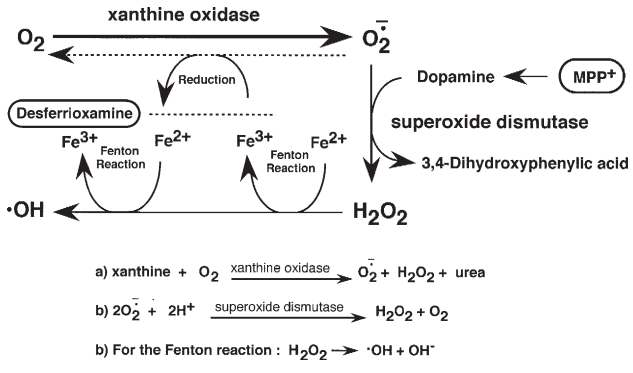

Fig. 5. The reaction pathway in rat heart illustrates the formation of hydroxyl radical in the presence of iron (III) and oxygen. Abbreviations: MPP^+, 1-methyl-4-phenylpyridinium ion; O_2^-, superoxide anion; •OH, hydroxyl radical.

in further formation of •OH. This is, perhaps, why desferrioxamine markedly increases •OH formation. These results suggest that iron (III) may reduce •OH formation by Fenton reaction in the rat brain. The present study demonstrated that the suppression of •OH formation by iron (III) might play a key role in protective effect of iron (III) on the rat brain. Based on the mass-action principle, an increase of iron (III) should reverse the Fenton reaction and reduce the •OH production.

In conclusion, •OH can arise from an interaction between hydrogen peroxide and O_2^- (Haber–Weiss reaction). However, iron (III) reduced the production of •OH generation, at least by measurement of 2,3-DHBA. The results of the present study may be useful in elucidating the actual mechanism of free radical formation in the pathogenesis of neurodegenerative brain disorders, including Parkinson's disease, Alzheimer's disease and traumatic brain injuries.

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