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Short communication

Selenium, an antioxidant, protects against methamphetamine-induced dopaminergic neurotoxicity

Syed Z. Imam [a], Glenn D. Newport [a], Fakhrul Islam [b], William Slikker Jr. [a], Syed F. Ali [a,c,*]

[a] *Neurochemistry Laboratory, Division of Neurotoxicology, National Center for Toxicological Research, FDA, Jefferson, AR, USA*
[b] *Neurotoxicology Laboratory, Department of Medical Elementology and Toxicology, Hamdard University, New Delhi, India*
[c] *Department of Neurology and Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR, USA*

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Abstract

Dopaminergic changes were studied in the caudate nucleus of adult female mice after pre- and post-treatment with an antioxidant, selenium, 72 h after the multiple injections of methamphetamine (METH, 4×10 mg/kg, i.p. at 2-h interval) or an equivalent volume of saline. Selenium treatment prevented the depletion of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in caudate nucleus resulting from the METH treatment. These data suggest that METH-induced neurotoxicity is mediated by free radical and selenium plays a protective role against METH-induced dopaminergic neurotoxicity.

Keywords: Methamphetamine; Neurotoxicity; Selenium; Dopamine; Caudate nucleus; Oxidative stress

Methamphetamine (METH) is a drug that is significantly abused worldwide. It causes neurotoxicity in rodents and non-human primates by producing long-term depletion of dopamine (DA) and its metabolites [28,12], decreasing the number of high affinity DA uptake sites [21] and decreasing the activity of tyrosine hydroxylase (TH) in striatum [19]. DA, TH and the DA transporter are reduced in the post-mortem striatum of chronic METH users [31]. Although the cellular and molecular mechanisms involved in METH-induced toxicity are not completely clarified, a role for oxygen-based radicals is well supported by several studies in the literature (for review, see Ref. [3]). The oxygen-based radical theory suggests that formation of toxic radicals from DA might be the main determinant of neurotoxicity of this illicit drug [3,9]. This concept is supported by results obtained from copper–zinc superoxide dismutase over expressed transgenic mice which are protected against the toxic effects of METH [4]. Furthermore, METH has also been reported to increase hydroxyl radicals in the brain in a time-dependent manner [14], produce oxidative stress [13] and produce free radicals [10]. METH-induced neurotoxic damage has also reported to be prevented by some known antioxidants like vitamin E or ascorbate [10]. In a recent study, it has been demonstrated that overexpression of glutathione peroxidase in PC12 cells resulted in protection against METH toxicity [17]. Also, repeated doses of METH injection has been found to decrease cortical and striatal glutathione peroxidase activity in mice [29].

Selenium (Se) is an essential dietary component for mammals, including humans. One of its well-understood functions is that it is present in the active center of glutathione peroxidase, a potent antioxidative enzyme that scavenges various peroxides and protects membrane lipids and macromolecules from oxidative damage [25]. Many neurotoxicants which give rise to specific degeneration of the dopaminergic nervous system are postulated to elicit their detrimental effects at least in part through the generation of various reactive oxygen species. Therapy with Se and other antioxidants results in positive clinical responses in various neurological degenerative diseases associated with increased oxidative damage [30,15]. The role of Se as an antioxidant agent as well as its presence in the active center of the enzyme glutathione peroxidase prompted us to investigate the effect of Se on METH-induced dopamin-

* Corresponding author. Neurochemistry Laboratory Division of Neurotoxicology, HFT-132 NCTR/FDA, Jefferson, AR 72079, USA. Fax: +1-870-543-7745; E-mail: sali@nctr.fda.gov

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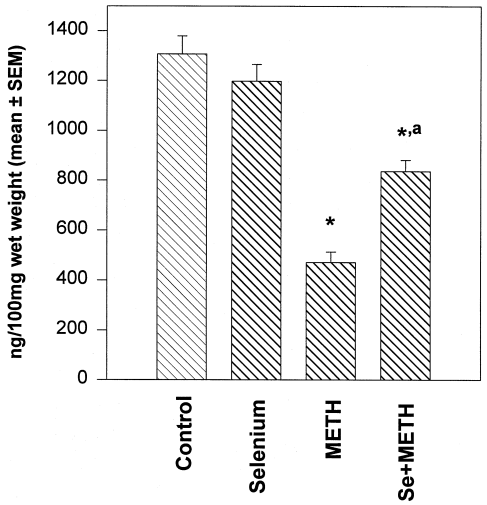

Fig. 1. Effects of METH (4×10 mg/kg, i.p.) on DA concentration in caudate nucleus of adult female C57BL/6N mice. Animals received selenium for 1 week before the first injection of METH and 1 week after the last injection of METH. Each value is mean ± S.E.M. derived from eight animals/group. * $p < 0.05$, significantly different from control group; [a] $p < 0.05$, significantly different from METH group.

ergic neurotoxicity which might result from oxidative stress. Therefore, in the present study we examined the effect of pre- and post-treatment of Se on the dopaminergic neurotoxicity in mice caudate nucleus induced by multiple doses of METH.

Adult female C57BL/6N mice weighing 25–30 g were used in this study. Animals were housed four per cage under controlled environmental conditions with food and water supplied ad libitum. Animals were divided into four groups with eight mice per group. Group I served as control and group II and IV were pre-treated with 0.5 mg Se/kg in the form of sodium selenite (Na_2SeO_3) in drinking water for 1 week. Average consumption of water per mouse per day was 5 ± 0.25 ml from a stock of 3 mg Se (6.57 mg Na_2SeO_3) per liter. Groups III and IV received 4 × 10 mg/kg METH, i.p. at 2 h. interval after 1 week of Se administration. Se supplementation continued for 1 more week to groups II and IV. Animals were sacrificed by decapitation 1 week after METH treatment and the brains were quickly removed and the caudate nucleus were dissected for neurochemical analyses.

The concentrations of DA and its metabolites, 3,4,-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were quantified in caudate nucleus by a modified HPLC method combined with electrochemical detection [1]. Tissues were weighed in a measured volume (20% w/v) of 0.2 M perchloric acid containing internal standard 3,4-dihydroxybenzylamine,100 ng/ml. The tissues were disrupted by ultrasonication and centrifuged at 4°C (15 000 × g; 7 min), and 150 μl of the supernatant was removed and filtered through a Nylon-66 microfilter (pore size, 0.2 μm; MF-1 centrifugal filter; Bioanalytical Systems, West Lafayette, IN, USA). Aliquots of 25 μl representing 2.5 mg of brain tissue were injected directly onto the HPLC/electrochemical detection system for separation of analytes. The amount of DA, DOPAC and HVA were calculated using standard curves that were generated by determining in triplicate the ratio between three different known amounts of the amine or its metabolites and a constant amount of internal standard. Analysis of differences between multiple treatment groups consisted of ANOVA followed by post-hoc comparison (Dunnett's test for control and drug group comparisons and Newman–Keuls test for comparison between groups). Significant differences were defined at $p < 0.05$.

Treatment with METH caused 64%, 65% and 38% decreases in DA (Fig. 1), DOPAC and HVA (Fig. 2), respectively, in caudate nucleus. No significant changes were found in DA, DOPAC or HVA in caudate nucleus of mice supplemented with Se alone for 2 weeks. However, in mice supplemented with Se and treated with multiple doses of METH, recovery of about 40% in DA (Fig. 1), 64% in DOPAC (Fig. 2) and almost 90% in HVA (Fig. 2) content were found in caudate nucleus.

In the present study, we demonstrated that METH-induced DA neurotoxicity in mice can be attenuated by an antioxidant Se. There are several reports in the literature which discuss the involvement of free radicals in METH-induced neurotoxicity. It has been reported that METH-induced DA depletion is attenuated in copper–zinc superox-

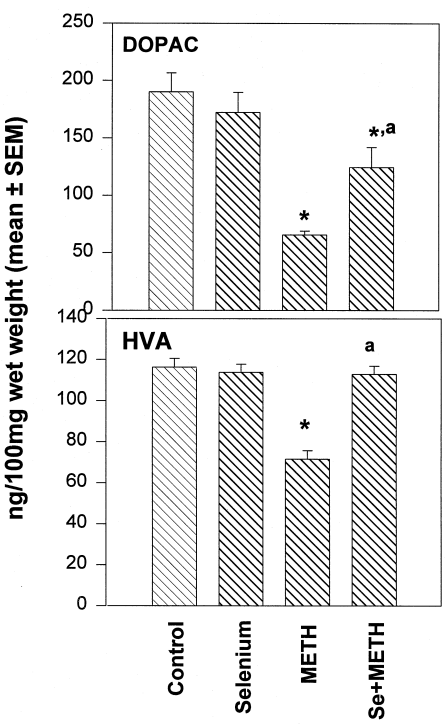

Fig. 2. Effects of METH (4×10 mg/kg, i.p.) on DOPAC (A) and HVA (B) concentration in caudate nucleus of adult female C57BL/6N mice. Animals received selenium for 1 week before the first injection of METH and 1 week after the last injection of METH. Each value is mean ± S.E.M. derived from eight animals/group. * $p < 0.05$, significantly different from control group; [a] $p < 0.05$, significantly different from METH group.

ide dismutase over expressed transgenic mice [5,4]. METH-induced neurotoxicity also results in a long-lasting decrease in DA levels and DA uptake sites in the striatum [22,27,6]. There are several other reports which support the correlation of free radicals [3,24] with the depletion of DA [8,18]. Recently, we and others have reported that a selective neuronal nitric oxide synthase inhibitor, 7-nitroindazole protects against the METH-induced dopaminergic neurotoxicity [22,11] which implicates the role of nitric oxide radicals in METH neurotoxicity. To further investigate, we also demonstrated that neuronal nitric oxide synthase knock-out mice are protected from METH-induced dopaminergic neurotoxicity [20] and an antioxidant, melatonin, can attenuate METH-induced dopaminergic neurotoxicity [21].

Se, an essential dietary element, has been shown to have antioxidant properties because of its presence in the active center of glutathione peroxidase [25]. A major defense system against the oxidative stress in brain is the glutathione reductase/glutathione peroxidase system, which utilizes glutathione. Glutathione itself provides neuroprotection in various neurodegenerative disorders associated with oxidative stress-mediated neuronal death, see review [2]. In the brain, glutathione peroxidase plays a predominant role in removing excess of some specific free radicals like hydroperoxides. Decreased glutathione peroxidase activity [16] and decreased levels of total and reduced glutathione [26] has been reported in the substantia nigra and striatum of Parkinson's patient. Decreased glutathione has also been found to cause the selective death of dopaminergic neurons by 1-methyl-4-phenylpyridinium [23]. Se-deficient diets have also been reported to enhance brain susceptibility, especially dopaminergic nerve terminals, to oxidative damage and result in an increased turnover of DA in the substantia nigra [7]. Since repeated doses of METH decrease glutathione peroxidase activity in brain [25] and Se supplementation increases its activity and scavenges free radicals, it is possible in the present study that the subchronic supplementation of Se might be acting through increasing the glutathione peroxidase activity and scavenging the free radicals and thus provides protection against the METH-induced dopaminergic neurotoxicity.

In the present study, we demonstrated the depletion of DA and its metabolites DOPAC and HVA in caudate nucleus after acute doses of METH, and also the effect of Se in protecting against the dopaminergic system in caudate nucleus. Significant recovery in DA and its metabolites in caudate nucleus by Se were observed in the animals treated with multiple acute doses of METH while Se alone produced no significant effect on the dopaminergic system. In summary, the present study demonstrates that selenium provides protection against METH-induced dopaminergic neurotoxicity. These studies further confirmed that METH-induced dopaminergic neurotoxicity is mediated via oxidative stress and can be protected by the antioxidant selenium.

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