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Zinc protects SK-N-SH cells from methamphetamine-induced α-synuclein expression

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Abstract

Methamphetamine (METH) is a well-known drug of abuse and neurotoxin that may cause temporary or permanent disturbances in the dopaminergic systems of the brain, predisposing individuals to Parkinsonism. Previously, we have shown that METH causes dopaminergic cell death by increasing the production of reactive oxygen species (ROS) and by depleting cellular ATP levels. These effects were abolished by pretreatment with $ZnCl_2$ which enhanced expression of the zinc binding protein, metallothionein. In the present study, the effects of $ZnCl_2$ on α-synuclein expression were examined further in METH-treated SK-N-SH cells in culture. We show that METH significantly increased α-synuclein expression in a dose-dependent manner after inducing oxidative stress. Pretreatment with $ZnCl_2$ (50 μM) reversed this stimulatory effect. We propose that zinc mediates this neuroprotective response via the production of metallothionein.

Keywords: α-synuclein; Methamphetamine; Parkinson's disease; Oxidative stress; Zinc; SK-N-SH cells; Metallothionein

Methamphetamine (METH) is a well-known drug of abuse and neurotoxin that may cause long-lasting changes in the dopaminergic pathways of the central nervous system (CNS) [6]. METH treatment is known to cause nigrostriatal damage in experimental animals as well as in humans [24]. Therefore, METH toxicity is frequently cited as a potential model of drug-induced Parkinsonism [9] similar to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a Parkinsonism-inducing environmental toxin. Chronic or intermittent amphetamine abuse may create temporary or permanent disturbances in the dopaminergic systems of the brain that may predispose individuals to Parkinsonism. Irreversible loss of dopaminergic function may increase the likelihood of developing Parkinsonism later in life [11].

Parkinson's disease (PD) is one of the most common movement disorders of the elderly. Its pathological characteristics include loss of dopaminergic neurons and the presence of intracellular inclusions called Lewy bodies (LBs). Wild-type α-synuclein is a major component of LBs in sporadic PD, dementia with LBs (a subtype of Alzheimer's disease (AD) known as the LB variant of AD), as well as in glial cytoplasmic inclusions in multiple system atrophy [17]. The mechanisms underlying neurodegeneration in PD are unknown. However, studies showing α-synuclein accumulation in LBs and mutations of α-synuclein in familial PD suggest that α-synuclein has an important role in the pathophysiology of PD [15]. An increasing number of studies have shown that oxidative stress is involved in the neurodegeneration. Oxidative modifications of α-synuclein and generation of stable oligomers may play an important role in the neurotoxicity of oxidative stress [18].

In our previous study [1], we showed that METH causes dopaminergic cell death by increasing reactive oxygen species (ROS) production and depleting cellular ATP levels. We

Abbreviations: AD, Alzheimer's disease; CNS, central nervous system; LB, Lewy bodies; METH, methamphetamine; PD, Parkinson's disease; ROS, reactive oxygen species

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proposed that metallothionein mediates a zinc-dependent neuroprotective effect against METH toxicity. Since the mechanism underlying the interaction between α-synuclein and oxidative stress has not been clearly elucidated, we have extended our study to further investigate the effects of METH on α-synuclein expression and to determine whether pretreatment with $ZnCl_2$ affects this response.

In this study, we present evidence that METH affects α-synuclein expression in a cultured dopaminergic cell line. Human SK-N-SH neuroblastoma cells that are normally used model for dopamine cells [1,21] were grown in media containing minimum essential media with 10% fetal bovine serum, 100 units/ml of penicillin and 100 μg/ml of streptomycin. The cells were maintained in a humidified incubator with 5% CO_2 in air at 37 °C. Cells were grown in 6-well culture plates and treated with various concentrations of METH (Sigma–Aldrich, St. Louis, MO) for 24 h. Some cells were pretreated with 50 μM $ZnCl_2$ for 12 h prior to METH exposure. Western blot analysis was employed to determine the amount of α-synuclein in the cells after different treatments using goat anti-α-synuclein antibody (R&D, Minneapolis, MN; 1:1000 in blocking buffer) and HRP-conjugated rabbit anti-goat IgG antibody (Chemicon, Tamecula, CA; 1:5000 in blocking buffer). Samples were visualized by enhanced chemiluminescence using ECL Plus™ Western Blotting Detection Reagents and exposed on Hyperfilm ECL (Amersham Biosciences, Piscataway, NJ).

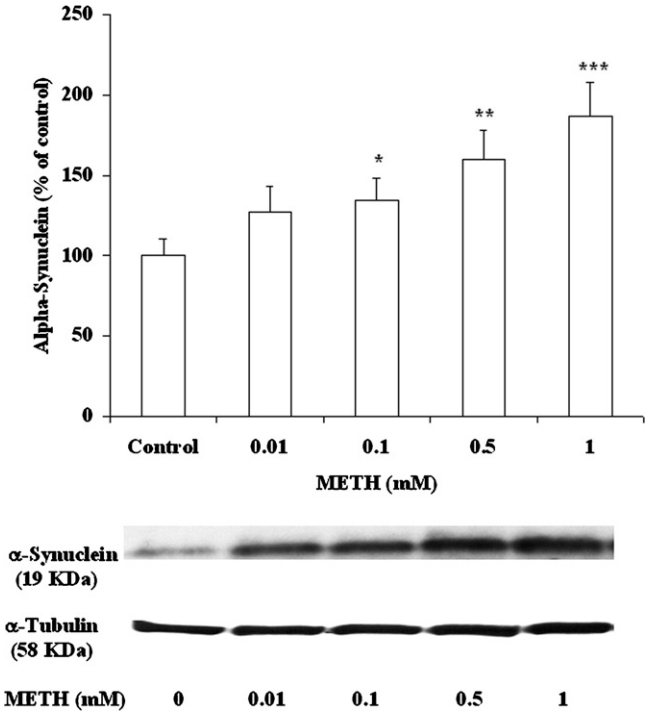

Fig. 1. Dose-dependent effects of METH on α-synuclein levels in cultured SK-N-SH cells. Western blot analysis was used to determine changes in α-synuclein expression after METH treatment. The bar graph represents the densitometric measurement of the α-synuclein normalized to α-tubulin levels in each lane on the blot. Cells were treated with increasing concentrations of METH for 24 h. Data represent mean ± S.E.M. of four independent experiments. The symbols (*), (**) and (***) indicate a significance between control and METH treated groups of $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

The immunoblots were quantified by measuring the density of α-synuclein normalized to α-tubulin levels of each lane using densitometry with the Scion image program (National Institutes of Health, Bethesda, MD). Protein determination was carried out by the Bradford method using Bio-Rad protein assay kit and bovine serum albumin as a standard. Experimental data were analyzed using ANOVA (analysis of variance) and Student's *t*-test to compare experimental and control groups. Sigma-Stat statistical software was employed. All values are represented as mean ± S.E.M. The significance was taken when *p*-values were 0.05 or less. The results show that after cells were exposed to 0.01, 0.1, 0.5 and 1 mM METH for 24 h, the level of α-synuclein increased significantly in a dose dependent manner to 127.1 ± 15.7%, 134.3 ± 14.2% ($p < 0.05$), 159.5 ± 18.8% ($p < 0.01$) and 187.0 ± 21.0% ($p < 0.001$) of the control values, respectively (Fig. 1).

In our previous study [1], we showed that pretreatment with zinc markedly decreased the loss of cell viability caused by METH. Zinc pretreatment increased the expression of metallothionein and prevented the generation of ROS and ATP depletion caused by METH. We suggested that metallothionein could be a potent free radical scavenger, thereby protecting neurons from several free radical-generating toxins. In order to determine whether zinc could protect against METH-induced α-synuclein expression, cells were pretreated with 50 μM $ZnCl_2$ for 12 h prior to treatment with various concentrations of METH for 24 h. The results show that the METH-induced α-synuclein expression was significantly attenuated by pretreating the cells with 50 μM of $ZnCl_2$ followed by 0.1, 0.5 and 1 mM METH to 101.72 ± 18.4% ($p < 0.01$), 133.3 ± 16.2% ($p < 0.05$) and

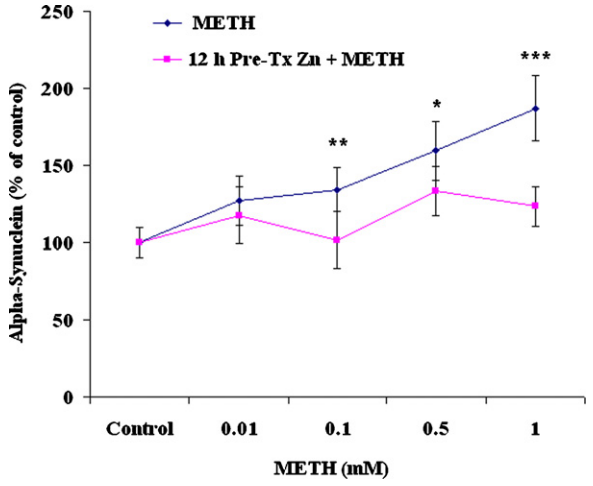

Fig. 2. The effect of $ZnCl_2$ on the METH-induced increase in α-synuclein expression in cultured SK-N-SH cells. Western blot analysis was used to determine changes in α-synuclein expression after $ZnCl_2$ and METH treatment. The curve represents densitometric measurement of the α-synuclein normalized to α-tubulin levels in each lane of the blot. Cells were treated with increasing concentrations of METH for 24 h, with or without pre-treatment with $ZnCl_2$ (50 μM for 12 h). The results are presented as percentage of untreated controls. Data represent mean ± S.E.M. of four independent experiments. The symbols (*), (**) and (***) denote significance between the METH-treated group and the METH + pre-incubation with 50 μM of $ZnCl_2$ group of $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.