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THE SUBCHRONIC EFFECTS OF 3,4-METHYLENDIOXYMETHAMPHETAMINE ON OXIDATIVE STRESS IN RAT BRAIN

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Abstract – The aim of the present study was to evaluate the subchronic effects of 3,4-methylenedioxymethamphetamine on several oxidative stress markers: index of lipid peroxidation (ILP), superoxide dismutase (SOD) activity, superoxide radical ($O_2^{\cdot-}$) levels, and reduced glutathione (GSH) levels in the frontal cortex, striatum and hippocampus of the rat. The study included 64 male Wistar rats (200-250 g). The animals were treated *per os* with of 5, 10, or 20 mg/kg of 3,4-methylenedioxymethamphetamine (MDMA) every day for 15 days. The subchronic administration of MDMA resulted in an increase in ILP, SOD and $O_2^{\cdot-}$, and a decrease in GSH, from which we conclude that oxidative stress was induced in rat brain.

Key words: 3,4-Methylenedioxymethamphetamine (MDMA, ecstasy); free radicals; oxidative stress; neurotoxicity.

INTRODUCTION

Oxidative stress and oxygen free radicals are thought to play an important role in both the acute and chronic effects of a number of neurotoxic processes. Superoxide radicals and hydrogen peroxide are produced during electron transport in the mitochondria (Fiaschi et al., 2010). Cells protect themselves against reactive oxygen intermediates (ROI) by using antioxidants such as the superoxide-scavenging enzyme superoxide dismutase (SOD) (Touati, 1988), catalase and glutathione peroxidase and other nonenzymatic agents such as glutathione, other thiols and vitamin E (Cadet, 1988).

3,4-Methylenedioxymethamphetamine (MDMA or "Ecstasy"), an amphetamine analog, is a popular recreational drug of abuse and has been shown to be potentially toxic to the serotoninergic nerve terminals of the brains of rodents and humans (Semple et al., 1999). MDMA-induced 5-HT toxicity is based on long-term biochemical effects such as a decrease in the tissue concentration of 5-HT (Turrillazi et al., 2010) and its major metabolite 5-hydroxyindolacetic acid, a decrease in the activity of tryptophan hydroxylase (Stone et al., 1987) and a reduction in the (3H)-paroxetine-labeled 5-HT reuptake sites (Bataglia et al., 1987). The mechanism of MDMA-induced 5-HT depletion has been proposed to involve the induction of oxidative stress (Sprague et al., 1998). In addition, treatments with antioxidants (Barbosa et al., 2012), as well as free radical scavengers, are neuroprotective against MDMA-induced 5-HT depletion.

Although the acute effect of MDMA on the brain has been well documented, the potential functional

consequences associated with long-term MDMA administration have not yet been thoroughly evaluated. In the present study, the neurochemical responses to repeated administration of MDMA were evaluated.

MATERIALS AND METHODS

Investigations were made on 64 6 weeks old male Wistar rats weighing about 250 g. The rats were divided into four groups (according to drug treatment) and each group consisted of 16 animals. The rats were housed in Makrolon cages, 4 per cage (Erath, FRG). Animals had free access to food and water. Average microclimate conditions were as follows: temperature (22±2°C), relative humidity (60-70%), and dark/light cycles (12 h). Food (commercial rat diet) and water were not restricted. MDMA was dissolved in distilled water and administered perorally at 5, 10 and 20 mg/kg once per day for 15 days. The control group received the same volume of distilled water. The animals were killed by decapitation 15 days after the treatment. Brain tissues of the ipsilateral forebrain cortex, hippocampus and striatum from individual animals were quickly isolated on ice and homogenized in an ice-cold buffer containing 0.25 mol sucrose, 1 mM EDTA, 10 mmol K-Na phosphate buffer, pH 7.0. Homogenates were centrifuged twice at 3 500 rpm for 15 min at 4°C. The supernatant obtained by this procedure was then frozen and stored at -70°C (Gurd et al., 1974).

Protein measurement

The content of protein in the rat brain homogenates was measured by the method of Lowry et al. (1974) using bovine serum albumin (Sigma) as standard.

Biochemical analysis

Superoxide dismutase (SOD) activity was determined as inhibition of epinephrine autooxidation at 480 nm. After adding 10mM of epinephrine (Sigma), the kinetics were monitored in sodium carbonate buffer (50 mM, pH 10.2; Serva) containing 0.1 mM EDTA (Sigma) (Sun et al., 1978).

The superoxide anion content was determined by the reduction of nitroblue-tetrazolium (Merck) in an alkaline, nitrogen-saturated medium. Analysis was performed at 515 nm (Auclair et al., 1985.).

The lipid peroxidation index was measured as malondialdehyde produced after stimulated peroxidation with 0.01 mM ferrosulfate (Merck) and 0.5 mM ascorbic acid (Serva). Thiobarbituric acid reagent (TBAR), consisting of trichloroacetic acid (Merck), thiobarbituric acid and HCl, reacts with malondialdehyde, the final product of polyunsaturated fatty acid peroxidation, measured at 533 nm (Villacara et al., 1989).

The content of reduced glutathione (GSH) was determined using 5,5-dithiobis-2-nitrobenzoic acid (DTNB, 36.9mg in 10ml of methanol) that reacts with aliphatic thiol compounds in tris-HCl buffer (0.4M, pH-8.9), producing a yellow-colored p-nitrophenol anion. Intensity of the color was used for spectrophotometrical measurement of GSH concentration at 412 nm (Anderson, 1986).

Data presentation and analysis

Data are expressed as means ± SD. Differences between groups were examined using a Student's independent t-test. Statistical significance was accepted at $p<0.05$.

RESULTS

As can be observed in Fig. 1, the daily peroral administration of a MDMA for 15 days caused a significant increase in SOD activity in all three brain regions examined in the rats. Fig. 2 shows no significant changes following administration of 5 mg/kg MDMA in the tissue concentration of $O_2^{\cdot -}$. The other MDMA-treated groups show increased levels of $O_2^{\cdot -}$. In MDMA-treated rats, the levels of ILP were significantly elevated relative to those for the controls (Fig. 3). MDMA caused slight decreases in GSH activity in the 5 mg/kg group, significant decreases in the 20 mg/kg group and no significant changes in the 10 mg/kg group (Fig 4).

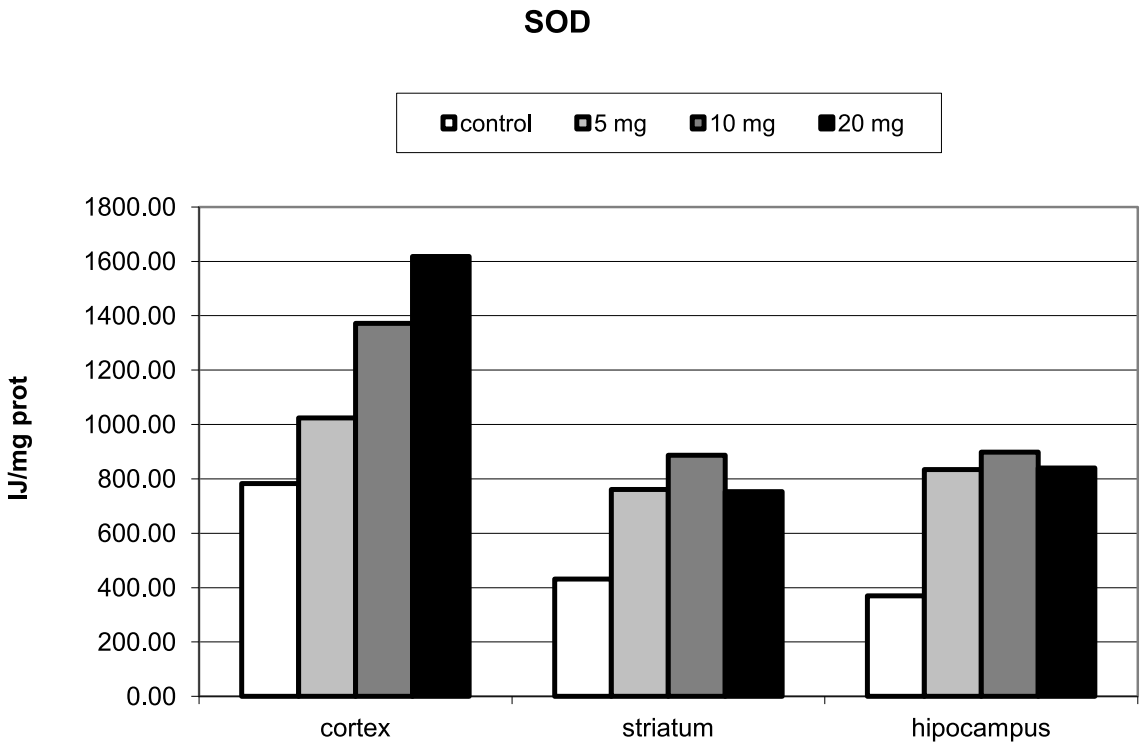

Fig. 1. Effects of methylendioxymetamphetamine on levels on SOD in forebrain cortex, hippocampus and striatum

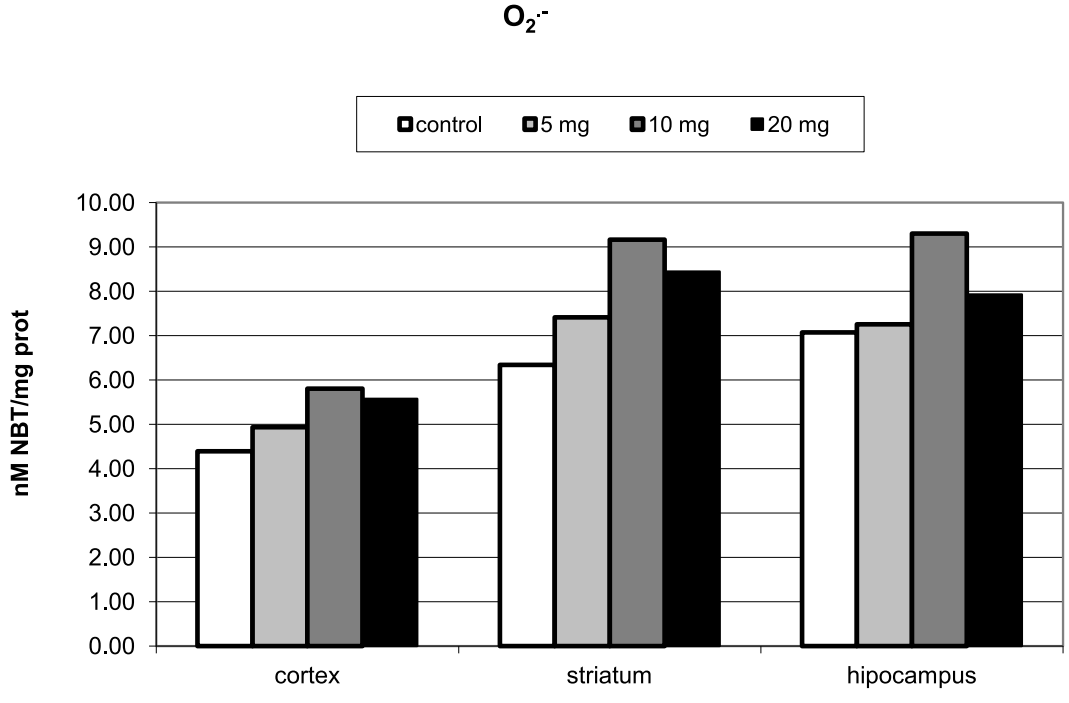

Fig. 2. Effects of methylendioxymetamphetamine on levels on $O_2^{\cdot-}$ in forebrain cortex, hippocampus and striatum

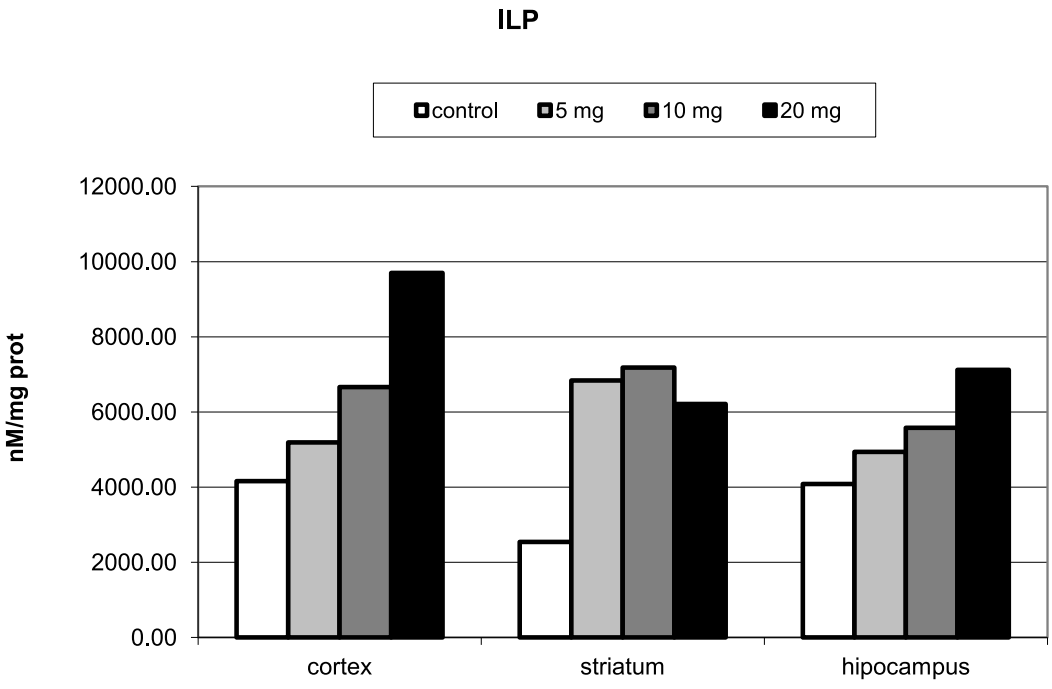

Fig. 3. Effects of methylendioxymetamphetamine on levels on ILP in forebrain cortex, hippocampus and striatum

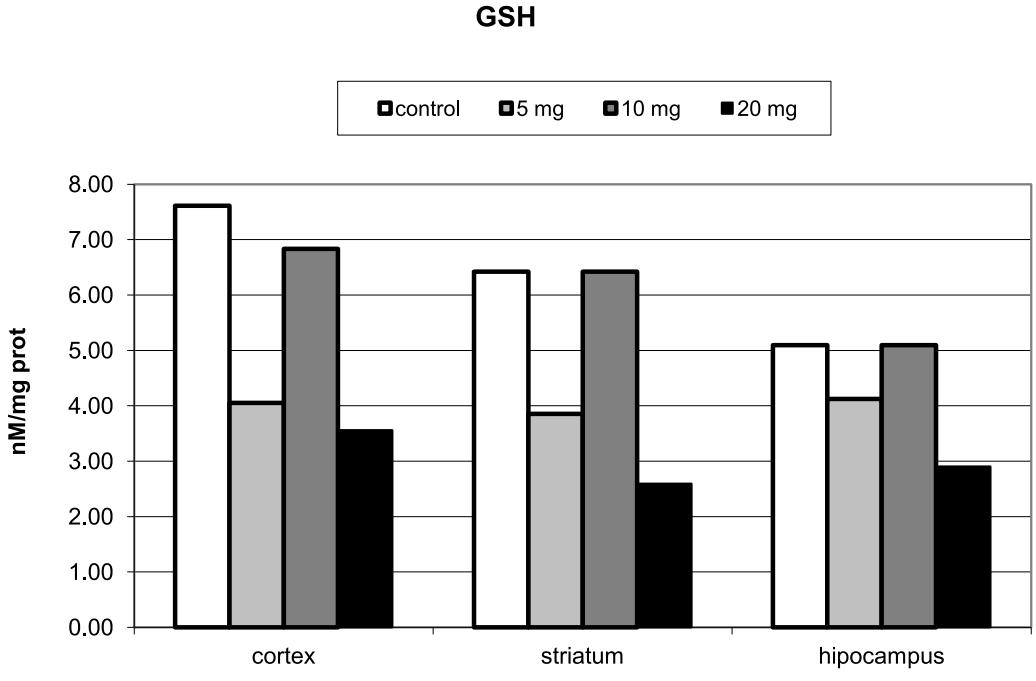

Fig. 4. Effects of methylendioxymetamphetamine on levels on GSH in forebrain cortex, hippocampus and striatum

DISCUSSION

Although the exact mechanism of MDMA-induced neurotoxicity is unknown, there is increasing evidence in support of the hypothesis that oxidative stress due to the formation of free radicals may be involved in MDMA-induced damage to 5-HT terminals. The findings that free-radical scavengers and antioxidants attenuate the MDMA-induced depletion of 5-HT (Gudelsky, 1996) provide indirect evidence of the involvement of free radicals in the mechanism of MDMA neurotoxicity.

Several theories have been proposed to explain the toxicity of MDMA. Colado et al. (1997) have postulated that free radicals produced by MDMA are formed within the 5-HT terminal and are generated by the oxidative quinone metabolites of MDMA itself. One possibility is that toxic 5-HT metabolite may be transported into the 5-HT axon and produce oxidative stress and terminal degeneration (Berger et al., 1992).

Excessive extracellular dopamine may give rise to the formation of reactive oxygen species or ROS (Cadet et al., 1998), and the MDMA-induced increase in hydroxyl radical formation may simply be a consequence of the increase in the extracellular concentration of dopamine. Huang et al. (1997) have reported that the administration of amphetamine alone does not increase hydroxyl radical formation. These results suggest that the generation of hydroxyl radicals is not simply the result of an increase in the extracellular concentration of dopamine. Nevertheless, there is recent for a role of dopamine in the MDMA-induced generation of hydroxyl radicals, as well as the long-term depletion of striatal 5-HT (Shankaran et al., 1999). Sprague et al. (1998) have speculated that dopamine released by MDMA may enter the 5-HT terminal through an activated 5-HT transporter and be oxidized by monoamine oxidase-B enzyme present within the 5-HT terminal, leading to the generation of free radicals.

It was shown that MDMA administration decreased the cytochrome oxidase complex IV of the electron transport chain in dopamine-rich areas (Burrows et al., 2000) It may be that the MDMA-induced release of dopamine compromised mitochondrial function because of auto-oxidation of dopamine metabolites to form quinones and reactive oxygen species. Quinones and ROS have been shown to inhibit mitochondrial enzymes (Ben-Schachar et al., 1995) and lead to the disruption of mitochondrial function and to an increase in intracellular calcium levels (Khodrow et al., 1999) and activation of both nNOS and eNOS (Stuehr et al., 1992).

The role of cell stress- and apoptosis-associated pathways in amphetamine neurotoxicity was also investigated (Stumm et al., 1999). Expression of the immediate early transcription factor, c-jun, and the translation initiation inhibitor, p97, are restricted to the non-methylated DA and MDA analogs and thereby to high neurotoxic potential.

Our data suggest that free radicals could be involved in MDMA-induced damage in rats. Over the 15 days following MDMA treatment, there were significant differences between the vehicle and drug-treated groups

The former (multiple-dose) protocol more reliably produces neurotoxicity. In monkeys that self-administer MDMA over several months, for a total drug exposure similar to a corresponding acute dosing protocol, no signs of neurotoxicity were observed (Fantegrossi et al., 2004). The data suggest that a critical threshold concentration of neurotoxic metabolites must be reached to produce a permanent neurotoxic response, with such a threshold only achievable either at very high doses or after repeated dosing regimens over a relatively short period of time.

It is not possible to exclude completely the possibility that pharmacokinetic tolerance contributes to the diminished functional responses to an acute injection (Simic et al., 2008) of MDMA following repeated administration of the drug. Three underlying mechanisms for chronic tolerance to psychoactive drugs are traditionally described as hepatic/metabolic, neurochemical and behavioral (Leonard,

1997; Julien, 1998). Following its administration, MDMA forms an intermediate metabolite complex with CYP2D6, leading to the almost complete loss of the enzyme for several days, then gradual recovery over the subsequent 2-3 weeks (Heydari et al., 2002). In terms of enzymatic induction, a literature review uncovered no repeated dose investigations. Also of potential concern are the 5-9% of Caucasians deficient in CYP2D6, and it has been suggested that they could be particularly susceptible to the adverse effects of MDMA (Tucker et al., 1994). Aguirre et al. (1995) found that an intensive repeated dose regimen (30 mg i.p. twice daily over 4 successive days), led to an increase in 5-HT1A receptor density in the frontal cortex, together with a parallel decrease in 5-HT1A receptor density in the dorsal raphe region. The frontal cortex post-synaptic receptor density changes were interpreted as possibly indicating 'adaptive changes to compensate for the loss of serotonin nerve terminals' (also in the hippocampus) (Aguirre et al., 1995). Because MDMA acutely stimulates 5-HT1A autoreceptors in the raphe region, equivalent neuroadaptive mechanisms were proposed to explain the decrease in somatodendritic autoreceptors after repeated dosing. Reneman et al. (2002) demonstrated a time-dependent increase in cortical 5-HT2A receptor density, which was strongly correlated with the extent of MDMA-induced serotonin loss; again, this was interpreted as compensatory upregulation.

In summary, the repeated administration of MDMA resulted in a decrease in the concentration of GSH and an increase in the generation of SOD, $O_2^{\cdot-}$ and ILP in the brain. These data support the conclusion that MDMA-induced neurotoxicity involves the induction of oxidative stress resulting from an increased generation of free radicals and a decreased antioxidant capacity of the brain. More studies are needed in order to further identify other key physiological and molecular events that are involved in the neurotoxic effects of the drug.

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