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The effects of single dose of methamphetamine on lipid peroxidation levels in the rat striatum and prefrontal cortex

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Abstract

The administration of methamphetamine to experimental animals results in damage to dopaminergic neurons. In the present study, we demonstrated that a single dose (15 mg/kg) of methamphetamine results in production of oxidative stress as demonstrated by increased thiobarbituric acid reactive substances levels in the rat striatum and prefrontal cortex. In conclusion, the results of present study provide further evidence in support of the notion that oxidative stress may play an important role in the methamphetamine-induced neurotoxicity.

Keywords: Dopamine; Methamphetamine; Oxidative stress; Lipid peroxidation; Striatum; Prefrontal cortex

1. Introduction

Methamphetamine (mAMPH) is a drug of abuse and is known to damage to dopaminergic neurons in rodents (Wagner et al., 1979) and nonhuman primates (Seiden et al., 1975). mAMPH causes the release of dopamine (DA), simultaneously prevents the degradation of DA by inhibiting monoamine oxidase, and inhibits DA uptake. In dopaminergic neurons, mAMPH interacts with synaptic vesicles and increases cytoplasmic DA levels (Cubells et al., 1994). The actions of mAMPH provide an environment which predisposes the autoxidation of DA. DA reacts with molecular oxygen to form quinones and semiquinones; reactive oxygen species (ROS), namely superoxide and hydroxyl free radicals and hydrogen peroxide, are also generated in the process (Graham, 1978).

Although the precise mechanisms of mAMPH-induced neurotoxicity are unknown, the previous findings suggest that oxidative stress might play an essential role in these changes (Cadet et al., 1994; Cubells et al., 1994; Giovanni et al., 1995; Hom et al., 1997). In our previous study, we have demonstrated that both the acute repeated and the chronic administration of mAMPH causes an increase in thiobarbituric acid reactive substances (TBARS) levels, an indicator of lipid peroxidation, in the rat striatum (Açikgöz et al., 1998). Moreover it has been demonstrated that the acute repeated administration of mAMPH causes lipid peroxidation, in striatum and frontal cortex (Jayanthi et al., 1998). However whether or not the single dose of mAMPH causes lipid peroxidation has not been known. The present study was carried out to determine the levels of TBARS following single dose of mAMPH in the striatum and prefrontal cortex. Additionally we examined antioxidant enzymes activities, superoxide dismutase (SOD) and glutathione peroxidase (GPx), to assess the effects of ROS.

2. Experimental procedures

Male Wistar rats, weighing 190–230 g, were used. Rats were treated with i.p. mAMPH (5, 10 or 15 mg/kg) or saline. Thirty minutes after the injection, rats were killed by cervical dislocation under ether anesthesia. Striatum and prefrontal cortex tissues were separated on an ice-cold

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Table 1
The effects of single dose of mAMPH on activities of SOD and GPx, and TBARS levels in the rat striatum[a]

Treatment	SOD (U/mg protein)	GPx (U/mg protein)	TBARS (nmol/mg protein)
Saline	2.15±0.32	0.018±0.01	0.073±0.02
5 mg/kg mAMPH	2.62±0.34	0.028±0.01	0.051±0.02
10 mg/kg mAMPH	2.42±0.47	0.015±0.01	0.090±0.04
15 mg/kg mAMPH	2.53±0.62	0.014±0.01	0.186±0.05*

[a] The values represent mean±S.E.M. of five animals per group.
* $P<0.05$, in comparison with all groups (by Tukey test).

surface. Tissue homogenates were prepared as described by Carrillo et al. (1991). An aliquot of the homogenate and supernatant was stored at −70°C until the determination of TBARS levels, SOD and GPx enzyme activities.

SOD and GPx activities were determined on supernatant using RANSOD and RANSEL kits (Randox labs., Crumlin, UK). Results were expressed as U/mg protein. TBARS levels were measured on homogenate according to the method of Rehncrona et al. (1980) and expressed as nmol/mg protein. Protein contents of supernatant and homogenate were determined as described by Markwell et al. (1978).

Results are presented as means±S.E.M. Statistical analysis of the data was performed by one-way analysis of variance followed by the Tukey test.

3. Results

The single dose of 15 mg/kg mAMPH caused a marked increase in TBARS levels ($P<0.05$) in the striatum. Administration of a lower dose (5 and 10 mg/kg) did not change TBARS levels. In this experiment, SOD and GPx activities were not affected by the single-dose of mAMPH (Table 1).

The single dose of 15 mg/kg mAMPH caused a marked increase in TBARS levels ($P<0.05$) in the prefrontal cortex. Administration of a lower dose (5 and 10 mg/kg) did not change TBARS levels. In this experiment, 10 mg/kg mAMPH caused an increase in SOD activity (Table 2).

4. Discussion

In the present study, we have demonstrated that single dose of mAMPH (15 mg/kg) causes an increase in TBARS levels, an indicator of lipid peroxidation in the striatum and prefrontal cortex. To our knowledge, this is the first report indicating that single dose of mAMPH causes lipid peroxidation in the rat brain. Our results confirm previous findings which show that mAMPH-induced dopaminergic neurotoxicity may be secondary to increased production of ROS. The resistance of SOD overexpressing transgenic mice to the neurotoxic effects of mAMPH suggest the involvement of superoxide radical in the resulting neurotoxicity of mAMPH (Cadet et al., 1994). Elevated expression of GPx which can reduce hydrogen peroxide, in PC12 cell line results in protection against mAMPH-induced neurotoxicity (Hom et al., 1997). It has been demonstrated that neurotoxic doses of mAMPH increase the hydroxyl radical concentration in striatum (Giovanni et al., 1995). In vitro data in DA neuronal cultures show that mAMPH accelerates the generation of ROS through DA oxidation (Cubells et al., 1994).

In the present study, we have shown that 15 mg/kg mAMPH caused an increase in TBARS levels in striatum and prefrontal cortex. However 5 and 10 mg/kg mAMPH did not cause an increase in TBARS levels. Amphetamine induced DA release is dose dependent. Increasing the dose of amphetamine causes a graded increase in maximal DA efflux (Butcher et al., 1988). During exposure to toxic doses of mAMPH, there is a large increase in the extracellular levels of DA, which is critical to the development of

Table 2
The effects of single dose of mAMPH on activities of SOD and GPx, and TBARS levels in the rat prefrontal cortex[a]

Treatment	SOD (U/mg protein)	GPx (U/mg protein)	TBARS (nmol/mg protein)
Saline	1.08±0.12	0.014±0.01	0.111±0.02
5 mg/kg mAMPH	1.40±0.22	0.014±0.01	0.061±0.03
10 mg/kg mAMPH	1.53±0.15**	0.015±0.01	0.080±0.02
15 mg/kg mAMPH	1.32±0.26	0.011±0.01	0.484±0.05*

[a] The values represent mean±S.E.M. of five animals per group.
* $P<0.05$, in comparison with all groups (by Tukey test).
** $P<0.05$, in comparison with saline-treated rats (by Tukey test).

mAMPH-induced dopaminergic neurotoxicity (O'Dell et al., 1991). High cytoplasmic or extracellular concentrations of DA can be toxic to the dopaminergic neuron because of the increased possibility that ROS are generated inside the cell or taken up into the nerve terminal. Therefore, increasing the dose of mAMPH may cause an increase formation of ROS, which leads to lipid peroxidation. Our results are consistent with previous findings which show that there is a significant correlation between the magnitude of mAMPH induced DA overflow and subsequent dopaminergic neurotoxicity (O'Dell et al., 1991).

DA levels in the striatum are much higher than in the frontal cortex. It may be expected that the increase of TBARS levels in the striatum after mAMPH administration was much higher than in the prefrontal cortex. However, in the present study, we have determined that the rise in TBARS levels in the striatum was lower than in the prefrontal cortex. It is important to note that striatum is particularly enriched in enzymes regulating the metabolism of ROS (Mizuno and Ohta, 1986). Our results appear to be consistent with these observations. Moreover, in saline-treated control groups, TBARS levels were higher in the prefrontal cortex compared with in the striatum. It has been demonstrated that administration of haloperidol results in lipid peroxidation, leading to increased production of hydrogen peroxide following metabolism of DA. Haloperidol-induced increase in TBARS levels in the cortex is much higher than in the striatum (Shivakumar and Ravindranath, 1992). Our results, much stronger increase in TBARS levels after mAMPH administration, in the prefrontal cortex, consistent with these observations.

The administration of mAMPH causes an increase in the extracellular levels of DA and glutamate, both of which are critical to the development of mAMPH-induced neurotoxicity (Stephans and Yamamoto, 1994). mAMPH triggers a series of events which could explain the role of ROS in the mAMPH-induced neurotoxicity. mAMPH causes high levels of extracellular DA, releasing DA from a cytosolic pool, simultaneously preventing the degradation of DA by inhibiting monoamine oxidase, and inhibiting DA uptake. DA can produce ROS. ROS lead to high levels of extracellular glutamate, inhibiting glutamate uptake (Berman and Hastings, 1997) and releasing glutamate (Gilman et al., 1993). Glutamate can cause an increase the release of DA (Clow and Jhamandas, 1989) and an additional ROS formation, interacting with specific receptors in dopaminergic terminals (Reynolds and Hastings, 1995). ROS cause lipid peroxidation, an indicator of neuronal damage.

The present study demonstrates that single dose of mAMPH (15 mg/kg) results in production of oxidative stress as demonstrated by increased TBARS levels in striatum and prefrontal cortex. In conclusion, the results of present study provide further evidence in support of the notion that oxidative stress may play an important role in the mAMPH-induced neurotoxicity.

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