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Research Report

Evidence for a role of energy dysregulation in the MDMA-induced depletion of brain 5-HT

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Abstract

Although the exact mechanism involved in the long-term depletion of brain serotonin (5-HT) produced by substituted amphetamines is not completely known, evidence suggests that oxidative and/or bioenergetic stress may contribute to 3,4-methylenedioxymethamphetamine (MDMA)-induced 5-HT toxicity. In the present study, the effect of supplementing energy substrates was examined on the long-term depletion of striatal 5-HT and dopamine produced by the local perfusion of MDMA (100 μM) and malonate (100 mM) and the depletion of striatal and hippocampal 5-HT concentrations produced by the systemic administration of MDMA (10 mg/kg i.p. ×4). The effect of systemic administration of MDMA on ATP levels in the striatum and hippocampus also was examined. Reverse dialysis of MDMA and malonate directly into the striatum resulted in a 55–70% reduction in striatal concentrations of 5-HT and dopamine, and these reductions were significantly attenuated when MDMA and malonate were co-perfused with nicotinamide (1 mM). Perfusion of nicotinamide or ubiquinone (100 μM) also attenuated the depletion of 5-HT in the striatum and hippocampus produced by the systemic administration of MDMA. Finally, the systemic administration of MDMA produced a 30% decrease in the concentration of ATP in the striatum and hippocampus. These results support the conclusion that MDMA produces a dysregulation of energy metabolism which contributes to the mechanism of MDMA-induced 5-HT neurotoxicity.

Theme: Disorders of the nervous system
Topic: Neurotoxicity

Keywords: MDMA; Nicotinamide; Ubiquinone; Serotonin; Neurotoxicity

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA), a ring substituted amphetamine analog, is widely abused as a recreational drug, and there is concern that the drug may damage 5-HT nerve terminals [19]. MDMA-induced neurotoxicity of 5-HT nerve terminals in rodents and non-human primates is evidenced by several biochemical and immunocytochemical findings such as depletion of tissue concentration of 5-HT and its major metabolite, 5-hydroxyindoleacetic acid [39,48], decrease in the activity of the enzyme tryptophan hydroxylase [40], reduction in the [^{3}H]-paroxetine labeled 5-HT reuptake sites [3] and reduced immunostaining of 5-HT terminals [31].

Although the exact mechanisms responsible for these long-term effects of MDMA on 5-HT axon terminals remain unknown, evidence suggests that oxidative stress and/or bioenergetic stress play an important role in this process. MDMA increases the formation of hydroxy radicals [11,12,42,43], increases lipid peroxidation [46] and reduces the concentration of the endogenous antioxidants vitamin E and ascorbic acid [44]. Furthermore, the administration of antioxidants prevents the MDMA-induced long-term depletion of 5-HT in the brain [10,20,44].

In addition to the role of reactive oxygen species, there is evidence for a role of reactive nitrogen species in the neurotoxicity produced by MDMA and other amphetamine

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analogs. MDMA increases nitric oxide and tissue nitrotyrosine in the rat striatum, and nitric oxide synthase (NOS) inhibitors provide significant neuroprotection against depletion of dopamine and/or 5-HT produced by these stimulants [1,13,15,23,24,49,56].

In addition to the potential role of oxidative stress in MDMA neurotoxicity, there is evidence for a role of bioenergetic stress in the toxicity produced by amphetamine analogs. Although the central administration of MDMA does not produce 5-HT toxicity [15,16,30,32], the concomitant administration into the striatum of the mitochondrial inhibitor malonate, which alone does not produce 5-HT depletion, and MDMA produces a significant reduction of tissue dopamine and 5-HT concentrations [15,30]. Moreover, the administration of MDMA or methamphetamine results in an inhibition of mitochondrial function [6], and amphetamine and methamphetamine have been shown to deplete ATP concentrations in the striatum [8,53]. Finally, administration of substrates of energy metabolism, such as nicotinamide and ubiquinone, has been shown to attenuate the neurotoxic effects of amphetamine and methamphetamine [47,53].

The systemic administration of amphetamine and its analogs, e.g., parachloroamphetamine, methamphetamine and MDMA, also has been shown to deplete brain glycogen [14,21,22]. In addition, it has been demonstrated that MDMA activates glycogen phosphorylase, an enzyme responsible for the breakdown of glycogen, in astroglial rich primary cultures [35].

Both nicotinamide and ubiquinone have been utilized previously to augment energy metabolism by enhancing mitochondrial oxidative phosphorylation. Nicotinamide, which is a precursor for the electron carrier molecule nicotinamide adenine dinucleotide, and ubiquinone, which is a component of the electron transport chain, enhance ATP production and have been shown to attenuate striatal lesions produced by the mitochondrial inhibitor malonate [4]. In the present study, the neuroprotective effects of nicotinamide and ubiquinone on the long-term 5-HT depletion produced by the systemic administration of MDMA or the concomitant intrastriatal administration of malonate and MDMA are documented. In addition, a depletion of ATP concentrations in the rat striatum and hippocampus following MDMA treatment is demonstrated.

2. Materials and methods

2.1. Animals

Adult male rats (200–275 gm) of the Sprague–Dawley strain (Charles River Laboratories, Portage, MI) were used in these studies. The animals were housed three per cage in a temperature (23 ± 1 °C) and humidity controlled room with a 12/12-h light/dark cycle and allowed food and water ad libitum. Animals undergoing surgery were housed one per cage, post-operatively. All procedures were in strict adherence to the National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee.

2.2. Drugs and drug treatments

(±)-3,4-Methylenedioxymethamphetamine hydrochloride (MDMA) was provided by the National Institute on Drug Abuse. Malonic acid disodium (malonate) was obtained from Acros (Morris Plains, NJ). Nicotinamide and decylubiquinone (ubiquinone) were obtained from Sigma Chemical Co. (St. Louis, MO). All the drugs administered by reverse dialysis into the striatum were dissolved in modified Dulbecco's phosphate-buffered saline containing 1.2 mM $CaCl_2$ and 5 mM glucose. MDMA was dissolved in 0.15 M NaCl for systemic administration. MDMA (100 μM) and malonate (100 mM) were perfused locally into the striatum through the microdialysis probe for 8 h, similar to the procedures described previously [15,30]. Nicotinamide (1 mM) was perfused into the striatum through the microdialysis probe beginning 2 h prior to and ending 6 h after the termination of the MDMA and malonate perfusion. In other experiments, rats received MDMA (10 mg/kg, i.p.) at 2 h intervals for a total of 4 injections. In these rats, nicotinamide (1 mM) or ubiquinone (100 μM) was perfused into the striatum beginning 2 h before the first MDMA or vehicle injection and continuing until 6 h after the last drug injection. The dosing regimen for nicotinamide and ubiquinone is similar to that described previously [47]. All drugs were administered at an ambient temperature of 23 ± 1 °C.

2.3. In vivo microdialysis

Rats were implanted with a stainless steel guide cannula under ketamine/xylazine (70/6 mg/kg, i.m.) induced anesthesia, 48–72 h prior to the insertion of the microdialysis probe. On the day of the dialysis experiment, a concentric style dialysis probe was inserted through the guide cannula into the striatum or hippocampus. The coordinates for the tip of the probe were A, 1.2 mm; L, 3.1 mm; V, −7.5 mm from bregma for the striatum and A, −5.5; L, 4.8; V, −7.0 for the hippocampus, according to the stereotaxic atlas [34]. The microdialysis probes were constructed as described previously [54]. The dialysis surface of the membrane (Spectra Por, 6000 MW cut-off, 210 μm outside diameter) for the striatum and hippocampus was 4.5 mm in length. The microdialysis probe was connected to an infusion pump set to deliver modified Dulbecco's phosphate buffered saline containing 1.2 mM $CaCl_2$ and 5 mM glucose at a constant flow rate of 1.8 μl/min. Drug perfusion was started after a 2-h equilibration period.

2.4. Biochemical measurements

2.4.1. Assay of tissue 5-HT and dopamine

Rats were killed by decapitation 5 days after drug treatment, and the brains were removed and frozen with

dry ice. For rats that had received drug only by reverse dialysis, a 400 μm section containing the probe tract was taken, and tissue directly adjacent to the probe tract was excised and stored at −80 °C until further analysis. The dimensions of the tissue excised were 4.5 mm (length) × 1.0 mm (width) × 0.4 mm (depth). In other experiments in which rats were perfused with nicotinamide or ubiquinone and injected systemically with MDMA or vehicle, tissue surrounding the probe tract was dissected together with a similarly sized sample of striatum from the contralateral side. This tissue from the contralateral side was exposed only to MDMA or vehicle and not nicotinamide or ubiquinone. It was determined in a separate experiment (n = 8) that physical trauma due to probe insertion per se did not alter the striatal concentration of 5-HT (probed tissue: 4.9 ± 0.4 ng/mg protein, unprobed tissue (contralateral side): 5.1 ± 0.6 ng/mg protein, P = 0.82) or dopamine (probed tissue: 121.6 ± 6.3 ng/mg protein, unprobed tissue (contralateral side): 125.0 ± 4.6 ng/mg protein, P = 0.68).

All tissue samples were homogenized in ice-cold 0.2 N perchloric acid and homogenates were centrifuged at 14,000 rpm for 5 min. Concentrations of dopamine and 5-HT were determined in 20 μl aliquots of the supernatants that were injected onto a C-18 reverse phase column connected to a LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN) equipped with a glassy carbon target electrode. The mobile phase for the separation of dopamine and 5-HT consisted of: 35 mM citric acid, 54 mM sodium acetate, 50 mg/l disodium ethylenediamine tetraacetate, 50 mg/l octane sulfonic acid sodium salt, 3% methanol, 3% acetonitrile, pH 4.1, pumped at a flow rate of 0.3 ml/min. Tissue dopamine and 5-HT concentrations are reported as ng/mg protein. Tissue protein content was determined using a Lowry protein assay.

2.4.2. Assay of tissue ATP

Rats were killed by decapitation and the heads were allowed to drop directly into liquid nitrogen in which they were maintained for 5 min. Decapitated heads were then stored at −80 °C until analysis. This procedure has been used by several investigators for the analysis of brain ATP [5,18,52]. A piece of striatum or ventral hippocampus (approximately 25 mg) was excised from the frozen brains and homogenized in 3 volumes of ice-cold 3 M perchloric acid. Ten volumes of ice-cold water were added to the tube and the contents were mixed. The homogenate was centrifuged at 10,000 × g for 15 min at 4 °C. The supernatant was neutralized with 2 M potassium carbonate and centrifuged at 10,000 × g for 15 min at 4 °C. ATP in the neutralized perchloric acid extracts was measured using the ATP bioluminescent kit obtained from Sigma-Aldrich. Aliquots (100 μl) of tissue extract were placed in the well of the microplate followed by the addition of 100 μl of the ATP mix containing firefly luciferin and luciferase. The luminescence signal was immediately measured in a luminometer (Polarstar Optima, BMG Labtechnologies, Chicago, IL). Tissue ATP values are reported as nmol/mg tissue.

2.5. Statistical analysis

The effects of drugs on tissue dopamine and 5-HT concentrations in all experiments were analyzed with a two-way ANOVA. The effect of MDMA on ATP concentrations was analyzed with a one-way ANOVA. Multiple pair wise comparisons were performed using the Student–Newman–Keuls test. Treatment differences for all the data were considered statistically significant at $P < 0.05$.

3. Results

The local perfusion of MDMA (100 μM) and malonate (100 mM) for 8 h into the striatum produced a significant ($P < 0.05$) 53% reduction of 5-HT and 69% reduction of dopamine concentrations. Perfusion of MDMA alone did not significantly alter the striatal concentration of dopamine (P = 0.92) or 5-HT (P = 0.78). Reverse dialysis of malonate alone into the striatum did not significantly alter 5-HT concentration (P = 0.93); however, it produced a significant 24% (P = 0.005) reduction of dopamine concentrations (Fig. 1). In rats perfused with nicotinamide (1 mM) together with MDMA and malonate, striatal 5-HT concentrations were not significantly depleted when compared to 5-HT values in vehicle-treated rats [$F(3,19) = 3.02$, P = 0.1] (Fig. 1A). Nicotinamide treatment also attenuated the depletion of striatal dopamine elicited by MDMA and malonate. Although striatal dopamine concentrations in MDMA and malonate dosed animals given nicotinamide were still depleted [$F(3,19) = 20.593$, P = 0.030], the magnitude of the reduction was significantly less ($P < 0.001$) than that produced in rats perfused with MDMA and malonate alone (Fig. 1A). Perfusion of nicotinamide alone did not alter the striatal concentration of 5-HT (P = 0.973) or dopamine (P = 0.32).

The effect of perfusion with nicotinamide (1 mM) or ubiquinone (100 μM) on the depletion of 5-HT in the striatum produced by the systemic administration of MDMA is depicted in Fig. 2. In rats treated systematically with MDMA (10 mg/kg, i.p. ×4), the striatal concentration of 5-HT was reduced 48% ($P < 0.05$) when compared to the vehicle treated group. In MDMA-treated rats, reverse dialysis with nicotinamide resulted in concentrations of 5-HT adjacent to the probe that were significantly [$F(1,21) = 2.86$, P = 0.006] greater than those in the contralateral side that were exposed only to MDMA (Fig. 2A). The tissue concentration of 5-HT in striatal tissue exposed to nicotinamide by reverse dialysis did not differ significantly [$F(1,21) = 6.77$, P = 0.53] from that in the untreated, contralateral side (Fig. 2A).

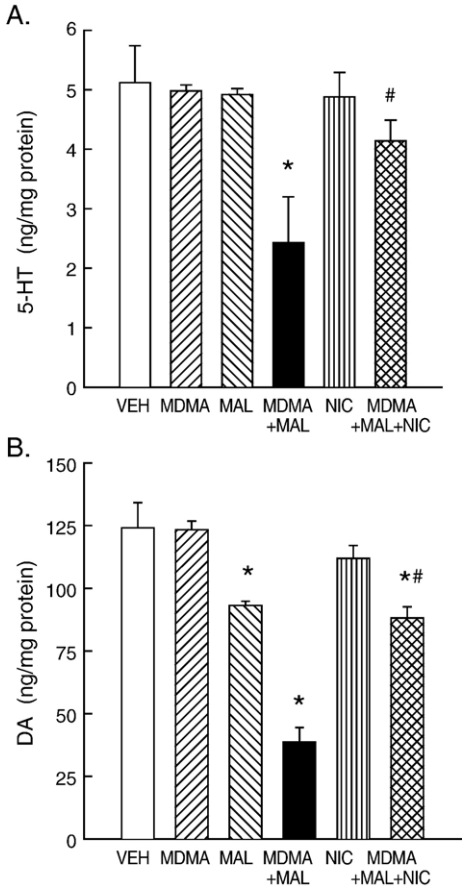

Fig. 1. Effect of nicotinamide (NIC) on MDMA + malonate (MAL)-induced 5-HT (A) and dopamine (B) depletion in the striatum. Vehicle (VEH) (n = 5), MDMA (100 μM, n = 6), MAL (100 mM, n = 5) or MDMA + MAL (n = 5) was perfused locally into the striatum for 8 h; control rats received only the dialysis buffer (VEH) (n = 5). Rats were perfused with NIC beginning 2 h prior to and ending 6 h after the perfusion of MDMA + MAL (n = 10). Control nicotinamide rats (n = 5) received only nicotinamide perfusion for 16 h. Rats were killed 5 days after drug perfusion. *P < 0.05 compared to the value for VEH-treated rats. $^{\#}P$ < 0.05 compared to the value for rats perfused with MDMA + MAL.

In a separate experiment, similar neuroprotection was obtained with ubiquinone (Fig. 2B). Whereas MDMA produced a significant 55% reduction of striatal 5-HT concentrations in control rats, no significant reduction in 5-HT concentrations was produced in MDMA-treated rats perfused with ubiquinone. Direct comparison of the striatal concentration of 5-HT in rats treated with MDMA or ubiquinone and MDMA revealed that tissue concentrations of 5-HT adjacent to the probe in ubiquinone-treated rats were significantly greater [$F(1,26) = 14.75$, $P < 0.001$] than those in tissue on the contralateral side exposed only to MDMA. Perfusion with ubiquinone alone did not alter the striatal concentration of 5-HT.

The ability of nicotinamide to prevent the MDMA-induced reduction of 5-HT in the hippocampus was also examined. Although nicotinamide alone did not have any effect on tissue 5-HT concentrations, it significantly attenuated the long-term depletion of hippocampal 5-HT produced by MDMA (Fig. 3). The concentration of 5-HT in hippocampal tissue of MDMA-treated rats adjacent to the probe through which nicotinamide was delivered was significantly [$F(1,26) = 8.59$, $P = 0.002$] greater than that in tissue on the contralateral side exposed only to MDMA.

Tissue concentrations of ATP in the striatum were significantly ($P < 0.001$) reduced by 30% and 26% at 12 h and 24 h, respectively, following the last injection of MDMA (10 mg/kg, i.p. ×4). No significant depletion (P = 0.18) of ATP was detected in animals killed 1 h following the last MDMA injection. The tissue concentration of ATP in the hippocampus also was significantly ($P < 0.001$)

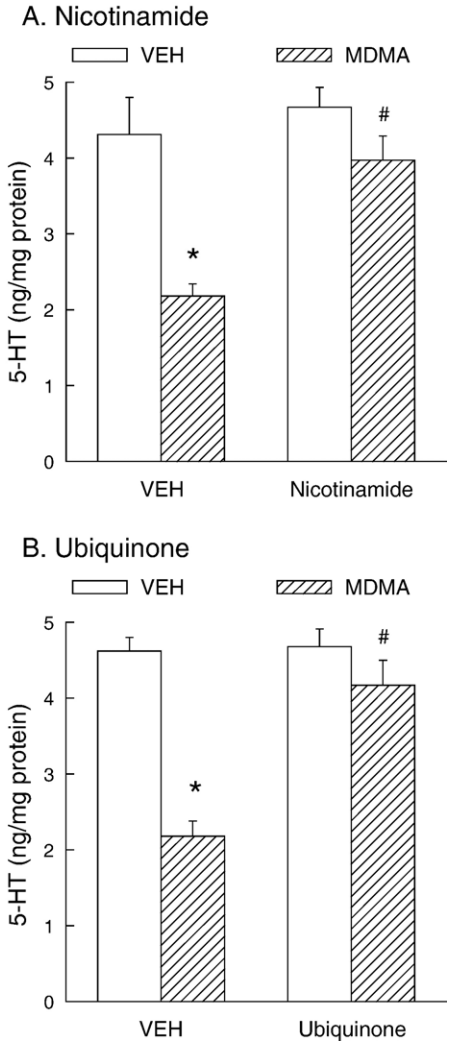

Fig. 2. Effect of energy substrates nicotinamide (A) and ubiquinone (B) on MDMA-induced long-term 5-HT depletion in the striatum. Nicotinamide (1 mM) or ubiquinone (100 μM) was perfused into the striatum, beginning 2 h prior to the first vehicle (VEH) (n = 6) or MDMA (10 mg/kg, i.p. every 2 h for a total of 4 injections) (n = 8) injection and ending 6 h after the fourth VEH or MDMA injection. Rats were killed 5 days after drug treatment. *P < 0.05 compared to the value for VEH-treated rats. $^{\#}P$ < 0.05 compared to the value for the vehicle + MDMA-treated rats.

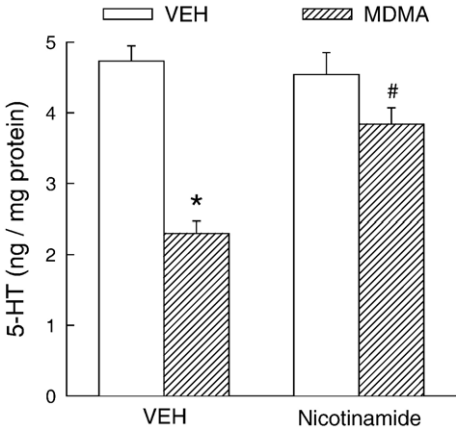

Fig. 3. Effect of nicotinamide on MDMA-induced long-term 5-HT depletion in the hippocampus. Nicotinamide (1 mM) was perfused into the hippocampus beginning 2 h before the first vehicle (VEH) ($n = 6$) or MDMA (10 mg/kg, i.p. every 2 h for a total of 4 injections) ($n = 8$) injection and ending 6 h after the fourth VEH or MDMA injection. Rats were killed 5 days after drug treatment. *$P < 0.05$ compared to the value for VEH-treated rats. $^{\#}P < 0.05$ compared to the value for the vehicle + MDMA-treated rats.

reduced by 33% 12 h following MDMA administration (Fig. 4).

4. Discussion

The results of the present study include the key findings that: (1) energy substrates attenuate MDMA-induced 5-HT depletion and the depletion of dopamine and 5-HT produced by the intrastriatal administration of malonate and MDMA, and (2) MDMA reduces striatal and hippocampal concentrations of ATP.

Previous studies have documented that the central administration of MDMA does not result in the long-term depletion of brain 5-HT [15,16,30,32]. However, the present results indicate that the central administration of MDMA can elicit 5-HT, as well as dopamine, toxicity in the presence of impaired mitochondrial function. The failure of the central administration of MDMA to elicit 5-HT toxicity has led to the proposition that MDMA must be converted into a neurotoxic metabolite [26,28,29]. Monks and colleagues have identified glutathione and *N*-acetylcysteine conjugates of α-methyl dopamine in the brain following MDMA administration, and these metabolites have been shown to generate free radicals [25] and produce 5-HT depletion [2,29]. Thus, it is conceivable that the glutathione and *N*-acetylcysteine metabolites of MDMA, rather than MDMA itself, impair mitochondrial function and promote long-term 5-HT depletion. However, although dopaminergic neurons have been shown to be vulnerable to the toxic effects of mitochondrial inhibition [7,55], the central administration of the neurotoxic metabolites of MDMA produce only a modest reduction of brain dopamine concentrations [26].

Alternatively, the lack of hyperthermia associated with the central administration of MDMA [30] may account for the lack of toxicity observed following direct, intracerebral administration of MDMA. Hyperthermia has been shown to contribute to MDMA-induced 5-HT depletion, although the exact role of hyperthermia in the process is unknown. However, exposure of brain tissue to high temperatures (i.e., hyperthermia) in vitro has been shown to reduce tissue ATP concentrations [27]. It can be envisioned that hyperthermia contributes to MDMA-induced bioenergetic stress (i.e., ATP depletion) that results in subsequent membrane ionic dysregulation, Ca^{2+} entry and additional free radical formation.

Administration of substrates of energy metabolism, such as nicotinamide and ubiquinone, has been shown to increase tissue ATP concentrations and attenuate the ATP depletion and the striatal lesions produced by the mitochondrial toxin malonate [4]. Nicotinamide is the precursor molecule for the electron carrier molecule NAD. Supplementation with nicotinamide increases the level of NADH available for oxidative phosphorylation, which then augments ATP production. Ubiquinone is an essential component of the electron transport chain, where it serves as an electron donor and acceptor and facilitates the movement of electrons along the chain, thereby enhancing ATP production.

In the present study, perfusion with nicotinamide or ubiquinone attenuated the long-term 5-HT depletion produced by the systemic administration of MDMA, as well as the depletion of striatal 5-HT and dopamine produced by the central administration of malonate and MDMA. The neuroprotection provided by administration of energy substrates against MDMA-induced neurotoxicity is consistent with the effect of nicotinamide and ubiquinone on the neurotoxicity produced by other amphetamine analogs. Perfusion of nicotinamide or ubiquinone has been reported to attenuate the long-term dopamine depletion produced by metham-

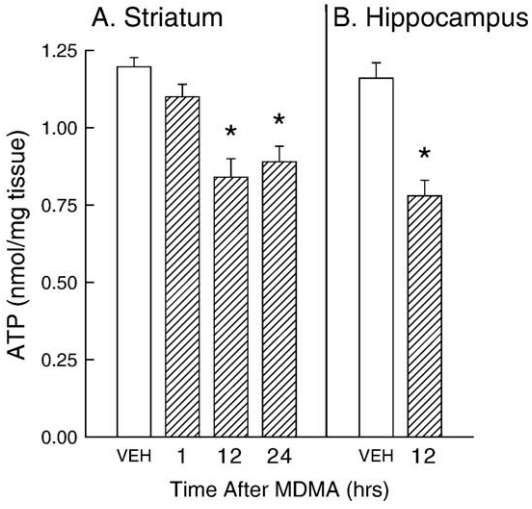

Fig. 4. Effect of MDMA on tissue ATP levels in the striatum and hippocampus. Rats received vehicle (VEH) ($n = 9$) or MDMA (10 mg/kg, i.p.) every 2 h for a total of 4 injections. Rats were killed either 1 h ($n = 6$), 12 h ($n = 7$) or 24 h ($n = 9$) after the last injection of MDMA. *$P < 0.05$ compared to the value for VEH-treated rats.

phetamine administration [47]. The systemic administration of nicotinamide has also been shown to attenuate the d-amphetamine-induced depletion of dopamine in the rat striatum [53]. The protection afforded by energy substrates against monoamine depletions produced by various amphetamines is supportive of the view that the neurotoxicity produced by these stimulants is associated with energy impairment. However, ubiquinone also exerts antioxidant properties [41] and malonate, as well as MDMA, has been shown to increase free radical production [17,33,42]. Hence, the possibility cannot be excluded that the neuroprotective effect of ubiquinone is due, in part, to scavenging of free radicals.

There is additional evidence consistent with the hypothesis that amphetamine analogs disrupt energy regulation. The administration of MDMA or methamphetamine produces a transient inhibition of mitochondrial function [6], and it has been reported that MDMA treatment reduces the activities of mitochondrial complexes II–III and I–III in the striatum [45]. In the present study, systemic administration of MDMA produced significant ATP depletion in the striatum and hippocampus. This is consistent with previous reports that have demonstrated a loss of striatal ATP following the administration of amphetamine [53] and methamphetamine [8]. Furthermore, the systemic administration of amphetamine and its derivatives parachloroamphetamine, methamphetamine and MDMA has been shown to deplete brain glycogen [14,21,22], which is the largest energy reserve in the brain [50].

Several mechanisms have been proposed which could account for a compromise in metabolic function following administration of amphetamine derivatives. Psychostimulants may increase neuronal energy utilization through sustained activation of monoamine transporters, hyperlocomotion and the production of hyperthermia [21]. Dysregulation of energy metabolism and energy depletion have been proposed to lead to an increase in intracellular Ca^{2+} in the mitochondria [21,38]. Binding of Ca^{2+} ions to calmodulin activates neuronal NOS and leads to a subsequent increased production of nitric oxide. The reaction of nitric oxide with superoxide forms the highly reactive and toxic peroxynitrite ion, which is capable of oxidizing lipid membranes and sulfhydryl moieties, as well as hydroxylating and nitrating aromatics [36,37,51]. Evidence indicates that activation of the NOS pathway and the subsequent release of nitric oxide occurs following MDMA administration [15]. Administration of NOS inhibitors has been found to provide neuroprotection against methamphetamine and MDMA-induced neurotoxicity [1,15,56]. Nitric oxide is a known inhibitor of mitochondrial complexes such as cytochrome oxidase [9] and nitric oxide-induced inhibition of mitochondrial complex activity may account for the MDMA-induced reduction in tissue ATP concentrations.

In conclusion, substrates of energy metabolism suppressed the depletion of 5-HT and dopamine produced by the central administration of MDMA and the mitochondrial inhibitor malonate, and the selective depletion of 5-HT following the systemic administration of MDMA. The systemic administration of MDMA also resulted in a significant depletion of striatal and hippocampal ATP. The present data support the hypothesis that MDMA produces a dysregulation of energy metabolism which contributes to MDMA-induced neurotoxicity.

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