A ^{31}P NMR Study of the Bioenergetic Effects of MDMA (Ecstasy) in Skeletal Muscle

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Introduction

Many users consider the club drug 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) to be harmless, case reports in the medical literature however suggest otherwise. Mortality as a result of ecstasy although unusual, but it occurs (1) and has been attributed to hyperthermia, acute myocardial infarctions and cerebral edema. Along with hyperthermia, one of the most commonly reported complications in human users of MDMA is rhabdomyolysis (2). Since agents that uncouple mitochondrial oxidative phosphorylation are known to cause severe hyperthermia and rhabdomyolysis (3) the present study was designed to biochemically define the effects of MDMA on mitochondrial uncoupling in relation to MDMA-mediated hyperthermia, rhabdomyolysis and ATP levels in skeletal muscle. In this abstract, we report ^{31}P NMR spectroscopic method used to assess mitochondrial energy coupling in skeletal muscle, noninvasively to assess rates of ATP synthesis in control and MDMA treated rats.

Methods

(±) MDMA was generously provided by the National Institute of Drug Abuse (NIDA). Male Sprague Dawley rats (175-300 g) were anesthetized with urethane (1.75-2g/kg sq) and were positioned on top of a water recirculating heating pad inside the NMR probe. An i.p. catheter was placed and connected to an extension tube to administer the drug. Fiber optic temperature probes were placed 1-2 cm deep into the rectum and into the lateral hind leg of the unstudied limb. A surface coil tunable to 106 MHz for ^{23}Na and 161.8 MHz for ^{31}P was placed directly over the lateral hind leg with a small bulb containing 400 mM phenyl phosphonic acid (PPA) on top of the surface coil as a reference. All ^{31}P NMR experiments were performed on a 9.4 T, 89 mm vertical bore magnet interfaced to a Varian INOVA console with following parameters: pw=10μs, np=3072, sw=15 kHz, nt=256 acquisition time= 4 min 44 sec for each spectrum. All NMR data were processed with NMR NUTS Software. FIDs were baseline corrected, zero-filling to 8K data points, multiplied by a single exponential corresponding to 25 Hz line-broadening and Fourier transformed. Calculations of all phosphate peak areas were done with respect to PPA signal area. Changes in PCr and β-ATP and temperatures in the NMR experiments were compared using repeated analysis of variance (ANOVA) with each time point compared back to baseline using a Dunnet's multiple comparison procedure. Muscle and core body temperatures along with PCr, β-ATP and inorganic phosphate (Pi) levels were monitored for 3 hrs 30 min. For MDMA groups (40 mg/kg in 0.2 ml saline), MDMA was injected 30 minutes after baseline acquisition. Intracellular pH was measured based on the chemical shift of P_i relative to PCr.

Results and Discussion

MDMA induced significant rise in both core body (37.7 to 39.4°C) and skeletal muscle temperatures (37.1 to 38.9°C) (**figure 1**). From ^{31}P NMR data, β-ATP signal areas showed significant ($p < 0.05$) reductions from the baseline values with the signal area decreasing 15% from baseline after MDMA treatment. Decreases in β-ATP signal area were accompanied by corresponding increases in inorganic phosphate (88% increase) and decrease in intracellular pH. The PCr signal also showed a small (8%) nonsignificant decrease after MDMA injection. An additional 3 rats as controls were examined under same experimental conditions. There were no significant changes in skeletal muscle and core body temperatures or PCr and ATP signal intensity over time for controls (**figures 2**). These data when viewed in light of the corresponding skeletal muscle hyperthermia strongly suggests impairment of mitochondrial oxidative phosphorylation. Myocytes rely on oxidative phosphorylation and the hydrolysis of high-energy phosphate bonds from phosphocreatine in the formation of ATP. As reported by Curry et al[4], any alterations in the concentration of either PCr or ATP can result in energy requirements exceeding ATP production ultimately leading to muscle cell breakdown and rhabdomyolysis. In summary, we have demonstrated that MDMA appears to interfere with oxidative phosphorylation *in vivo* resulting in reductions in both ATP and PCr with subsequent development of hyperthermia and rhabdomyolysis.

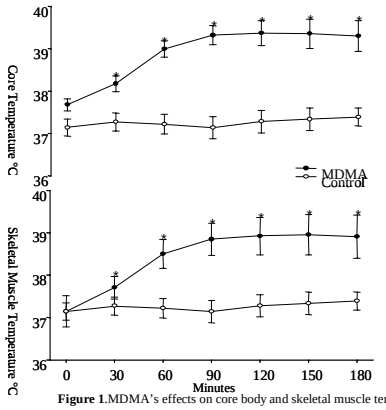

Figure 1.MDMA's effects on core body and skeletal muscle temperatures

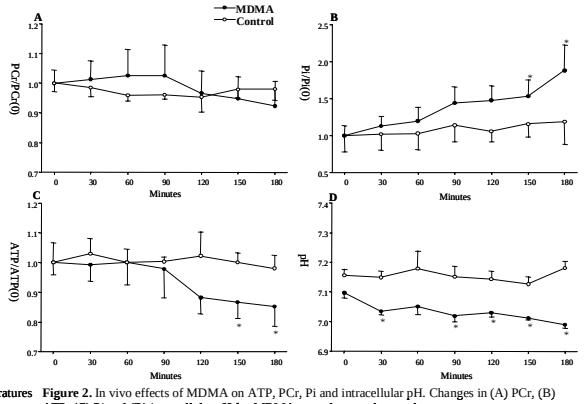

Figure 2. In vivo effects of MDMA on ATP, PCr, Pi and intracellular pH. Changes in (A) PCr, (B) ATP, (C) Pi and (D) intracellular pH for MDMA treated rats and controls.

References

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