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Research Report

Treadmill running restores MDMA-mediated hyperthermia prevented by inhibition of the dorsomedial hypothalamus

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ABSTRACT

The contribution of exercise to hyperthermia mediated by MDMA is not known. We recently showed that inhibiting the dorsomedial hypothalamus (DMH) attenuated spontaneous locomotion and hyperthermia and prevented deaths in rats given MDMA in a warm environment. The goal of this study was to confirm that restoring locomotion through a treadmill would reverse these effects thereby confirming that locomotion mediated by the DMH contributes to MDMA-mediated hyperthermia. Rats were randomized to receive bilateral microinjections, into the region of the DMH, of muscimol (80 pmol/100 nl) or artificial CSF followed by a systemic dose of either MDMA (7.5 mg/kg, i.v.) or saline. Immediately after the systemic injection, rats were placed on a motorized treadmill maintained at 32 °C. Rats were exercised at a fixed speed (10 m/min) until their core temperature reached 41 °C. Our results showed that a fixed exercise load abolished the decreases in temperature and mortality, seen previously with inhibition of the DMH in freely moving rats. Therefore, locomotion mediated by neurons in the DMH is critical to the development of hyperthermia from MDMA.

1. Introduction

Deaths involving the drug 3,4-methylenedioxymethamphetamine (MDMA) often occur at dance parties where the ambient temperature may be elevated, and users commonly engage in vigorous exercise. Previous research has shown that exercise increases the toxicity and hyperthermia of MDMA (Duarte et al., 2005; Tao et al., 2014) but despite this, locomotion and exercise have been discounted as major contributors to hyperthermia from MDMA. If locomotion is a major contributor to heat-related deaths from MDMA, then understanding how stimulants evoke locomotion and allow persons to exert themselves to the point of heat stroke is an important step in developing prevention strategies.

Abbreviations: MDMA, 3,4-methylenedioxymethamphetamine; DMH, dorsomedial hypothalamus; NE, norepinephrine; D_1, dopamine 1 receptor; 5-HT-2_A, serotonin 2A receptor

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We have previously shown that inhibiting neurons in the region of the dorsomedial hypothalamus (DMH) attenuates spontaneous locomotion, hyperthermia, and prevents mortality in rats given MDMA in a warm environment (Zaretsky et al., 2014). Interestingly, inhibiting neurons in the region of the DMH had no effect on increases in brown adipose tissue (iBAT) thermogenesis, or on decreases in cutaneous blood flow mediated by MDMA (Zaretsky et al., 2014). This suggests that locomotion may by itself be a significant contributor to the development of hyperthermia from MDMA.

The DMH, however, is involved in numerous physiologic processes that might also contribute to hyperthermia from MDMA: shivering (Nakamura and Morrison, 2011), stress-evoked increases in heart rate and adrenocorticotropic hormone (DiMicco et al., 2002), and behavioral thermoregulation (Almeida et al., 2006). Although there is a correlation between decreases in locomotion and decreases in hyperthermia from MDMA, it is unknown if it is locomotion or another effect produced by neurons in the DMH that contributes to hyperthermia from MDMA.

The objective of this study was to confirm that locomotion mediated by the DMH is an important contributor to hyperthermia from MDMA given to rats in a warm environment. To do this we used treadmills to isolate and control the amount of locomotion produced by rats given MDMA. We hypothesized that decreases in MDMA-mediated hyperthermia and mortality seen previously with inhibition of the DMH, would be abolished when locomotion was restored using a treadmill in DMH-inhibited rats given MDMA in a warm environment.

2. Results

2.1. *Temperature curves*

Running at an ambient temperature of 32 °C quickly increased the body temperature of rats (Fig. 1A). Independent of their group, all the rats reached body temperatures of 41 °C by 40 min. Rats who were injected with MDMA (aCSF/MDMA and muscimol/MDMA), however, reached critical temperatures 16 min faster ($p<0.05$) than rats given saline (aCSF–Saline and Muscimol/Saline groups). There were no differences within the Saline or MDMA groups. This is in contrast to our previous paper where we showed that inhibiting the DMH significantly decreased the temperature of MDMA-treated rats (Zaretsky et al., 2014). Therefore equalizing the amount of locomotion between groups of rats treated with MDMA reverses the temperature effects seen with inhibiting the DMH.

2.2. *Heat accumulation*

MDMA, compared to saline, also increased the rate of heat accumulation (Fig. 1B). There were no differences within groups given MDMA (aCSF/MDMA vs. Musc/MDMA) or within groups given Saline (aCSF/Sal vs. Musc/Sal). Furthermore, this data shows that of the individual contributions to heat accumulation in rats treated with MDMA locomotion accounts for ~50%. The other half is likely the combination of impaired heat dissipation (through vasoconstriction) and non-shivering thermogenesis (through iBAT).

3. Discussion

When viewed in light of our previous work, the findings of this study demonstrate that decreases in the MDMA-mediated hyperthermia seen after inhibition of neurons in the DMH are due to reductions in locomotion.

In two previous papers we showed that inhibiting neurons in the DMH decreased spontaneous locomotion and hyperthermia produced by MDMA (Zaretsky et al., 2014; Rusyniak et al., 2008). It was not known, however, if decreases in locomotion were the cause of attenuated temperatures or merely associated with them. As we show in this paper, restoring locomotion in DMH-inhibited rats also restores hyperthermia thereby confirming locomotion as a significant contributor to MDMA-mediated hyperthermia. It is important to note that non-locomotor effects are also important contributors to hyperthermia mediated by MDMA. The differences (~0.1 °C/min) in the rate of heat accumulation between rats given MDMA and saline (Fig. 1B) are likely from non-locomotor contributions to heat accumulation such as iBAT thermogenesis and cutaneous vasoconstriction (Zaretsky et al., 2014; Blessing et al., 2003, 2006). In our previous paper we demonstrated that inhibition of the DMH increased survival but did not change iBAT thermogenesis or cutaneous vasoconstriction (Zaretsky et al., 2014). Therefore, while locomotor and non-locomotor components of heat accumulation from MDMA are approximately equal, inhibition of locomotion alone is sufficient to prevent hyperthermia and mortality from MDMA. Furthermore, MDMA users cannot control changes in non-shivering thermogenesis or cutaneous blood flow. They can however volitionally decrease or stop locomotion. While it seems intuitive that locomotion would contribute to heat production, our study is the first to conclusively show this with MDMA, and is contrary to what many authors have previously asserted (Dafters, 1995; Docherty and Green, 2010; Green et al., 2004; O'Shea et al., 2005; Rusyniak et al., 2007).

The contribution of exercise to hyperthermia in users of MDMA is of particular clinical relevance, as many people use MDMA and stimulants to facilitate dancing in hot crowded environments (MtB and Engels, 2005). This may be why the majority of published cases of hyperthermia from MDMA continue to occur in persons at dance venues. An important question arising from this is why users of MDMA do not stop exercising when their core temperature becomes elevated. Under normal conditions in rodents and humans, exhaustion develops when core temperatures reach a critical threshold (Noakes, 2012). In humans this is ~40 °C (González-Alonso et al., 1999) while in rodents it is between 41–42 °C (Walters et al., 2000). Exhaustion is thought to be a protective mechanism preventing heat stroke (Noakes, 2012). Therefore drugs that prevent exhaustion may also increase the risk of developing drug-induced hyperthermia. It has been previously shown that drugs that increase central concentrations of dopamine and norepinephrine (NE), in both rats and humans, delay the development of exhaustion in a warm environment (Roelands et al., 2008; Roelands and Meeusen, 2010; Watson