Winter 12-11-2017

Host Microbiome Regulation of Hyperthermia Mediated by 3,4-Methylenedioxymethamphetamine (MDMA, Molly)

Emily Ridge
eridge@bgsu.edu

Host Microbiome Regulation of Hyperthermia Mediated by 3,4-Methylenedioxymethamphetamine (MDMA, Molly)

Emily Ridge

Honors Project 2017

Abstract

Hyperthermia is one of the most acute and life-threatening consequences of 3,4-methylenedioxymethamphetamine (MDMA) use. The hyperthermia induced by MDMA involves a complex interaction between heat generation and loss of heat dissipation. Recent studies have demonstrated a role for gut microbiome in the regulation of body weight and temperature. Here, we investigated the potential role of the gut microbiome in MDMA-mediated hyperthermia. For fourteen days prior to treatment with MDMA (20 mg/kg, sc) male, Sprague-Dawley rats were provided regular drinking water or drinking water laced with the non-absorbable antibiotics, bacitracin (0.5 mg/mL), neomycin (2mg/mL), and vancomycin (0.2mg/mL). Antibiotic (ABX) treatment reduced gut bacteria and increased cecal size. MDMA-induced a hyperthermic response that resulted in a maximal temperature change (ΔT_{max}) of 4.6 ± 0.1 °C and only a 50% survival rate 60 minutes after treatment. Conversely, ABX treatment prior to MDMA attenuated the hyperthermic response with a ΔT_{max} of 3.4 ± 0.6 °C and a 100% survival rate 60 minutes after treatment. An acute intraperitoneal injection of ABX 30 minutes before MDMA had no effect on the hyperthermic response, eliminating the possibility of a pharmacodynamics interaction between ABX and MDMA. Overall, these findings demonstrate that the gut microbiome contributes to the hyperthermia mediated by MDMA.

Introduction

3,4-Methylenedioxymethamphetamine (MDMA, Figure 1) is a synthetic drug more commonly known as "Ecstasy" or "Molly." Although MDMA was used for a short time as an adjunct to psychotherapy in the 1970's, illicit use of the drug did not become popular until the emergence of the rave scene in the 1980's (Dar and McBrien, 1996). Like other substituted amphetamines, MDMA acts as a sympathomimetic agent increasing norepinephrine levels centrally and peripherally (Sprague et al., 2007). MDMA-induced hyperthermia has been associated with a combination of peripheral vasoconstriction (Pedersen and Blessing, 2001) and activation of mitochondrial uncoupling proteins (for a review, see Mills et al., 2003). Norepinephrine binds to the α_1-adrenergeric receptor in vascular smooth muscle, resulting in vasoconstriction and attenuated heat dissipation. MDMA induced release of norepinephrine also indirectly activates uncoupling proteins (UCPs), through the release of free fatty acids (Mills et al., 2007). When activated, UCPs dissipate the proton gradient across the inner mitochondrial membrane, resulting in increased proton conductance and the release of energy as heat (Cannon and Nedergaard, 2004). Free fatty acids in brown adipose tissue and skeletal muscle mitochondria serve as ligand activators for UCP facilitated proton leak (Echtay et al., 2001; Brand and Esteves, 2005). Zietak et al. (2016) demonstrated that changes in intestinal microbiota and bile acids occur when ambient temperature is reduced, in parallel with UCP induction. Skakun (1958) noted a marked increase in the total amount of bile under the influence of amphetamine. Based on these studies and previous knowledge of UCP-regulated thermogenesis, we hypothesize that intestinal bacteria may play a role in MDMA-induced hyperthermia.

Results

Knockdown of Intestinal Bacteria using Non-Absorbable Antibiotics. To determine if changes in intestinal microbiota influence the thermogenic responses to MDMA, animals were given a cocktail of antibiotics, including bacitracin, neomycin, and vancomycin, via their drinking water, for 14 days before MDMA treatment. Daily fluid intake and body weight did not differ between groups over the course of the experiment. However, prolonged treatment with antibiotics did result in markedly enlarged ceca (Fig. 2), as has been previously described (Kiraly et al. 2016).

Effects of Gut Microbiota on MDMA-Induced Hyperthermia. Following the subcutaneous (SC) administration of saline in both the antibiotic and water control groups, core body temperature did not significantly change over the 60 min monitoring period. MDMA significantly increased core body temperature in both the antibiotic and water experimental groups at all time points compared to the two control groups ($p < 0.0001$, Fig. 3A). The ABX MDMA group showed a marked attenuated thermogenic response compared to that of the H_2O MDMA group ($p=0.006$, Fig. 3B). Total area under the curve (TAUC) was also calculated, which provided additional evidence for the diminished thermogenic responses of the ABX MDMA group when compared to the H_2O MDMA group ($p=0.004$, Fig. 3C). Additionally, only 50% of the control group treated with MDMA survived after 60 min post-treatment, while the antibiotic group treated with MDMA had a survivorship of 100% (Fig. 3D).

Acute Antibiotic Treatment. In order to confirm that the effects of the oral antibiotic treatment on MDMA-induced hyperthermia were due to local actions within the gut and not caused by a pharmacodynamics interaction between MDMA and the antibiotics, we

injected the animals with a single IP dose of antibiotics (1.67 mg/kg vancomycin, 20 mg/kg neomycin, and 293 U/kg bacitracin in PBS) thirty minutes before MDMA. These animals did not display an attenuated thermogenic response to MDMA. Instead, these animals displayed a similar maximum change in temperature as the control group ($p=0.4525$, Fig. 4).

Discussion

In this study, we found that the depletion of gut microbiota resulted in a marked attenuation of core body temperature in animals treated with MDMA. By 60 min post-injection, both the H_2O MDMA and the ABX MDMA had displayed thermogenic responses to the drug administration. However, the average core body temperature of the control group treated with MDMA was about a full degree Celsius higher than that of the ABX MDMA group, both at 60 min and 90 min post-treatment. Additionally, TAUC analysis and ΔT_{max} information provided further evidence that the ABX MDMA treatment group had a significantly smaller thermogenic response to MDMA administration compared to that of the H_2O MDMA treatment group. Together, this data suggests that the host gut microbiome plays a role in the mechanism by which MDMA mediates hyperthermia.

While this is the first study to directly examine the role of the gut microbiome in MDMA-mediated hyperthermia, it is not the first to analyze the role of intestinal bacteria in UCP-regulated thermogenesis. A previous study demonstrated that changes in ambient temperature led to differences in gut microflora profiles, which directly affected UCP-regulated thermogenesis and adiposity levels (Zietak et al., 2016). However, this study

did not utilize antibiotics to produce a marked change in the host gut microbiome. In our study, we saw an attenuated thermogenic response to MDMA administration only when antibiotics were given orally and thus were directly applied to the gut microbiota. Intraperitoneal injections of the antibiotics did not lead to a significant decrease in core rectal temperature, suggesting that it was the reduced gut bacteria that led to the attenuated thermogenic response.

While the mechanism by which the host microbiome mediates MDMA-induced hyperthermia remains unknown, we demonstrated here that there is a relationship between these two biological systems. Although further work is needed to understand these underlying mechanisms, our results help define potential routes for future research. In particular, it will be important to define how alterations in the gut microbiome interact with bile acids to modify MDMA-mediated hyperthermia. While much remains to be discovered, there are potentially important therapeutic effects from these results, including the identification of points of intervention in the development of acute sympathomimetic-mediated hyperthermia. It could also lead to more personalized medical treatments, tailored to an individual's specific gut microbiome.

Methods

Animals. Male Sprague-Dawley Rats (284.6 ± 2.4g, Envigo, Indianapolis, IN) were used. Animals were housed one per cage ($21.0 \times 41.9 \times 20.3$ cm^3), maintained on a 12:12h light/dark schedule, and provided access to food and water ad libitum. Animals were sustained on a minimum 10% fat diet and housed in a room kept at $24 - 26$ ℃ in

order to maximize a thermogenic response (Mills et al., 2007, Dafters et al., 1994). Animal maintenance and research were conducted in accordance with the eighth edition of the Guide for the Care and Use of Laboratory Animals as adopted and circulated by the National Institutes of Health, and protocols were approved by the Bowling Green State University Animal Care and Use Committee. All animals were allowed to acclimate to the facility for one week prior to the start of any treatments.

Study Design. Animals were randomly assigned to four treatment groups (n=6): H_2O Control, ABX Control, H_2O MDMA, and ABX MDMA. Antibiotics were administered via the drinking water for fourteen days in accordance with the methods described in the study by Kiraly et al. (2016). Antibiotic doses were: Neomycin 2mg/mL, Vancomycin 0.2mg/mL, and Bacitracin 0.5 mg/mL. Fluid intake was measured daily and body weight was measured every five days. On the fourteenth day, animals were injected subcutaneously with either saline or MDMA (20 mg/kg). Temperatures were taken rectally just prior to treatment (baseline) and every 30 min for an hour post-treatment, using a Physiotemp Thermalert TH-8 termocouple (Physitemp Instruments, Clifton, NJ) attached to a RET-2 rectal probe. The animals were then sacrificed and brown adipose tissue (BAT), skeletal muscle tissue (SKM), and cecum were dissected out for qPCR analysis of bacterial count (cecum), UCP 1&3 and TGR5 quantification (BAT and SKM). In order to rule out the systemic effects of the antibiotics, animals were injected intraperitoneally with 1.67 mg/kg vancomycin, 20 mg/kg neomycin, and 293 U/kg bacitracin in PBS (Kiraly et al. 2015) thirty minutes before the administration of MDMA (20 mg/kg). Temperatures were taken rectally just prior to antibiotic administration (baseline) and every 30 minutes for an hour post-treatment of MDMA.

Statistical Analysis. GraphPad InStat v.6.0 software was used to complete all statistical analyses of data. Temperatures between treatment groups were compared using one-way ANOVA with a Student-Newman-Keuls post-hoc test. Additionally, temperatures within a treatment group were compared using a one-way ANOVA with a Dunnett's post-hoc test. Maximum temperature change (ΔT_{max}) was calculated by comparing the maximum increase in core temperature to the animal's baseline temperature. An unpaired, two-tailed t-test was used to compare the maximum temperature changes between the ABX MDMA and H2O MDMA groups. Significance was set at the 95% confidence level ($p < 0.05$).

Figures

Figure 1

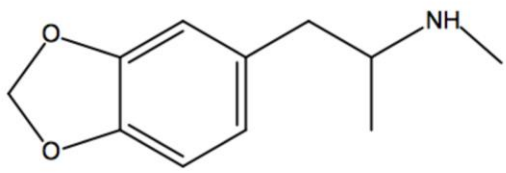
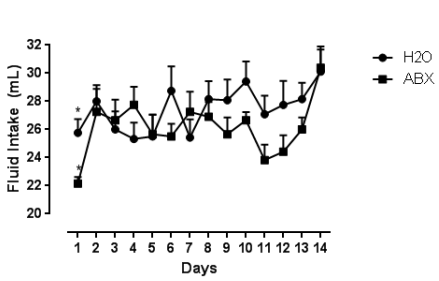
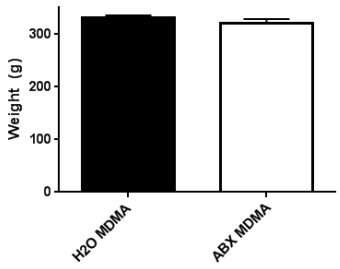


Figure 1. Chemical structure of 3,4-methylenedioxymethamphetamine (MDMA).

Figure 2



A.

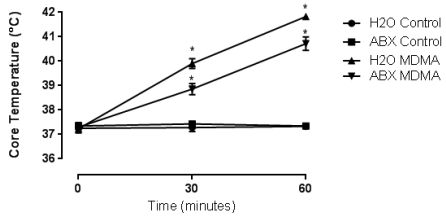


B.

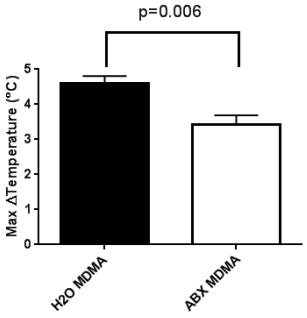
C.

Figure 2. Daily fluid intake for both antibiotic-treated (ABX) and control (H_2O) groups (A). Mean body weight for both antibiotic-treated and control groups (B). Gross histology of the cecum from control (left) and antibiotic-treated (right) animals (C).

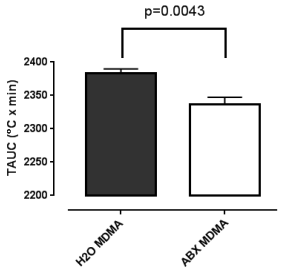
Figure 3



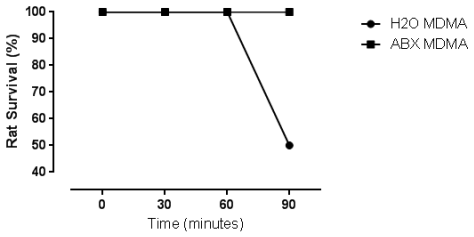
A.



B.



C.



D.

Figure 3. Changes in core temperature following the administration of MDMA (A). Maximum change in temperature following the administration of MDMA (B). Temperature-area under the curve (TAUC) following the administration of MDMA (C). Animal survivorship following the administration of MDMA (D). * indicates significant difference from all other treatment groups ($p<0.05$).

Figure 4

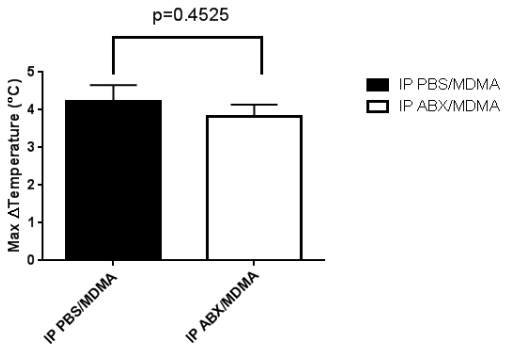


Figure 4. Maximum change in temperature following intraperitoneal administration of antibiotics.

References

Brand MD, and Esteves TC (2005) Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. *Cell Metab* 2:85-93.

Cannon B, and Nedergaard J (2004) Brown adipose tissue: function and physiological significance. *Physiol Rev 84*:277-359.

Dafters, R.I. (1994). Effect of ambient temperature on hyperthermia and hyperkinesis induced by 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") in rats. *Psychopharmacology 114*: 505-508.

Dar, KJ, McBrien, ME (1996). MDMA induced hyperthermia: report of a fatality and review of current therapy. *Intensive Care Med 22:* 995-996.

Echtay KS, Winkler E, Frischmuth K, and Klingenberg M (2001) Uncoupling proteins 2 and 3 are highly active H^+ transporters and highly nucleotide sensitive when activated by coenzyme Q (ubiquione). *Proc Natl Acad Sci USA 98*:1416-1421.

Kiraly, D.D., Walker, D.M., Calipari, E.S., Labonte, B., Issler, O., Pena, C.J., Ribeiro, E.A. Russo, S.J. Nestler, E.J. (2016). Alterations of the host microbiome affect behavioral responses to cocaine. *Scientific Reports 6:* 1-12.

Mills EM, Banks ML, Sprague JE, and Finkel T (2003) Pharmacology: uncoupling the agony from ecstasy. *Nature 426*:403-404.

Mills, E.M., Weaver, K.L., Abramson, E., Pfeiffer, M., and Sprague, J.E. (2007). Influences of dietary fats on ecstasy-induced hyperthermia. *Br. J. Pharma: 151,* 1103-1108.

Pedersen NP, and Blessing WW (2001) Cutaneous vasoconstriction contributes to hyperthermia induced by 3,4-methylenedioxymethamphetamine (Ecstasy) in conscious rabbits. *J Neurosci 21*:8648–8654.

Skakun NP (1958) The Effect of Adrenalin and Sympathomimetic Amines (Ephedrine, Amphetamine) on Bile Secretion of the Liver. *Bulletin of Experimental Biology and Medicine 45*:474-479.

Zietak M, Chabowska-Kita A, Kozak LP (2017) Brown fat thermogenesis: stability of developmental programming and transient effects of temperature and gut microbiota in adults. *Biochimie 134*:93-95.