Published in final edited form as:

Toxicol Lett. 2011 October 10; 206(2): 234–237. doi:10.1016/j.toxlet.2011.07.025.

Identification of a Possible Role for Atrial Natriuretic Peptide in MDMA-induced hyperthermia

Sandra L Hrometz¹, Karen E Thatcher¹, Jeremy A Ebert¹, Edward M Mills², and Jon E Sprague^{1,*}

¹The Department of Pharmaceutical and Biomedical Sciences, The Raabe College of Pharmacy, Ohio Northern University, Ada, OH 45810, USA

²The Division of Pharmacology and Toxicology, College of Pharmacy, University of Texas at Austin, Austin, TX 78712, USA

Abstract

MDMA (3,4-methylenedioxymethamphetamine) induces thermogenesis in a mitochondrial uncoupling protein 3-dependent manner. There is evidence that this hyperthermia is mediated in part by the lipolytic release of free fatty acids, that subsequently activate uncoupling protein 3 in skeletal muscle mitochondria. We hypothesize that atrial natriuretic peptide (ANP), a strong lipolytic mediator, may contribute to the induction and maintenance of MDMA-induced thermogenesis. The specific aims of this study were to 1) determine if ANP is released following MDMA administration, and 2) use the ANP receptor antagonist, Anantin, to ascertain the role of ANP in MDMA-induced hyperthermia. ANP levels were measured in plasma at baseline, 10, 20, 30 and 60 min following MDMA (40 mg/kg, sc) administration in 16 male Sprague-Dawley rats. A robust increase in ANP was seen within ten min of MDMA administration. ANP levels returned to baseline at 20 min and then gradually rose over the 60 min monitoring period. The administration of Anantin (40 mg, ip), 15 min before and after MDMA, significantly attenuated the MDMA-induced hyperthermia. We conclude that ANP signaling contributes to the hyperthermia induced by MDMA.

Keywords

Hyperthermia; 3,4-methylenedioxymethamphetamine (MDMA, “Ecstasy”); Atrial Natriuretic Peptide; Anantin

1. Introduction

Hyperthermia is the most widely known acute adverse effect that can follow ingestion of MDMA. MDMA induced hyperthermia involves both central thermoregulation (Rusyniak et al., 2008; Docherty & Green 2010) and peripheral changes in blood flow and thermogenesis.

© 2011 Elsevier Ireland Ltd. All rights reserved.

*Corresponding author: Jon E Sprague, PhD Professor of Pharmacology & Dean Ohio Northern University The Raabe College of Pharmacy 525 S. Main Street Ada, OH 45810, USA Phone: +1-419-772-2277 Fax: +1-419-772-3554 j-sprague@onu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest statement

The authors declare that there are no conflicts of interest.

Peripherally, the increased body temperature seen with MDMA is due in part to a combination of peripheral vasoconstriction (which prevents heat dissipation (Pedersen et al., 2001)) and activation of mitochondrial uncoupling proteins (UCP) that generate heat in skeletal muscle (SKM) and adipose tissues (Mills et al., 2003; Mills et al., 2004; Kelly et al., 2011). Free fatty acids (FFA) activate mitochondrial UCP3 and induce proton leak across the inner mitochondrial membrane. This process, referred to as mitochondrial uncoupling, dissociates the mitochondrial proton gradient from ATP synthesis, and releases the free energy as heat (Lowell et al., 2000). The strength of the relationship between increased plasma FFA levels and an increased thermogenic response to MDMA has been shown previously in rats fed a high-fat diet (Mills et al., 2007).

Although peak thermogenesis does not occur until 60 min after MDMA administration, the significant increases in plasma norepinephrine (NE), FFA and insulin occur within 30 min of administration (Sprague et al., 2007; Banks et al., 2009). The 35-fold increase in plasma NE levels (Sprague et al., 2007) stimulates peripheral α_1 , β_2 and β_3 adrenergic receptors (AR) (Kuusela et al., 1997; Zhao et al., 1997; Himms-Hagen et al., 1978). Activation of α_1 AR mediates peripheral vasoconstriction. Activation of β_2 and β_3 AR stimulates lipolysis and ultimately increases FFA production through protein kinase A (PKA) action (Zimmermann et al., 2004; Haemmerle et al., 2002).

Atrial natriuretic peptide (ANP), acting on the natriuretic peptide receptor (NPR) type A (NPR-A) stimulates lipolysis and FFA production through activation of protein kinase G (PKG). Intracerebroventricular administration of ANP induces a dose dependent increase in body temperature (Pataki et al., 1999). However, the lipolysis induced by ANP occurs through a separate pathway (protein kinase G mediated) than that of catecholamine-induced lipolysis (protein kinase A mediated) (Sengenès et al., 2000). Both catecholamines (via a cAMP-PKA signaling pathway) and ANP (via a cGMP-PKG signaling pathway) phosphorylate and activate hormone sensitive lipase (HSL) and perilipins that hydrolyze stored triglycerides (TG) to yield FFAs (Sengenès et al., 2003). The signal transduction pathway stimulated by ANP to promote lipolysis is strictly connected to an increase in intracellular cGMP concentration and does not cause a change in cAMP in human adipocytes (Sengenès et al., 2000, 2003; Moro, et al., 2004). With respect to potency, the lipolysis occurring with ANP is comparable to that of the non-selective β agonist isoproterenol (Sengenès et al., 2000, 2003). Plasma insulin levels, also increased following MDMA administration (Banks et al., 2009). Insulin stimulates uptake of FFAs into SKM by recruiting FFA transporters to the plasma membrane (Luiken et al., 2002). Unlike NE, insulin inhibits lipolysis. However, it has been shown in vivo that insulin's antilipolytic effects do not affect ANP-induced lipolysis (Sengenès, et al., 2000; Moro et al., 2004, 2005). As the movement of FFA is essential in this sympathomimetic model of thermogenesis, we hypothesized that ANP may play a role in MDMA-induced hyperthermia.

2. Material and methods

2.1 Animals

The plasma ANP time course experiment was performed in adult male Sprague-Dawley rats inserted with jugular venous catheters (JVC). JVC animals were individually housed and used in the studies examining plasma ANP levels. The JVC animals weighed 125-175 g on the day of the experiments. The thermogenesis experiment was performed using adult male Sprague-Dawley rats weighing 180-210 g. Rats utilized for the thermogenesis experiment were housed in groups of 3. All animals were purchased from Charles Rivers (Wilmington, ME, USA) and given ad libitum access to standard 15 % kcal fat lab chow (Harlan Teklad 22/5 Rodent Diet, Harlan, Indianapolis, IN, USA) and water. Cage dimensions for all animals were 21.0 × 41.9 × 20.3 cm. Housing conditions were maintained at 21-22°C with a

12:12 hour light-dark cycle. Rats were allowed a two day acclimation period before starting treatments. An ambient temperature of 24°C was maintained for all experiments.

2.2 Drugs and chemicals

Anantin was obtained from Ray Biotech (Norcross, GA, USA), AssayMax Rat ANP ELISA Kit from AssayPro (St. Charles, MO, USA), BSA from Fisher Scientific (Pittsburgh, PA, USA) and MDMA was generously donated by Dr. David E. Nichols of Purdue University (West Lafayette, IN, USA). Unless specified, all other chemicals and reagents were obtained from Sigma Aldrich Chemical (St. Louis, MO, USA).

2.3 Temperature assessment

Core (rectal) temperature was measured in all animals before injections and after MDMA administration using a Thermalert TH-8 (Physitemp Instruments, Clifton, NJ, USA) monitor with a rectal probe (RET-2) attached to the thermocoupler. White petrolatum was applied to the probe before insertion. The probe was inserted 3 cm into the rectum while the rat was gently restrained. A steady readout was obtained within 30 sec of probe insertion. Change from baseline temperature was calculated 60 min after MDMA treatment. Sixty min has been shown as the peak thermogenic response time associated with MDMA in the rodent model (Sprague et al., 2007).

2.4 ANP Studies

2.4.1 Study design for measuring ANP release following MDMA—Sixteen JVC rats were given MDMA (40 mg/kg, sc). Blood samples were taken at baseline, then 10, 20, 30, and 60 min following administration of MDMA. To minimize the number of animals used and the amount of blood taken from each animal, blood samples were obtained from all animals at baseline and 60 min post-MDMA and at the randomly allocated times of 10, 20 or 30 min post drug challenge. At each time point, approximately 200-300 uL of blood was drawn from the catheters. The same volume was replaced with 0.9% sodium chloride, followed by lumen lock solution (500 IU heparin in 0.9% sodium chloride). Blood samples were then inverted 8 - 10 times to initiate coagulation then centrifuged for 10 min at 6300 RPM ($2000 \times g$) at 20°C. Plasma was collected, immediately placed on ice, then stored at -80 °C until analyzed for ANP.

2.4.2 ANP determination—Plasma samples were analyzed for ANP using AssayMax Rat ANP ELISA Kit according to kit procedure. Briefly, plasma samples were placed in supplied wells, washed, combined with biotinylated rANP antibody and allowed to incubate for two hours. Samples were then washed and incubated with streptavidin-peroxidase conjugate for 30 min. Next, samples were washed and incubated with chromogen substrate for ten min. Once color density appeared, stop solution was added and absorbance was immediately read at 450 nm.

2.4.3 Statistical analysis for ANP concentrations post-MDMA—Data was analyzed using standard curve interpolation using log-log curve fit. ANP levels were then analyzed in Graphpad Instat using Dunnet's change from baseline tests.

2.5 Anantin

2.5.1 Study design for Anantin with MDMA-induced hyperthermia—Anantin is a peptide ANP receptor antagonist isolated from a strain of *Streptomyces coeruleus* (Weber et al., 1991). In the present study, the dose, route and administration pattern of anantin was based upon previous literature (Dodd-o et al., 2008). Twenty-four male Sprague-Dawley rats were randomly allocated into 4 treatment groups: Saline/Saline/Saline (control), Anantin /

Saline/Anantin (Anantin only), Saline/MDMA/Saline (MDMA only) and Anantin/MDMA/Anantin (Anantin/MDMA). On the day of the experiment, animals received Anantin (40 mg, ip) 15 min prior to and 15 min after MDMA (40 mg/kg, sc) administration. Baseline temperatures were taken before the first dose of Anantin (15 min prior to MDMA). Administration of MDMA was considered time zero. Rectal temperatures were taken at baseline, 15, 30, 45, and 60 min post-MDMA.

2.5.2 Statistical analysis for temperature measurements—The temperature measurements were converted to temperature area under the curve (TAUC) in a fashion similar to those described elsewhere (Miller and O'Callaghan, 2003). TAUC is a composite measurement of temperature and was determined for each animal by taking the temperatures measured at baseline (time of MDMA administration), 15, 30, 45 and 60 min after MDMA administration. This composite measure represents the area under the curve of a plot of temperature (1°C) versus time (min), and is expressed as °C × min. All data is reported as mean ± SEM. TAUC were compared between treatment groups with an ANOVA with a Student Newman-Kuels post hoc test. Statistical significance was set *a priori* at $p \leq 0.05$.

3.0 Results

3.1 Effects of MDMA on ANP release

Plasma ANP levels were significantly increased from 360 ng/mL to 1100 ng/mL (> 300 %) when measured ten min after MDMA administration ($p < 0.01$). ANP levels returned to baseline by 20 min post-MDMA and then significantly peaked again at 60 min ($p < 0.01$; Figure 1).

3.2 Effects of Anantin on MDMA-induced hyperthermia

Core body temperatures increased in all rats following MDMA challenge from $37.02 \pm 0.07^\circ\text{C}$ to a peak temperature of $40.28 \pm 0.32^\circ\text{C}$ ($p < 0.001$). No significant change in core body temperature was noted from immediately prior to anantin challenge ($36.85 \pm 0.13^\circ\text{C}$) through the 60 minute duration of the experiment. Furthermore, rats pretreated with anantin before MDMA experienced a significantly attenuated rise in core temperatures compared to those receiving MDMA only. Control and anantin only animals remained normothermic throughout the duration of the experiment (Figure 2A). The TAUC data demonstrate that Anantin significantly reduced the overall rise in temperature induced by MDMA ($p < 0.01$; Figure 2B). Both MDMA only and Anantin/MDMA had a significantly elevated temperature compared to the control and Anantin only treatments ($p < 0.01$).

4. Discussion

Lipolysis has traditionally been regarded to be controlled exclusively by catecholamines and insulin via a protein kinase A- (PKA)-dependent signaling pathway. ANP has emerged as an important mediator of lipolysis via a PKG pathway that is independent of catecholamine-signaling (Sengenès et al., 2003). ANP is released from cardiac myocytes in response to hypertension and stretch of the atria and/or ventricles. As a sympathomimetic, MDMA increases heart rate and blood pressure (Broadley, 2010), which likely accounts for MDMA-induced ANP release. As the release of FFA are essential in the MDMA-induced thermogenic model (Sprague, et al., 2007), release of ANP would contribute to this increase in FFA release. The present findings demonstrate that MDMA significantly increases plasma ANP levels. Pollack et al., (1997) demonstrated a dramatic increase in ANP release subsequent to volume expansion, atrial stretch and endothelin. ANP was measured 35 min after volume expansion and showed a steady increase that became significantly increased at 65 min. Pollock et al., documented robust surge in ANP and steady increase over 60 minutes

is consistent with our present findings. The initial significant rise in ANP occurred within ten min of MDMA administration (Figure 1). From a pharmacodynamic perspective, this “spike” in ANP at 10 min occurs chronologically at an appropriate time point to contribute to the peak elevation in FFA seen 30 min after MDMA treatment (Sprague et al., 2004, 2007). Subsequent antagonism of NPR-A with Anantin significantly attenuated the rise in temperature seen with MDMA (Figure 2).

There are three natriuretic peptide receptors (NPR): NPR-A, NPR-B and NPR-C. NPR-A and NPR-B possess guanylate cyclase activity that ultimately leads to the activation of PKG. NPR-C is thought to act as a clearance receptor to influence ANP half-life. ANP has high affinity for both NPR-A and NPR-B (for a review see Potter, et al., 2006). NPR-A has the highest affinity for ANP and is expressed in adipose and cardiovascular tissues. In rat adipocytes, Jeandel et al. (1988) reported a 3-fold increase in basal cGMP levels in response to ANP. Sengenès et al. (2002) however reported that ANP-induced lipolysis occurs predominantly in humans and primates adipose tissue and to a lesser extent in other mammals such as rats, mice, rabbits, and dogs. Their explanation for the unresponsiveness of rat adipocytes to ANP-induced lipolysis is the low expression of biological NPR-A, that would therefore be insufficient to promote the cGMP levels required to reach the set-point for HSL activation (Sengenès et al., 2002). The significance of ANP-induced lipolysis in rat adipocytes may only contribute a small amount of cGMP; however, antagonism of NPR-A with anantin significantly decreased MDMA-induced hyperthermia in our rodent model. Because of these differences between rodents and humans with regards to NPR-A mediated lipolysis in adipose tissue, the role of ANP in sympathomimetic-induced hyperthermia may well be greater in humans.

Acknowledgments

This study was supported in part by grants from the National Institutes of Health (1R15 DA022712-01A1) and from the Bower, Bennett & Bennett Endowment Fund. MDMA was generously donated by Dr. David E. Nichols of Purdue University

Abbreviations

ANP	atrial natriuretic peptide
AR	adrenergic receptor
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
FFA	free fatty acid
HSL	hormone sensitive lipase
JVC	jugular venous catheter
MDMA	3,4-methylenedioxymethamphetamine
NE	norepinephrine
NPR	natriuretic peptide receptor
PKA	protein kinase A
PKG	protein kinase G
SKM	skeletal muscle
TAUC	temperature area under the curve

UCP3 uncoupling protein 3

References

- Abumrad NA, el-Maghrabi MR, Amri EZ, Lopez E, Grimaldi PA. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. *J. Biol. Chem.* 1993; 268:17665–17668. [PubMed: 7688729]
- Banks ML, Buzard SK, Gehret CM, Monroy AN, Kenaston MA, Mills EM, Sprague JE. Pharmacodynamic characterization of insulin on MDMA-induced thermogenesis. *Eur J Pharmacol.* 2009; 615:257–261. [PubMed: 19482019]
- Bonen A, Luiken JJ, Arumugam Y, Glatz JF, Tandon NN. Acute regulation of fatty acid uptake involves the cellular redistribution of fatty acid translocase. *J. Biol. Chem.* 2000; 275:14501–14508. [PubMed: 10799533]
- Bonen A, Chabowski A, Luiken JJ, Glatz JF. Mechanisms and regulation of protein-mediated cellular fatty acid uptake: molecular, biochemical, and physiological evidence. *Physiol.* 2007; 22:15–28.
- Broadley KJ. The vascular effects of trace amines and amphetamines. *Pharmacol Ther.* 2010; 125:363–375. [PubMed: 19948186]
- Campbell SE, Tandon NN, Woldegiorgis G, Luiken JJ, Glatz JF, Bonen A. A novel function for fatty acid translocase (FAT)/CD36: involvement in long chain fatty acid transfer into the mitochondria. *J. Biol. Chem.* 2004; 279:36235–36241. [PubMed: 15161924]
- De Leon H, Bonhomme M-C, Thibault G, Garcia R. Localization of atrial natriuretic factor receptors in the mesenteric arterial bed. Comparison with angiotensin II and endothelin receptors. *Circ. Res.* 1995; 77:64–72. [PubMed: 7788883]
- Docherty JR, Green AR. The role of monoamines in the changes in body temperature induced by 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) and its derivatives. *Br. J. Pharmacol.* 2010; 106(5):1029–1044. [PubMed: 20590597]
- Dodd-o JM, Hristopoulos ML, Kibler K, Gutkowska J, Mukaddam-Daher S, Gonzalez A, Welsh-Servinsky LE, Pearse DB. The role of natriuretic peptide receptor-A signaling in unilateral lung ischemia-reperfusion injury in the intact mouse. *Am J Physiol Lung Cell Mol Physiol.* 2008; 294:714–723.
- Echtay KS, Winkler E, Frischmuth K, Klingenberg M. Uncoupling proteins 2 and 3 are highly active H^+ transporters and highly nucleotide sensitive when activated by coenzyme Q (ubiquione). *Proc. Natl. Acad. Sci. U.S.A.* 2001; 98:1416–1421. [PubMed: 11171965]
- Goke R, Goke B, Noll B, Richter G, Christoph-Fehmann H, Arnold R. Receptors for atrial natriuretic peptide on isolated rat adipocytes. *Biomed. Res.* 1989; 10:463–467.
- Haemmerle G, Zimmermann R, Hayn M, Theussl C, Waeg G, Wagner E, Sattler W, Magin TM, Wagner EF, Zechner R. Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle, and testis. *J. Biol. Chem.* 2002; 277:4806–4815. [PubMed: 11717312]
- Harmon CM, Luce P, Beth AH, Abumrad NA. Labeling of adipocyte membranes by sulfo-N-succinimidyl derivatives of long-chain fatty acids: inhibition of fatty acid transport. *J. Membr. Biol.* 1991; 121:261–268. [PubMed: 1865490]
- Himms-Hagen J, Cerf J, Desautels M, Zaror-Behrens G. Thermogenic mechanisms and their control. *Experientia. Suppl.* 1978; 32:119–134. [PubMed: 348485]
- Hirsch IB. Insulin Analogues. *N. Engl. J. Med.* 2005; 352:174–183. [PubMed: 15647580]
- Holloway GP, Luiken JJ, Glatz JF, Spriet LL, Bonen A. Contribution of FAT/CD36 to the regulation of skeletal muscle fatty acid oxidation: an overview. *Acta Physiol.* 2008; 194:293–309.
- Houseknecht KL, Baile CA, Matteri RL, Spurlock ME. The biology of leptin: a review. *J. Anim. Sci.* 1998; 76:1405–1420. [PubMed: 9621947]
- Jeandel L, Okamura H, Belles-Isles M, Chabot JG, Dihl F, Morel G, Kelly PA, Heisler S. Immunocytochemical localization, binding, and effects of atrial natriuretic peptide in rat adipocytes. *Mol. Cell. Endocrinol.* 1988; 62:69–78. [PubMed: 2545486]

- Jezek P, Engstova H, Zackova M, Vercesi AE, Costa AD, Arruda P, Garlid KD. Fatty acid cycling mechanism and mitochondrial uncoupling proteins. *Biochim. Biophys. Acta.* 1998; 1365:319–327. [PubMed: 9693744]
- Kasiske BL, O'Donnell MP, Keane WF. The Zucker rat model of obesity, insulin resistance, hyperlipidemia, and renal injury. *Hypertension.* 1992; 19:1110–15. [PubMed: 1730447]
- Kelly OM, McNamara YM, Manzke LH, Meegan MJ, Porter RK. The Preservation of in vivo Phosphorylated and Activated Uncoupling Protein 3 (UCP3) in Isolated Skeletal Muscle Mitochondria following Administration of 3,4-Methylenedioxymeth-amphetamine (MDMA aka Ecstasy) to Rats/Mice. *Mitochondrion.* 2011 Article in Press, Corrected Proof.
- Kuusela P, Rehnmark S, Jacobsson A, Cannon B, Nedergaard J. Adrenergic stimulation of lipoprotein lipase gene expression in rat brown adipocytes differentiated in culture: mediation via beta3- and alpha1-adrenergic receptors. *Biochem. J.* 1997; 321:759–767. [PubMed: 9032464]
- Lafontan M, Moro C, Berlan M, Crampes F, Sengenès C, Galitzky J. Control of lipolysis by natriuretic peptides and cyclic GMP. *Trends Endocrinol. Metab.* 2008; 19:130–137. [PubMed: 18337116]
- Lafontan M, Moro C, Sengenès C, Galitzky J, Crampes F, Berlan M. An unsuspected metabolic role for atrial natriuretic peptides: the control of lipolysis, lipid mobilization, and systemic nonesterified fatty acids levels in humans. *Arterioscler. Thromb. Vasc. Biol.* 2005; 25:2032–2042. [PubMed: 16123323]
- Lowell BB, Spiegelman BM. Towards a molecular understanding of adaptive thermogenesis. *Nature.* 2000; 404:652–660. [PubMed: 10766252]
- Luiken JJ, Schapp FG, van Nieuwenhoven FA, van der Vusse GJ, Bonen A, Glatz JF. Cellular fatty acid transport in heart and skeletal muscle as facilitated by proteins. *Lipids.* 1999; 34:S169–S175. [PubMed: 10419138]
- Luiken JJ, Turcotte LP, Bonen A. Protein-mediated palmitate uptake and expression of fatty acid transport proteins in heart giant vesicles. *J. Lipid Res.* 1999; 40:1007–1016. [PubMed: 10357832]
- Luiken JJ, Dyck DJ, Han XX, Tandon NN, Arumugam Y, Glatz JF, Bonen A. Insulin induces the translocation of the fatty acid transporter FAT/CD36 to the plasma membrane. *Am. J. Physiol. Endocrinol. Metab.* 2002; 282:E491–E495. [PubMed: 11788383]
- Luiken JJ, Koonen DP, Willems J, Zorzano A, Becker C, Fischer Y, Tandon N, van der Vusse GJ, Bonen A, Glatz JF. Insulin stimulates long-chain fatty acid utilization by rat cardiac myocytes through cellular redistribution of FAT/CD36. *Diabetes.* 2002; 51:3113–3119. [PubMed: 12351456]
- Miller DB, O'Callaghan JP. Elevated environmental temperature and methamphetamine neurotoxicity. *Environ. Res.* 2003; 92:48–53. [PubMed: 12706754]
- Mills EM, Banks ML, Sprague JE, Finkel T. Pharmacology: uncoupling the agony from ecstasy. *Nature.* 2003; 426:403–404. [PubMed: 14647371]
- Mills EM, Rusyniak DE, Sprague JE. The role of the sympathetic nervous system and uncoupling proteins in the thermogenesis induced by 3,4-methylenedioxymethamphetamine. *J. Mol. Med.* 2004; 82:787–799. [PubMed: 15602689]
- Mills EM, Weaver KL, Abramson E, Pfeiffer M, Sprague JE. Influence of dietary fats on Ecstasy-induced hyperthermia. *Br. J. Pharmacol.* 2007; 151:1103–1108. [PubMed: 17533413]
- Moro C, Galitzky J, Sengenès C, Crampes F, Lafontan M, Berlan M. Functional and pharmacological characterization of the natriuretic peptide-dependent lipolytic pathway in human fat cells. *J. Pharmacol. Exp. Ther.* 2004; 308:984–992. [PubMed: 14634036]
- Moro C, Polak J, Richterova B, Sengenès C, Pelikanova T, Galitzky J, Stich V, Lafontan M, Berlan M. Differential regulation of atrial natriuretic peptide- and adrenergic receptor-dependent lipolytic pathways in human adipose tissue. *Metabolism.* 2005; 54:122–132. [PubMed: 15562390]
- Park BY, Lee Y, Walton M, Duplomb L, Unger RH. Demonstration of reverse fatty acid transport from rat cardiomyocytes. *J. Lipid. Res.* 2004; 45:1992–1999. [PubMed: 15342682]
- Pataki I, Jaszberenyi M, Telegdy G. Hyperthermic effect of centrally administered natriuretic peptides in the rat. *Peptides.* 1999; 20:193–197. [PubMed: 10422874]
- Pedersen NP, Blessing WW. Cutaneous vasoconstriction contributes to hyperthermia induced by 3,4-methylenedioxymethamphetamine (Ecstasy) in conscious rabbits. *J. Neurosci.* 2001; 21:8648–8654. [PubMed: 11606652]

- Potter LR, Abbey-Hosch A, Dickey DM. Natriuretic Peptides, Their Receptors, and cyclic Guanosine Monophosphate-dependent Signaling Functions. *Endocrine Reviews*. 2006; 27(1):47–72. [PubMed: 16291870]
- Rusyniak DE, Ootsuka Y, Blessing WW. When administered to rats in a cold environment, 3,4-methylenedioxymethamphetamine reduces brown adipose tissue thermogenesis and increases tail blood flow: effects of pretreatment with 5-HT1A and dopamine D2 antagonists. *Neuroscience*. 2008; 154:1619–1626. [PubMed: 18534763]
- Sengenès C, Berlan M, De Glisezinski I, Lafontan M, Galitzky J. Natriuretic peptides: a new lipolytic pathway in human adipocytes. *FASEB. J.* 2000; 14:1345–1351. [PubMed: 10877827]
- Sengenès C, Zakaroff-Girard A, Moulin A, Berlan M, Bouloumie A, Lafontan M, Galitzky J. Natriuretic peptide-dependent lipolysis in fat cells is a primate specificity. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2002; 283:R257–R265. [PubMed: 12069952]
- Sengenès C, Bouloumie A, Hauner H, Berlan M, Busse R, Lafontan M, Galitzky J. Involvement of a cGMP-dependent pathway in the natriuretic peptide-mediated hormone-sensitive lipase phosphorylation in human adipocytes. *J. Biol. Chem.* 2003; 278:48617–48626. [PubMed: 12970365]
- Sprague JE, Mallet NM, Rusyniak DE, Mills E. UCP3 and thyroid hormone involvement in methamphetamine-induced hyperthermia. *Biochem Pharmacol.* 2004; 68:1339–1343. [PubMed: 15345323]
- Sprague JE, Yang X, Sommers J, Gilman TL, Mills EM. Roles of norepinephrine, free fatty acids, thyroid status, and skeletal muscle uncoupling protein 3 expression in sympathomimetic-induced thermogenesis. *J. Pharmacol. Exp. Ther.* 2007; 320:274–280. [PubMed: 17012607]
- Weber W, Fischli W, Hochuli E, Kupfer E, Weibel EK. Anantin-a peptide antagonist of the atrial natriuretic factor (ANF). I. Producing organism, fermentation, isolation and biological activity. *J. Antibiotics*. 1991; 44(2):164–171. [PubMed: 1849131]
- Zhao J, Cannon B, Nedergaard J. α 1-Adrenergic stimulation potentiates the thermogenic action of β 3-adrenoreceptor-generated cAMP in brown fat cells. *J. Biol. Chem.* 1997; 272:32847–32856. [PubMed: 9407062]
- Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, Lass A, Neuberger G, Eisenhaber F, Hermetter A, Zechner R. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science*. 2004; 306:1383–1386. [PubMed: 15550674]

Highlights

1. A robust increase in ANP was seen within 10 min of MDMA administration.
2. ANP levels returned to baseline at 20 min & then gradually rose over 60 min.
3. Anantin (ANP receptor antagonist) significantly attenuated MDMA hyperthermia.
4. ANP contributes to the induction and maintenance of MDMA-induced thermogenesis.

Figure 1. Time course representation of ANP release following MDMA (40 mg/kg, sc) administration. Each point represents the mean $\pm$ SEM (n = 4 – 16). *Denotes significantly greater than baseline ($p < 0.01$).

Figure 2.

Effect of Anantin (40 mg, ip) on MDMA (40 mg/kg, sc)-induced hyperthermia. Anantin was administered 15 min before and 15 min after MDMA (time 0). A) Each point represents the mean $\pm$ SEM (n = 9). *Denotes significantly different than all other treatment groups (p<0.05). B) Each bar represents the mean $\pm$ SEM (n = 9). *Denotes significantly different than all other treatment groups (p < 0.01).