Published in final edited form as:

Neurosci Lett. 2011 July 8; 498(2): 162–166. doi:10.1016/j.neulet.2011.05.006.

The role of Orexin-1 receptors in physiologic responses evoked by microinjection of PgE2 or muscimol into the medial preoptic area

Daniel E. Rusyniak^{a,b,*}, Dmitry V. Zaretsky^a, Maria V. Zaretskaia^a, and Joseph A. DiMicco^b

^aDepartment of Emergency Medicine, Indiana University School of Medicine, Indianapolis, IN 46202 USA

^bDepartment of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46202 USA

Abstract

The medial preoptic area (mPOA) of the hypothalamus has long been thought to play an important role in both fever production and thermoregulation. Microinjections of prostaglandin E2 (PgE2) or the GABA_A agonist muscimol into the mPOA cause similar increases in body temperature, heart rate, and blood pressure. Microinjections of these compounds however evoke different behavioral responses with muscimol increasing and PgE2 having no effect on locomotion. The purpose of this study was to determine the role of orexin-1 receptors in mediating these dissimilar responses. Systemic injections of the orexin-1 receptor antagonist SB-334867 reduced temperature and cardiovascular responses produced by microinjections of muscimol, but had no effect on either response produced by PgE2. SB-334867 did not significantly decrease locomotion evoked by microinjections of muscimol into the mPOA. These data suggest that there are two central nervous system circuits involved in increasing body temperature, heart rate and blood pressure: one circuit activated by muscimol, involving orexin neurons, and a separate orexin-independent circuit activated by PgE2.

Keywords

microinjection; orexin; muscimol; prostaglandin E2; hypothalamus

Introduction

The preoptic area (POA) of the mammalian hypothalamus is often referred to as the thermoregulatory center of the brain [1, 15]. Research has shown this brain region is involved in mediating thermogenesis to a variety of stimuli, including infection and cold exposure. Microinjecting the GABA_A antagonist bicuculline into the POA blocks

© 2011 Elsevier Ireland Ltd. All rights reserved.

*Corresponding author: Daniel Rusyniak, 1050 Wishard Blvd, RG R2200, Indianapolis, Indiana 46202, Phone: (317) 630-7276, Fax: (317) 656-4216.

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.

Contributions: DVZ, DER, and JAD designed the experiments; DVZ, MVZ, JAD and DER conducted the experiments; DER wrote the manuscript; DER, DVZ, MVZ, and JAD edited the manuscript.

thermogenesis produced by systemic injections of lipopolysaccharide (LPS), a laboratory model of sepsis, and thermogenesis produced by cold exposure [7, 19–22]. Additionally, LPS increases prostaglandin E2 (PgE2) levels in the POA [32], and cold exposure increases GABA concentrations in the medial preoptic area (mPOA) [7]. Therefore, many investigators have come to use microinjections of either PgE2 or GABA agonists (e.g. muscimol) into the mPOA as a laboratory model of thermogenesis; microinjection of either compound evokes a similar cardiovascular and thermogenic response [6, 38]. As neurons in the POA are largely GABAergic, microinjections of inhibitory compounds, such as PgE2 or muscimol, are thought to induce thermogenesis by removing tonic inhibitory tone on downstream thermogenic and cardiogenic brain areas [16, 18, 36].

Despite similarities in the cardiovascular and thermogenic responses seen after microinjections of muscimol and PgE2, these compounds cause opposite behavioral responses: Microinjection of muscimol increases locomotion [5] while microinjection of PgE2 decreases locomotion [9, 10, 38]. Similar responses are seen with the systemic injections of LPS, which reduces locomotion, and exposure to cold which raises locomotion [4, 5, 10]. While the mechanisms by which mPOA microinjections of PgE2 and muscimol produce differing behavioral responses are not known, one possibility may be the selective activation of orexin-containing neurons.

Orexins are neuropeptides made exclusively in the hypothalamus (the lateral hypothalamus [LH] and the dorsomedial hypothalamus [DMH] are the primary locations of orexin-containing neurons) [3]. Similar to microinjections of muscimol into the mPOA, the central administration of orexin-A increases body temperature, heart rate, plasma levels of ACTH, and locomotion [11–14, 24, 30, 35]. As orexin neurons have been shown to receive afferent projections from the POA [27], and to express c-Fos when muscimol is perfused into the mPOA [31], they seem likely neuronal candidates to play a role in the effects mediated by microinjections of muscimol. Unlike microinjections of muscimol, systemic injections of LPS suppress c-Fos expression in orexin neurons [4]. Therefore, we hypothesized that opposite reactions to microinjections of PgE2 and muscimol into the mPOA are, at least in part, mediated by the neuropeptide orexin. To address this, we studied the effects of the specific orexin-1 (OX₁R) receptor antagonist SB-334867 on the cardiovascular, thermal, and behavioral responses generated by microinjections of muscimol and PgE2 into the mPOA.

Methods

Animals

Male Sprague-Dawley rats (300 ± 10 g) from Harlan (Indianapolis, IN) were maintained in 12-h light-dark cycle (lights on at 7am) and fed *ad libitum*. All procedures conformed to guidelines set forth by the NIH and were approved by the Institutional Animal Care and Use Committee. All experiments were performed in a laboratory where ambient temperature was maintained between 24–25°C and humidity maintained between 30–50%. A total of 12 animals were used for this study.

Surgical preparation

Rats were anesthetized with ketamine/xylazine mixture (80 mg/kg ketamine; 12 mg/kg xylazine, i.p.) and placed in a stereotaxic apparatus with the incisor bar set 3.3 mm below the interaural line. A single guide cannula targeting the medial preoptic area was then positioned and secured using previously reported methodology [6, 36, 38]. In addition to a guide cannula, femoral venous catheters (for drug delivery) and intraperitoneal telemetric transmitters (for the measurement of temperature, locomotion, heart rate and blood pressure)

were placed using methods previously described [26, 37, 38]. After the surgery, the rats were allowed to recover in their home cages for at least seven days before experimentation.

Microinjection technique

The procedure for microinjections in both experiments involved connecting a microinjector (33 gauge, Plastics One Inc., Roanoke, VA) to a 10 μ l Hamilton syringe with Teflon FEP tubing (ID=0.12 mm; OD= 0.65 mm; BAS, USA) and loading it with vehicle or with a solution of the drug to be injected. The Hamilton syringe was then mounted in an infusion pump (KDS-200, KD Scientific Inc.) that was used to deliver a volume of 100 nL of over 30 s. The dummy cannula, placed in the guide cannula at the time of surgery, was then removed and the rat was left undisturbed for an additional 40–60 min. At the time of the experiment, a microinjector was inserted into the guide cannula and the drug or vehicle injected. After the microinjection, the injector was left in place for one minute. The microinjection was considered successful if, when tested, immediately after removal flow appeared from the removed injector within 5 s after the pump was reactivated. In addition, injection sites were later confirmed using fluorescent microspheres added to the injection solutions.

Histologic identification of injection sites

Rats were deeply anesthetized with pentobarbital (100 mg/kg i.p.) and perfused transcardially with 60 ml of cold 0.9% saline followed by 60 ml of 4% paraformaldehyde in phosphate-buffered saline (pH 7.4). Brains were removed and post-fixed in the same fixative overnight and then submerged in PBS containing 25% sucrose for at least 24 h at + 4 °C for complete saturation. Subsequently, frozen coronal sections were cut at a nominal thickness of 40 μ m using a Leica cryostat. Two sets of slides were created as consecutive sections and were mounted and processed separately. One was stained with 1% neutral red, cleared in xylene, and coverslipped with Entellan mounting medium (Merck, Darmstadt, Germany). Another was left unstained and coverslipped with water-based Vectashield Hard Set mounting media for fluorescence (Vector Laboratories, Burlingame, CA). Injection sites were determined using both sets of slides by identifying the location of the fluorescent beads using a conventional and fluorescent microscope according to the atlas of Paxinos and Watson by an observer blinded to group allocation as has been previously described (See Figure 1 for a representative example) [23, 25].

Experiment

At the start of the experiments, rats were placed in their home cages on the telemetry receiver plates. A Dataquest telemetry system (Data Sciences, St. Paul, MN) was used to measure heart rate, blood pressure, core body temperature as well as activity of conscious freely moving rats.

Via intravenous catheter, animals in the muscimol group (n=8) were administered either SB-334867 (10 mg/kg) or vehicle. Then, 30 min later, muscimol was microinjected into the mPOA. After a 2-day washout period, animals previously given SB-33486 were given vehicle, and vice versa, followed by microinjection of muscimol into the mPOA. The order of treatment was randomized and an identical procedure was followed for animals in the PgE2 group (n=4) with the exception that PgE2, not muscimol, was microinjected into the mPOA. Doses and volumes of muscimol and PgE2 were 100 pmol/100 nl and 150 pmol/100 nl respectively. The dose of PgE2 was based on our previous work in this lab showing it produces responses similar to those produced by the concentrations of muscimol in this study [38]. The dose of muscimol in this work was chosen to maximize locomotor activity. In our previous paper a dose of 80 pmol, but not 20 pmol, was shown to significantly increase locomotion (comparing units of locomotion in this study with our prior work

requires multiplying the current units by a factor of 10). Increases in temperature, heart rate, mean arterial pressure, and locomotion evoked by 100 pmol of muscimol, demonstrated in this study, are similar to those produced with 80 pmol [38].

Materials

Muscimol (Sigma Aldrich, St.Louis, MO) was dissolved in sterile isotonic saline [4mM], and aliquots were stored at -20°C until the time of the experiment. Prostaglandin E2 (Cayman Chemical, Ann Arbor, MI) was dissolved in 1-methyl-2-pyrrolidinone [0.5M], then aliquoted and stored under argon until use. Muscimol and prostaglandin E2 were reconstituted with sterile isotonic saline immediately before microinjection. SB-334867 (Tocris Bioscience, St.Louis, MO) was prepared immediately before injection by dissolving 10mg of SB-334867 in 40 μl DMSO, 60 μl 1M HCl, and 900 μl of 10% (2-hydroxypropyl)- β -cyclodextrin (Sigma Aldrich, St.Louis, MO) in saline. This resulted in a clear yellow solution. The vehicle was prepared the same way without drug.

Data analysis

Results are expressed as 10 min means $\pm$ SEM. Statistical analyses of physiologic parameters were performed using Statistica for Windows (StatSoft Inc., Tulsa, OK). Data for each group (muscimol and PgE2) were analyzed by analysis of variance (ANOVA) with repeated measures. The within-subject factors were treatment and time. Data were plotted using Prism (GraphPad Software Inc., La Jolla, CA)

Results and Discussion

Systemic injections of the orexin-1 receptor antagonist SB-334867 significantly blunted heart rate, mean arterial pressure, and core temperature caused by microinjections of muscimol into the region of the mPOA (Figure 2). Our findings, partly, corroborate neuroanatomical data. Neurons in the mPOA heavily innervate orexin-synthesizing neurons [27, 34] and perfusing muscimol into the mPOA significantly increases the expression of c-Fos in orexin-synthesizing neurons [31]. Further support for a connection between the mPOA and orexin neurons comes from physiologic studies. Microinjections of the GABA_A-antagonist bicuculline in the lateral hypothalamus, which removes GABAergic tone in the region where the majority of orexin-neurons reside, evoke responses similar to those produced by microinjecting muscimol in the POA: increases in interscapular brown adipose tissue (iBAT) sympathetic nerve activity, iBAT temperature, heart rate, and mean arterial pressure [2]. Central injections of orexin-A likewise increase iBAT sympathetic nerve activity, iBAT temperature, heart rate, and mean arterial pressure [12, 13, 17, 28, 30]. These data, suggest that GABAergic neurons in the POA provide tonic inhibitory tone to orexin-synthesizing neurons and that removing this tone contributes to increases body temperature, heart rate, and blood pressure.

Despite these findings, and contrary to our hypothesis, SB-334867 did not significantly reduce locomotion mediated by microinjections of muscimol. While there was a trend ($p=0.11$, Figure 2) toward a reduction in locomotion, the results did not reach the level of statistical significance. This may be the result of the large amount of variability in locomotor responses seen between individual animals.

It is also possible that locomotor responses mediated by muscimol involve a separate pathway which does not involve orexin neurons. A more plausible explanation, however, is that a significant amount of locomotion induced by orexin involves the orexin-2 receptor (OX₂R). This possibility is supported by recent data from Samson et al. who showed that a specific OX₂R receptor agonist induced locomotor behavior, and that this behavior was not

blocked by an OX₁R receptor antagonist [29]. Furthermore, Samson et al. showed locomotion evoked by the central administration of orexin-A is only partially affected by an OX₁R receptor antagonist [29]—a finding similar to ours.

In contrast to its effects with muscimol, SB-334867 did not attenuate cardiovascular or temperature effects mediated by microinjections of PgE2 into the mPOA (Figure 2). This data is in contrast to anatomic data which show that EP3 receptor-expressing neurons project to neurons in the LH [18]. Despite these neuroanatomic connections, microinjections of muscimol into the LH has no effect on PgE2-induced increases in iBAT sympathetic nerve activity, iBAT temperature, heart rate, and core body temperature [18]. Furthermore, while Monda et al showed that systemic injections of salicylate decreased orexin-A-induced tachycardia, iBAT, and core body temperature, these effects were not evident until 1.5 to 2.0 h after the central injection of orexin-A [12]; these authors suggested that prostaglandins were not involved in initiating temperature effects from orexin [12]. Our data would likewise suggest that orexin neurons are not involved in mediating the early effects of PgE2 microinjections. While not specifically tested, it is unlikely that OX₂R receptors play a significant role on PgE2-induced changes in heart rate, blood pressure, or body temperature. Previous work by others have shown that cardiovascular and temperature effects in response to the central administration of orexin-A are blocked by OX₁R receptors antagonists [28] and that central administration of orexin-B (which is more specific to OX₂R receptors) does not produce increases in body temperature [8]. Although not specifically studied, it is possible that orexin-B, and therefore OX₂R, may play a role in cardiovascular effects mediated by PgE2 as the central administration of orexin-B has been shown to increase heart rate and mean arterial pressure [33].

Together our findings, that the OX₁R receptor antagonist SB-334867 attenuated the cardiovascular and thermogenic effects evoked by microinjections of muscimol, but not PgE2, suggest that inhibition of GABAergic neurons in the mPOA activate two circuits: one which involves orexin neurons and one which does not. Furthermore, our data implies that inhibiting the subset of GABAergic neurons in the mPOA expressing EP3 receptors activates the orexin-independent circuit.

Abbreviations

DMH	Dorsomedial hypothalamus
iBAT	interscapular brown adipose tissue
LH	lateral hypothalamus
LPS	lipopolysaccharide
mPOA	medial preoptic area
OX₁R	orexin-1
OX₂R	orexin-2
PAO	preoptic area
PgE2	prostaglandin E2

Acknowledgments

This work was supported by USPHS grants DA020484 and NS 19883. In addition, the authors would like to acknowledge the valuable editorial assistance of Pamela J. Durant.

References

1. Boulant JA. Role of the preoptic-anterior hypothalamus in thermoregulation and fever. *Clin. Infect. Dis.* 2000; 31:S157–S161. [PubMed: 11113018]
2. Cerri M, Morrison SF. Activation of lateral hypothalamic neurons stimulates brown adipose tissue thermogenesis. *Neuroscience*. 2005; 135:627–638. [PubMed: 16125857]
3. de Lecea L, Kilduff TS, Peyron C, Gao X-B, Foye PE, Danielson PE, Fukuhara C, Battenberg ELF, Gautvik VT, Bartlett FS, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG. The hypocretins: Hypothalamus-specific peptides with neuroexcitatory activity. *Proc. Natl. Acad. Sci. U. S. A.* 1998; 95:322–327. [PubMed: 9419374]
4. Gaykema RPA, Goehler LE. Lipopolysaccharide challenge-induced suppression of Fos in hypothalamic orexin neurons: Their potential role in sickness behavior. *Brain. Behav. Immun.* 2009; 23:926–930. [PubMed: 19328847]
5. Girardier L, Clark MG, Seydoux J. Thermogenesis associated with spontaneous activity: an important component of thermoregulatory needs in rats. *J. Physiol.* 1995; 488(Pt 3):779–787. [PubMed: 8576867]
6. Hunt JL, Zaretsky DV, Sarkar S, Dimicco JA. Dorsomedial hypothalamus mediates autonomic, neuroendocrine, and locomotor responses evoked from the medial preoptic area. *Am. J. Physiol.* 2010; 298:R130–R140.
7. Ishiwata T, Saito T, Hasegawa H, Yazawa T, Kotani Y, Otokawa M, Aihara Y. Changes of body temperature and thermoregulatory responses of freely moving rats during GABAergic pharmacological stimulation to the preoptic area and anterior hypothalamus in several ambient temperatures. *Brain Res.* 2005; 1048:32–40. [PubMed: 15913569]
8. Jones DNC, Gartlon J, Parker F, Taylor SG, Routledge C, Hemmati P, Munton RP, Ashmeade TE, Hatcher JP, Johns A, Porter RA, Hagan JJ, Hunter AJ, Upton N. Effects of centrally administered orexin-B and orexin-A: a role for orexin-1 receptors in orexin-B-induced hyperactivity. *Psychopharmacology (Berl)*. 2001; 153:210–218. [PubMed: 11205421]
9. Konsman JP, Parnet P, Dantzer R. Cytokine-induced sickness behaviour: mechanisms and implications. *Trends Neurosci.* 2002; 25:154–159. [PubMed: 11852148]
10. Kozak W, Conn CA, Kluger MJ. Lipopolysaccharide induces fever and depresses locomotor activity in unrestrained mice. *Am. J. Physiol.* 1994; 266:R125–R135. [PubMed: 8304533]
11. Kuru M, Ueta Y, Serino R, Nakazato M, Yamamoto Y, Shibuya I, Yamashita H. Centrally administered orexin/hypocretin activates HPA axis in rats. *Neuroreport*. 2000; 11:1977–1980. [PubMed: 10884055]
12. Monda M, Viggiano A, Mondola P, De Luca V. Inhibition of prostaglandin synthesis reduces hyperthermic reactions induced by hypocretin-1/orexin A. *Brain Res.* 2001; 909:68–74. [PubMed: 11478922]
13. Monda M, Viggiano A, Viggiano A, Viggiano E, Messina G, Tafuri D, De Luca V. Sympathetic and hyperthermic reactions by orexin A: Role of cerebral catecholaminergic neurons. *Regul. Pept.* 2007; 139:39–44. [PubMed: 17134769]
14. Monda M, Viggiano AN, Viggiano A, Viggiano E, Lanza A, De Luca V. Hyperthermic reactions induced by orexin A: role of the ventromedial hypothalamus. *Eur. J. Neurosci.* 2005; 22:1169–1175. [PubMed: 16176359]
15. Morrison SF, Nakamura K, Madden CJ. Central control of thermogenesis in mammals. *Exp. Physiol.* 2008; 93:773–797. [PubMed: 18469069]
16. Nakamura K, Morrison SF. Central efferent pathways mediating skin cooling-evoked sympathetic thermogenesis in brown adipose tissue. *Am. J. Physiol.* 2007; 292:R127–R136.
17. Nakamura T, Uramura K, Nambu T, Yada T, Goto K, Yanagisawa M, Sakurai T. Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. *Brain Res.* 2000; 873:181–187. [PubMed: 10915829]
18. Nakamura Y, Nakamura K, Matsumura K, Kobayashi S, Kaneko T, Morrison SF. Direct pyrogenic input from prostaglandin EP3 receptor-expressing preoptic neurons to the dorsomedial hypothalamus. *Eur. J. Neurosci.* 2005; 22:3137–3146. [PubMed: 16367780]

19. Osaka T. Blockade of prostaglandin E2-induced thermogenesis by unilateral microinjection of GABAA receptor antagonist into the preoptic area. *Brain Res.* 2008; 1230:107–114. [PubMed: 18662676]
20. Osaka T. Cold-induced thermogenesis mediated by GABA in the preoptic area of anesthetized rats. *Am. J. Physiol.* 2004; 287:R306–R313.
21. Osaka T. Lipopolysaccharide-induced thermogenesis mediated by GABA in the preoptic area of anesthetized rats. *J. Therm. Biol.* 2006; 31:229–234.
22. Osaka T. Prostaglandin E2 fever mediated by inhibition of the GABAergic transmission in the region immediately adjacent to the organum vasculosum of the lamina terminalis. *Pflügers Arch.* 2008; 456:837–846. [PubMed: 18188586]
23. Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. New York: Academic Press; 1998.
24. Russell SH, Small CJ, Dakin CL, Abbott CR, Morgan DG, Ghatei MA, Bloom SR. The central effects of orexin-A in the hypothalamic-pituitary-adrenal axis in vivo and in vitro in male rats. *J. Neuroendocrinol.* 2001; 13:561–566. [PubMed: 11412343]
25. Rusyniak DE, Zaretskaia MV, Zaretsky DV, DiMicco JA. 3,4-Methylenedioxymethamphetamine- and 8-hydroxy-2-di-n-propylamino-tetralin-induced hypothermia: role and location of 5-hydroxytryptamine 1A receptors. *J. Pharmacol. Exp. Ther.* 2007; 323:477–487. [PubMed: 17702902]
26. Rusyniak DE, Zaretskaia MV, Zaretsky DV, DiMicco JA. Microinjection of muscimol into the dorsomedial hypothalamus suppresses MDMA-evoked sympathetic and behavioral responses. *Brain Res.* 2008; 1226:116–123. [PubMed: 18586013]
27. Sakurai T, Nagata R, Yamanaka A, Kawamura H, Tsujino N, Muraki Y, Kageyama H, Kunita S, Takahashi S, Goto K, Koyama Y, Shioda S, Yanagisawa M. Input of orexin/hypocretin neurons revealed by a genetically encoded tracer in mice. *Neuron.* 2005; 46:297–308. [PubMed: 15848807]
28. Samson WK, Bagley SL, Ferguson AV, White MM. Hypocretin/orexin type 1 receptor in brain: role in cardiovascular control and the neuroendocrine response to immobilization stress. *Am. J. Physiol.* 2007; 292:R382–R387.
29. Samson WK, Bagley SL, Ferguson AV, White MM. Orexin receptor subtype activation and locomotor behaviour in the rat. *Acta Physiologica.* 2010; 198:313–324. [PubMed: 19889100]
30. Samson WK, Gosnell B, Chang JK, Resch ZT, Murphy TC. Cardiovascular regulatory actions of the hypocretins in brain. *Brain Res.* 1999; 831:248–253. [PubMed: 10412003]
31. Satoh S, Matsumura H, Fujioka A, Nakajima T, Kanbayashi T, Nishino S, Shigeyoshi Y, Yoneda H. FOS expression in orexin neurons following muscimol perfusion of preoptic area. *Neuroreport.* 2004; 15:1127–1131. [PubMed: 15129159]
32. Sehic E, Székely M, Ungar AL, Oladehin A, Blatteis CM. Hypothalamic prostaglandin E2 during lipopolysaccharide-induced fever in guinea pigs. *Brain Res. Bull.* 1996; 39:391–399. [PubMed: 9138749]
33. Shirasaka T, Nakazato M, Matsukura S, Takasaki M, Kannan H. Sympathetic and cardiovascular actions of orexins in conscious rats. *Am. J. Physiol.* 1999; 277:R1780–R1785. [PubMed: 10600926]
34. Yoshida K, McCormack S, España RA, Crocker A, Scammell TE. Afferents to the orexin neurons of the rat brain. *J. Comp. Neurol.* 2006; 494:845–861. [PubMed: 16374809]
35. Yoshimichi G, Yoshimatsu H, Masaki T, Sakata T. Orexin-A regulates body temperature in coordination with arousal status. *Exp. Biol. Med.* 2001; 226:468–476.
36. Zaretskaia MV, Zaretsky DV, DiMicco JA. Role of the dorsomedial hypothalamus in thermogenesis and tachycardia caused by microinjection of prostaglandin E2 into the preoptic area in anesthetized rats. *Neuroscience Letters.* 2003; 340:1–4. [PubMed: 12648744]
37. Zaretskaia MV, Zaretsky DV, Shekhar A, DiMicco JA. Chemical stimulation of the dorsomedial hypothalamus evokes non-shivering thermogenesis in anesthetized rats. *Brain Res.* 2002; 928:113–125. [PubMed: 11844478]
38. Zaretsky DV, Hunt JL, Zaretskaia MV, DiMicco JA. Microinjection of prostaglandin E2 and muscimol into the preoptic area in conscious rats: Comparison of effects on plasma

adrenocorticotrophic hormone (ACTH), body temperature, locomotor activity, and cardiovascular function. *Neurosci. Lett.* 2006; 397:291–296. [PubMed: 16406311]

Figure 1.

Photomicrographs of coronal rat brain sections from a representative medial preoptic area microinjection experiment. The distribution of red (A) and green (B) fluorescent microspheres marks the site of injections of muscimol (100pmol/100nl) into the mPOA of a single animal on two separate occasions (once pretreated with intravenous saline and once with intravenous SB-334867). The corresponding neutral red counterstained section (C) shows the anatomic landmarks used for marking injection sites. A schematic representing the area of injections for all the animals is shown in figure D. The schematic was adapted from the atlas of Paxinos and Watson [23]. MnPO, median preoptic area; och, optic chiasm; VOLT, vascular organ of the lamina terminalis; aca, anterior commissure; 3V, third ventricle.

Figure 2.

Effect of SB-334867 on cardiovascular, thermogenic and locomotor responses to 150 pmol/100nl of PgE2 (left column, n=4) and 100 pmol/100nl muscimol (right column, n=8) microinjected into medial preoptic area of conscious rats. SB-334867 (closed circles) or vehicle (open circles) was administered i.p. 30 minutes before microinjection. There were no statistically significant differences between any of the baseline values between groups before microinjection. When tested by ANOVA with repeated measures, the effect of SB-334867 was significantly different ($p < 0.05$, denoted by *) in Muscimol group for heart rate ($F(1,7)=9.7$, $p=0.017$), mean arterial pressure ($F(1,7)=10.4$, $p=0.015$), and temperature ($F(1,6)=11.4$, $p=0.012$). SB-334867 had no effect on locomotion produced by muscimol ($F(1,7)=3.3$, $p=0.11$) or any parameter in PgE2 treated animals ($p > 0.1$).