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Title Page

5-HT_{2A} receptor agonist-induced hyperthermia is induced via vasoconstriction by peripheral 5-HT_{2A} receptors and brown adipose tissue thermogenesis by peripheral serotonin loss at a high ambient temperature

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Running Title Page

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Abbreviations

BAT, Brown adipose tissue

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BBB, Blood brain barrier

iBAT, Intrascapular brown adipose tissue

MDMA, 3,4-methylenedioxymethamphetamine

PBS, Phosphate buffered saline

UCP1, Uncoupling protein-1

TPH, Tryptophan hydroxylase

25B-NBOMe,

2-(4-Bromo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine

5-HT, 5-Hydroxytryptamine

5,7-DHT, 5,7-dihidroxytryptamine

6-OHDA, 6-hydroxydopamine

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Abstract

Recreational drugs, such as 3,4-methylenedioxymethamphetamine (MDMA) and cocaine, induce hyperthermia, which is affected by ambient temperature. 2-(4-Bromo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine (25B-NBOMe), a selective agonist of 5-HT_{2A} receptor used as a recreational drug, reportedly induces hyperthermia. This study aimed to verify whether 25B-NBOMe induces ambient temperature-dependent hyperthermia and to clarify its mechanism. Eight-week-old male Sprague–Dawley rats were administered intraperitoneal injection of 25B-NBOMe at an ambient temperature of 23°C or 29°C. 25B-NBOMe administration at 23°C did not change the core body temperature of the rats, whereas administration at 29°C induced significant hyperthermia 30–120 min post-administration. Tail surface temperature temporarily decreased 30 min post-administration, indicating heat storage by peripheral vasoconstriction despite a high ambient temperature. Because 25B-NBOMe-induced-hyperthermia was suppressed by sarpogrelate, but not by destruction of central noradrenaline or serotonin neurons, peripheral 5-HT_{2A} receptors were considered to contribute to the development of hyperthermia at a high ambient temperature, independently from central neurons. The temperature of brown adipose tissue (BAT) increased 60–120 min post-administration of 25B-NBOMe at 29°C, indicating thermogenesis. Previous studies have reported that peripheral

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serotonin contributes to the inhibition of BAT thermogenesis. Decreased plasma serotonin levels were observed at 29°C, and serotonin administration partially suppressed 25B-NBOMe-induced hyperthermia at a high ambient temperature, suggesting that decreased levels of peripheral serotonin induced BAT thermogenesis. Our findings indicate that 25B-NBOMe induces hyperthermia at a high ambient temperature via vasoconstriction regulated by 5-HT_{2A} receptors and BAT thermogenesis mediated by decreased levels of plasma serotonin. Thus, peripheral serotonin partially plays an important role in thermoregulation.

Introduction

Recreational drugs, such as 3,4-methylenedioxymethamphetamine (MDMA) or cocaine, are known to induce hyperthermia, which is affected by ambient temperatures (Parrot, 2012; Auger et al., 2017). Freedman et al. verified that ambient temperature-dependent hyperthermia is induced by MDMA administration in 10 participants, and reported that body temperature among the participants following drug-administration at 30°C was significantly higher than that at 18°C (Freedman et al., 2005). Many studies have reported ambient temperature-dependent drug-induced hyperthermia in animal models. Gonzalez demonstrated that hyperthermia occurred in rats administered cocaine at 27°C, whereas cocaine administered at 20°C induced hypothermia (Gonzalez, 1993). Malberg and Seiden studied MDMA-induced changes in the body temperature of rats at every 2°C change in a strictly controlled ambient temperature and demonstrated that ambient temperatures of ≥24°C and ≤22°C induce hyperthermia and hypothermia, respectively (Malberg and Seiden., 1998), suggesting that MDMA attenuates thermoregulation. However, the precise mechanism underlying ambient temperature-dependent hyperthermia remains unknown.

Thermal information is perceived by the transient receptor potential family in the skin. At high ambient temperatures, signals from the thermoregulatory central neural

pathways of the preoptic area, medial preoptic area, and dorsal hypothalamic area as well as the rostral raphe pallidus nucleus are transmitted to the peripheral thermoregulatory organs, resulting in heat loss through vasodilation and evaporative cooling through sweating or reduced thermogenesis in skeletal muscles and brown adipose tissue (BAT) (Morrison and Nakamura, 2011). Certain serotonin neurons have been reported to play specific roles in the central neural thermoregulation system (Hodges and Richerson, 2010), and central serotonin neurons in the thermoregulatory system have been the key focus of drug-induced hyperthermia studies because many drugs affecting serotonergic neurons induce hyperthermia (Musselman and Saely, 2013). Lin et al. have reported that the 5-HT_2 receptor agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) injected into the rat hypothalamus increased intracellular serotonin levels, leading to hyperthermia, whereas 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) injections exerted an adverse hypothermic effect (Lin et al., 1998). Some studies have reported that DOI induced peripheral vasoconstriction and non-shivering thermogenesis in the intrascapular BAT (iBAT) in rabbits and rats via sympathetic neural stimulation (Blessing and Seaman, 2003; Ootsuka and Blessing, 2006).

Serotonin is a neurotransmitter, but at the same time, it also acts as a peripheral hormone in various organs (Gamoh et al., 2013; Herr et al., 2017). Separated by the

blood-brain barrier (BBB), central serotonin is synthesized by tryptophan hydroxylase (TPH) 2 in the brain stem, while peripheral serotonin is synthesized by TPH1 in the gut (Walther and Bader, 2003); hence, central and peripheral serotonins are considered to be independent from each other. Currently, the roles of peripheral serotonin and its receptors in the thermoregulatory system have been gaining attention.

5-HT_{2A} receptors are distributed in cardiovascular smooth muscle cells and platelets and contribute to vasoconstriction and aggregation, respectively (Kaumann et al., 2006). Rat adipose tissue has also been reported to bear 5-HT_{2A} receptors, which suppress lipolysis by β-adrenergic stimulation (Hansson et al., 2016). Recently, Crane et al. formulated a novel theory regarding the association between BAT thermogenesis and peripheral serotonin levels (Crane et al., 2015). They demonstrated that peripheral serotonin reduced by TPH1 inhibition increases the expression of uncoupling protein-1 (UCP1) in the mitochondrial membrane and iBAT thermogenesis induced by noradrenergic stimulation, indicating a peripheral serotonin thermoregulatory system independent from central signaling; however, the association of the theory with drug-induced hyperthermia or whether it is affected by ambient temperature remains unclear.

Drug-induced hyperthermia has also been reported in cases using

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2-(4-bromo-2,5-dimethoxyphenyl)-*N*-(2-methoxybenzyl)ethanamine (25B-NBOMe), a substance abused as a recreational drug (Poklis et al., 2014; Yoshida et al., 2015). NBOMes are compounds formed by the addition of a 2-methoxybenzyl group to a phenethylamine, 4-bromo-2,5-dimethoxyphenylamine (2C-B), and act as agonists of 5-HT_{2A} and 5-HT_{2C} receptors. In particular, 25B-NBOMe has been reported to show a high affinity for 5-HT_{2A} receptors (Juncosa Jr. et al., 2013). Intravenous (Ettrup et al., 2013) or intraperitoneal (Shintani-Ishida et al., 2018) 25B-NBOMe injections show that the compound is heavily localized in the lungs and multiple peripheral organs, as well as the brain.

In this study, we confirmed that intraperitoneal 25B-NBOMe administration induces hyperthermia in rats at a high ambient temperature, which is similar to the effects of MDMA and cocaine. Moreover, we aimed to verify the contribution of the peripheral thermoregulatory system to ambient temperature-dependent 25B-NBOMe-induced hyperthermia in an animal model.

Materials and Methods

Eight-week-old male Sprague–Dawley rats (n = 131) corresponding to young adults in human (Sengupta, 2013) were purchased from Shimizu Laboratory Supplies Company (Kyoto, Japan). Postoperatively, the rats were individually housed in cages

with wood-chip bedding at 23°C ± 1°C ambient temperature, 12/12-h light/dark cycle, with free access to water and food. The experiments were performed with the rats under freely moving conditions in the same cage.

All experiments were started at 2:30 PM, considering the influence of the circadian rhythm. The ambient temperature was controlled by a room air conditioner and was maintained (23°C or 29°C) from 1 h before the start of the experiment.

The core body temperature was measured by implanting a logger “DST milli-T” (Star-Oddi, Gardabaer, Iceland), which was set up to gather samples at 5-min intervals during the experiment, into the abdominal cavity. Implantation was performed under general anesthesia (isoflurane inhalation, 2.0%–2.5%) at least 4 days before the start of the experiment for recovery. Data were retrieved after the experiment and analyzed using Mercury Software (Star-Oddi). The same method was used to measure BAT temperature by implanting the logger into the intrascapular area (Cannon et al., 2004). Tail surface temperature was measured using an infrared thermometer FHT-P2 Avantek (Claybox Ltd., Hong Kong) at 10-min intervals during the experiment. Before the experiment, the accuracy of the infrared thermometer was confirmed to be less than 1.76% at 23°C–29°C ambient temperatures by measuring water at 34–50°C.

25B-NBOMe hydrochloride (PubChem CID: 76965389; Kim et al., 2016) was

obtained from Lipomed (Arlesheim, Switzerland), and 1 mg of the compound was dissolved in 10 µl dimethyl sulfoxide and 90 µl methanol and subsequently diluted to 0.25 mg/ml with phosphate-buffered saline (PBS) for intraperitoneal administration. Serotonin hydrochloride (CID: 160436) was purchased from Nakalai Tesque (Kyoto, Japan) and was dissolved in PBS to achieve a concentration of 0.05 mg/ml or 0.1 mg/ml. Sarpogrelate hydrochloride (CID: 444005) was obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan), and 1.0 mg of the compound was dissolved in 10 µl dimethyl sulfoxide and subsequently diluted to 5.0 mg/ml with PBS. Sarpogrelate hydrochloride concentration was determined via a previously described method (Rajesh et al., 2006). 6-Hydroxydopamine (6-OHDA) hydrobromide (CID: 176170) and 5,7-dihidroxytryptamine (5,7-DHT) hydrobromide (CID: 35781) were obtained from Sigma-Aldrich (St. Louis, MO) and Adipogen Life Sciences, Inc. (San Diego, CA), respectively. Ten milligram of each compound was diluted in 400 µl saline containing 0.1% ascorbic acid. Escitalopram oxalate (CID: 146571) and desipramine hydrochloride (CID: 65327) were obtained from Sigma-Aldrich and Wako Pure Chemical Industries, Ltd, respectively. Escitalopram was diluted to 5.0 mg/ml with PBS and desipramine was diluted to 25.0 mg/ml with PBS.

Intraventricular administration of 6-OHDA or 5,7-DHT was carried out as previously described (Reader and Gauthier, 1984; Tanaka et al., 2016). Rats were pretreated

intraperitoneally with 5.0 mg/kg of escitalopram 30 min before 6-OHDA infusion, or 25.0 mg/kg of desipramine similarly before 5,7-DHT infusion. Under general anesthesia (isoflurane inhalation, 2.5%–3.0%), 6-OHDA (200 μg/8 μl) or 5,7-DHT (200 μg/8 μl) was infused into the bilateral ventricle (0.80 mm posterior, 1.5 mm lateral, 4.0mm depth from bregma and skull; Paxinos and Watson, 1986) using a 23-gauge needle and a mycrosyringe infusion pump over a period of 10 min. The rats had at least 5 days to recover before 25B-NBOMe was administered. The effects of 6-OHDA and 5,7-DHT were confirmed by measuring catecholamine and serotonin levels in brain tissue using high-performance liquid chromatography (Loftis et al., 2010).

Platelet-poor plasma was prepared from rats 1 h post 25B-NBOMe administration to measure noradrenaline, adrenaline, dopamine, and serotonin levels. The rats were killed by collecting total blood by cardiac centesis under general anesthesia. Blood was transferred into a plastic tube containing Na_2EGTA and centrifuged within 30 min from collection to separate the plasma: Plasma levels of three different catecholamines and serotonin were measured at LSI Medience Corporation (Tokyo, Japan) using high-performance liquid chromatography and an enzyme-linked immunosorbent assay, respectively.

Temperature data are presented as the mean ± S.E. Biochemical levels are shown as

the median with 75% confidence intervals. Statistical analyses were performed using "EZR (Easy R)" on R Commander version 1.31 (based on R Commander version 2.2-3) (Kanda, 2014).

Animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Animal Experiments Committee of Kyoto Prefectural University of Medicine (approval number: M28-417).

Results

25B-NBOMe induced hyperthermia at a high ambient temperature

Intraperitoneal and tail surface temperatures of rats were monitored following the administration of either 0.25 mg/kg of 25B-NBOMe or vehicle at 23°C or 29°C ambient temperature. The administration of 25B-NBOMe or vehicle at 23°C did not increase core body temperature (Fig 1). However, at 29°C, the administration of 25B-NBOMe significantly increased the core body temperature 30–120 min post-administration compared with the administration of vehicle (one-way ANOVA, followed by Tukey multiple comparison tests, $P < 0.05$) (Fig 1).

25B-NBOMe impaired heat loss through vasodilation via 5-HT_{2A} receptors, resulting in hyperthermia at a high ambient temperature

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The tail surface temperature of the rats reflects peripheral vasoconstriction or vasodilation (El Bitar et al., 2014). Before the experiments, the tail temperatures of rats at 29°C were higher than those at 23°C (Fig 2), indicating vasodilation, as previously reported (El Bitar et al., 2014). At 29°C ambient temperature, 25B-NBOMe administration temporarily decreased tail surface temperature with a peak at 30 min post-administration compared with the vehicle group (one-way ANOVA, followed by Tukey multiple comparison test, $P < 0.05$). At 23°C ambient temperature, the tail surface temperature was not significantly different between the 25B-NBOMe and vehicle groups (Fig 2).

To investigate whether hyperthermia during the early phase of 30 min post-administration was due to vasoconstriction induced by 25B-NBOMe, 5.0 mg/kg sarpogrelate was intraperitoneally administered 30 min before the administration of 0.25 mg/kg 25B-NBOMe to the animals at 29°C. Sarpogrelate hydrochloride is a selective antagonist of 5-HT_{2A} receptors (Pertz and Elz, 1995) that cannot pass the BBB (Nitanda et al., 2005). The 25B-NBOMe-induced decrease in tail surface temperature was controlled in sarpogrelate pre-administered animals to an insignificant level compared with that in the vehicle group (Fig 3A). Thus, sarpogrelate pre-administration restrained 25B-NBOMe-induced hyperthermia at a high ambient temperature (Welch's t-test, $P < 0.05$) (Fig 3B).

25B-NBOMe induced BAT thermogenesis during the late phase at a high ambient temperature

The tail surface temperatures of animals administered 25B-NBOMe at 29°C ambient temperature returned to baseline 60 min post-administration, but the core body temperature remained higher until 120 min post-administration. The temperature of the intrascapular area, where BAT is distributed in rats (Al-Noori et al., 2016; Crane et al., 2015), was monitored to evaluate BAT thermogenesis as another indicator of hyperthermia. The BAT temperature was elevated at 30 min post-administration, which was similar in both the vehicle and 25B-NBOMe groups (Fig 4). BAT temperature in the vehicle group gradually returned to the baseline after 30 min (Fig 4). In contrast, the BAT temperature in the 25B-NBOMe group continued to increase at 60–90 min post-administration (Fig 4), showing a similar trend to the changes in core body temperature (Fig 1).

Plasma serotonin level decreased at a high ambient temperature

Plasma catecholamine and serotonin levels were measured 1 h post-administration, when the BAT temperature significantly increased in the 25B-NBOMe group at 29°C ambient temperature (Fig 4). Both the 25B-NBOMe and vehicle groups showed lower

plasma serotonin levels at 29°C than those at 23°C (Kruskal–Wallis rank sum test followed by Steel Dwass test, $P < 0.05$; 23°C vehicle vs. 29°C vehicle, 23°C vehicle vs. 29°C 25B-NBOMe, 23°C 25B-NBOMe vs. 29°C vehicle, and 23°C 25B-NBOMe vs. 29°C 25B-NBOMe) (Fig 5). Noradrenaline, adrenaline, and dopamine levels tended to be higher in the 25B-NBOMe group than in the vehicle group at both 23°C and 29°C ambient temperatures although the difference was not statistically significant (data not shown).

Pre-injection of serotonin prevented the prolongation of 25B-NBOMe-induced hyperthermia at a high ambient temperature

To investigate whether decreased serotonin levels contribute to 25B-NBOMe-induced hyperthermia, 0.05 mg/kg or 0.1 mg/kg serotonin was intraperitoneally administered to the animals at 29°C 30 min before the administration of 0.25 mg/kg 25B-NBOMe to compensate for the decreased plasma serotonin. In a pretreatment with 0.1 mg/kg serotonin, the core body temperature started to increase post 25B-NBOMe administration, but began to decrease after 60 min, and was significantly lower at 90 min than that in the 25B-NBOMe group alone (Welch's t-test, $P < 0.05$) (Fig 6A). Pretreatment with 0.05 mg/kg serotonin slightly suppressed 25B-NBOMe-induced hyperthermia at 60–120 min, indicating a dose-dependent effect (Fig 6A). BAT

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hyperthermia was also diminished by pretreatment with 0.1 mg/kg serotonin although this was not statistically significant (Fig 6B).

Destruction of central 5-HT neurons or central noradrenaline neurons did not attenuate 25B-NBOMe-induced hyperthermia at a high ambient temperature

Intraventricular administration of 6-OHDA, a selective neurotoxin that destroys noradrenaline neurons prior to dopamine neurons depending on the dose (Uretsky and Iversen, 1970), decreased brain noradrenaline levels (Supplemental Data). On the other hand, intraventricular administration of 5,7-DHT, which destroys serotonin neurons (Reader and Gauthier, 1983), decreased brain serotonin levels (Supplemental Data). Rats were given 0.25 mg/kg 25B-NBOMe at 29°C ambient temperature. The administration of 25B-NBOMe induced hyperthermia in the animals pretreated with 6-OHDA as well as in sham-operated animals. The core body temperatures in those pretreated with 5,7-DHT showed significant elevation 30–150 min post-administration of 25B-NBOMe compared with the sham group, resulting in three fatalities: two rats died 90 min post-administration; another died after 150 min (Fig 7).

Discussion

In this study, we successfully demonstrated that 25B-NBOMe induced hyperthermia in rats, which has been observed among patients abusing 25B-NBOMe compounds. Our study was conducted with 0.25 mg/kg 25B-NBOMe intraperitoneal administration, which corresponds to a moderate to high dose in humans (Ettrup et al., 2013; Papoutsis et al., 2015). However, hyperthermia was induced only at a high ambient temperature (Fig 1). High ambient temperatures have been reported to enhance hyperthermia induced by adrenergic substances, such as MDMA and cocaine (Parrot, 2012; Gonzales, 1993); however, our study showed that other drugs, including serotonergic agents, can mediate a similar phenomenon.

25B-NBOMe-induced hyperthermia in rats at 29°C lasted 120 min, with two failures in peripheral thermoregulation. The first failure was observed during the early phase of hyperthermia, at 30 min post 25B-NBOMe administration. Increased tail surface temperature due to vasodilation at a high ambient temperature was temporarily suppressed by 25B-NBOMe (Fig 2). A previous report indicated that 5-HT_{2A} receptor agonist induces rat tail vasoconstriction and increases core body temperature (Blessing and Seamann, 2003). This mechanism has been recognized to contribute to the maintenance of thermoneutral conditions at a low ambient temperature or to be an action toward pain (El Bitar et al., 2014). In our study, temporal vasoconstriction was not observed in the vehicle group; therefore, vasoconstriction may result from

5-HT_{2A} receptor activation by 25B-NBOMe rather than an action toward pain caused by the intraperitoneal injection. 25B-NBOMe administration at 23°C did not show significant vasoconstriction, perhaps because ambient temperature affects vasoconstriction to a certain extent (Ootsuka and Blessing, 2006). Sarpogrelate hydrochloride, a selective 5-HT_{2A} receptor blocker, inhibited 25B-NBOMe-induced vasoconstriction at 29°C (Fig 3A), resulting in the control of the early-phase hyperthermia (Fig 3B). Blessing et al. reported that the administration of DOI reduces cutaneous blood flow in rabbits and rats and simultaneously increases their core body temperatures. Because DOI-induced vasoconstriction was reduced in rabbits with cervical sympathetic trunk cuts, they posited that peripheral vasoconstriction by DOI is regulated by sympathetic neural stimulation (Blessing and Seamann, 2003). However, because sarpogrelate cannot pass the BBB (Nitanda et al., 2005), 5-HT_{2A} receptors in the peripheral organs, such as vascular smooth muscle, must contribute to thermoregulation, in addition to the central or sympathetic nerves.

We considered another mechanism for the prolongation of hyperthermia in the late phase because the decrease in tail surface temperature was transient. High iBAT temperature was observed 90 min post 25B-NBOMe administration, indicating iBAT thermogenesis, only in the 25B-NBOMe-administered rats at a high ambient temperature (Fig 4). Crane et al. demonstrated that serotonin attenuates

isoproterenol-stimulated cyclic AMP in iBAT cells, thus suggesting that peripheral serotonin controls BAT thermogenesis triggered by β-adrenergic stimulation (Crane et al., 2015). Moreover, Oh et al. demonstrated that 5-HT_3 receptor activation by 1-(m-chlorophenyl)-biguanide (m-CPBG) in brown adipocytes suppresses β-adrenergic thermogenesis (Oh et al., 2015). In our study, a high ambient temperature reduced plasma serotonin level in rats despite 25B-NBOMe administration (Fig 5), and compensation for the decreased serotonin level via pre-injection with a 0.1 mg/kg dose controlled the elevation of core body temperature in the late phase and shortened the duration of hyperthermia (Fig 6A). Because the supplied serotonin neither controls early-phase hyperthermia nor passes the BBB (Gershon and Tack, 2007), we propose that the decreased plasma serotonin levels contributed to the prolongation of hyperthermia in the late phase. BAT thermogenesis in the study by Crane et al. might have contributed to late-phase hyperthermia, and the supplied serotonin might have controlled BAT thermogenesis via 5-HT_3 receptors. However, the contribution made by the peripheral regulation of BAT thermogenesis during drug-induced hyperthermia may in part be because serotonin pre-injections only slightly restrained elevated iBAT temperature (Fig 6B). Although previous reports have shown that central 5-HT_{2A} stimulation increases central dopamine or peripheral adrenaline levels (Bagdy et al., 1989; Gobert and Millan, 1999), we could not increase

peripheral catecholamine levels through 25B-NBOMe administration. This result also supported the theory of the partial contribution of β-adrenergic thermogenesis in iBAT during drug-induced hyperthermia at a high ambient temperature.

Surprisingly, the destruction of central noradrenergic pathways by 6-OHDA and that of 5-HT neurons by 5,7-DHT did not attenuate 25B-NBOMe induced hyperthermia at 29°C. On the contrary, 25B-NBOMe administration at a high ambient temperature induced further hyperthermia in animals pretreated with 5,7-DHT compared with the sham operated group (Fig 7) and several rats died after their core body temperatures exceeded 42°C. Myers reported previously that microinjections of 5,6-dihydroxytryptamine to the anterior hypothalamus caused impaired thermoregulation in exposure to warm (35°C) or cold (8°C) temperatures in rats (Myers, 1975). In our study, neurotoxin pretreated animals had the same core body temperatures at 29°C before 25B-NBOMe administration as per the sham operated group; however, 25B-NBOMe-induced hyperthermia was higher among the animals. Our results may indicate that the destruction of serotonin neurons by 5,7-DHT reduces the control of 25B-NBOMe-induced hyperthermia at high ambient temperatures, which is evoked by peripheral reaction.

In conclusion, we showed that 25B-NBOMe-induced hyperthermia was affected by ambient temperatures. Hyperthermia at a high ambient temperature was induced by

vasoconstriction mediated by peripheral 5-HT_{2A} receptors, which generally dilate for heat loss. However, because vasoconstriction was only transient, long-lasting hyperthermia may be associated with decreased peripheral serotonin levels at high ambient temperature, which allow further thermogenesis in the peripheral organs, including BAT. Importantly, the peripheral administration of both sarpogrelate and serotonin successfully controlled drug-induced hyperthermia, and the destruction of central noradrenergic or serotonergic neurons did not prevent drug-induced hyperthermia. Our findings may be useful for the development of novel peripheral treatments for drug-induced hyperthermia.

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Authorship Contributions

Participated in research design: Nakamura and Shintani-Ishida

Conducted experiments: Nakamura and Shintani-Ishida

Contributed new reagents or analytic tools: Nakamura and Shintani-Ishida

Performed data analysis: Nakamura

Wrote or contributed to the writing of the manuscript: Nakamura, Shintani-Ishida, and Ikegaya

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Footnotes

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Legends for Figures

Fig 1. Core body temperatures of rats following the administration of 0.25 mg/kg 25B-NBOMe or vehicle at 23°C or 29°C ambient temperature. Values = means, Error bar = S.E.; One-way ANOVA, followed by Tukey multiple comparison test, *P < 0.05, 29°C vehicle vs. 29°C 25B-NBOMe; n = 6 for 23°C vehicle and 23°C 25B-NBOMe, n = 5 for 29°C vehicle and 29°C 25B-NBOMe.

Fig 2. Tail surface temperature of rats following the administration of 0.25 mg/kg 25B-NBOMe or vehicle at 23°C or 29°C ambient temperature. Values = means, Error bar = S.E.; One-way ANOVA, followed by Tukey multiple comparison test, *P < 0.05; 29°C vehicle vs. 29°C 25B-NBOMe; n = 5.

Fig 3. (A) Tail Δ temperature of rats following the administration of vehicle, 0.25 mg/kg 25B-NBOMe alone, or with the pre-infusion of 5.0 mg/kg sarpogrelate hydrochloride at 29°C ambient temperature. Values = means, Error bar = S.E.; One-way ANOVA, followed by Tukey multiple comparison test, *P < 0.05; 29°C vehicle vs. 29°C 25B-NBOMe, n.s. 29°C vehicle vs. 29°C 25B-NBOMe + sarpogrelate; n = 5. (B) Core Δ temperature of rats following the administration of 0.25 mg/kg 25B-NBOMe alone or with the pre-infusion of 5.0 mg/kg sarpogrelate hydrochloride at 29°C ambient temperature. Values = means, Error bar = S.E.; Welch's t-test, *P < 0.05, **P < 0.01; n = 6.

Fig 4. iBAT Δ temperature of rats following the administration of 0.25 mg/kg 25B-NBOMe or vehicle at 29°C ambient temperature. Values = means, Error bar = S.E.; Welch's t-test, *P < 0.05; n = 8.

Fig 5. Serum serotonin levels 1 h post-administration of 0.25 mg/kg 25B-NBOMe or vehicle at 23°C or 29°C ambient temperature. Dots = median, error bar = 75% confidence interval; Kruskal–Wallis rank sum test, followed by Steel Dwass test, *P < 0.05 for 23°C vehicle and $^{\dagger}P$ < 0.05 for 23°C 25B-NBOMe, respectively. n = 4 for 23°C vehicle, n = 5 for 23°C 25B-NBOMe, n = 7 for 29°C vehicle, and n = 6 for 29°C 25B-NBOMe.

Fig 6. (A) Core Δ temperature of rats following the administration of 0.25 mg/kg 25B-NBOMe alone or with the pre-infusion of 0.05 mg/kg or 0.1 mg/kg serotonin hydrochloride at 29°C ambient temperature. Values = means, Error bar = S.E.; Welch's t-test, *P = 0.05; 25B-NBOMe alone vs pre-infusion of 1.0 mg/kg serotonin hydrochloride; n = 6. (B) iBAT Δ temperature of rats following the administration of 0.25 mg/kg 25B-NBOMe alone or with the pre-infusion of 1.0 mg/kg serotonin hydrochloride at 29°C ambient temperature. Values = means, Error bar = S.E.; Welch's t-test, n.s.

Fig 7. Core body temperatures of rats pretreated with 5,7-DHT, 6-OHDA, or sham operated following the administration of 0.25 mg/kg 25B-NBOMe at 29°C ambient

temperature. Values = means, Error bar = S.E.; One-way ANOVA, followed by Tukey multiple comparison test, *$P < 0.05$, 5,7-DHT group vs. sham operated group; n = 5, †a, 2 rats of 5,7-DHT pretreated group died 90 min post-administration, †b, 1 rat of 5,7-DHT pretreated group died 150 min post-administration.

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Figures

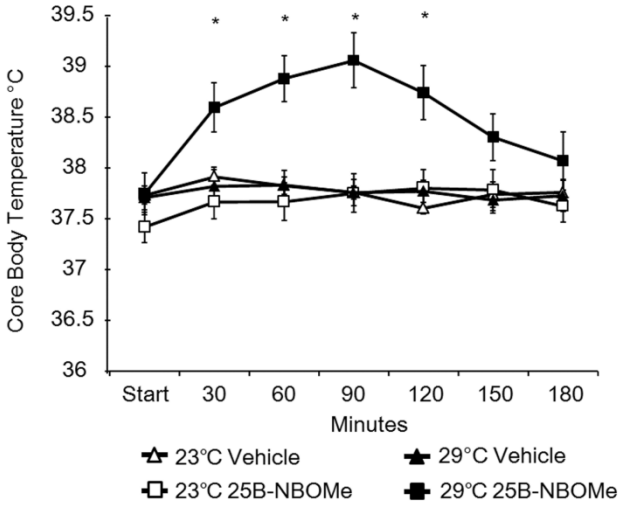

Fig 1.

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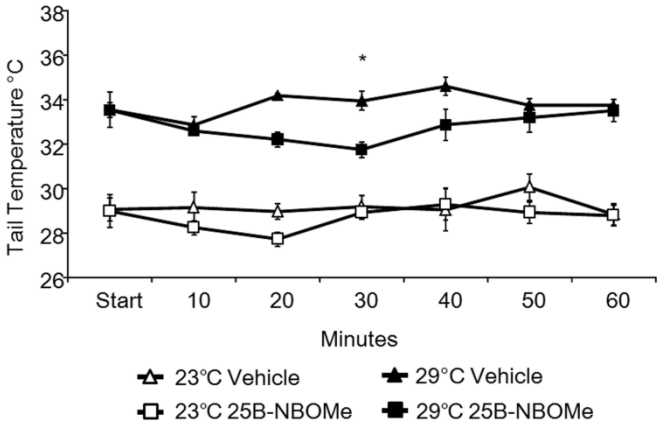

Fig 2.

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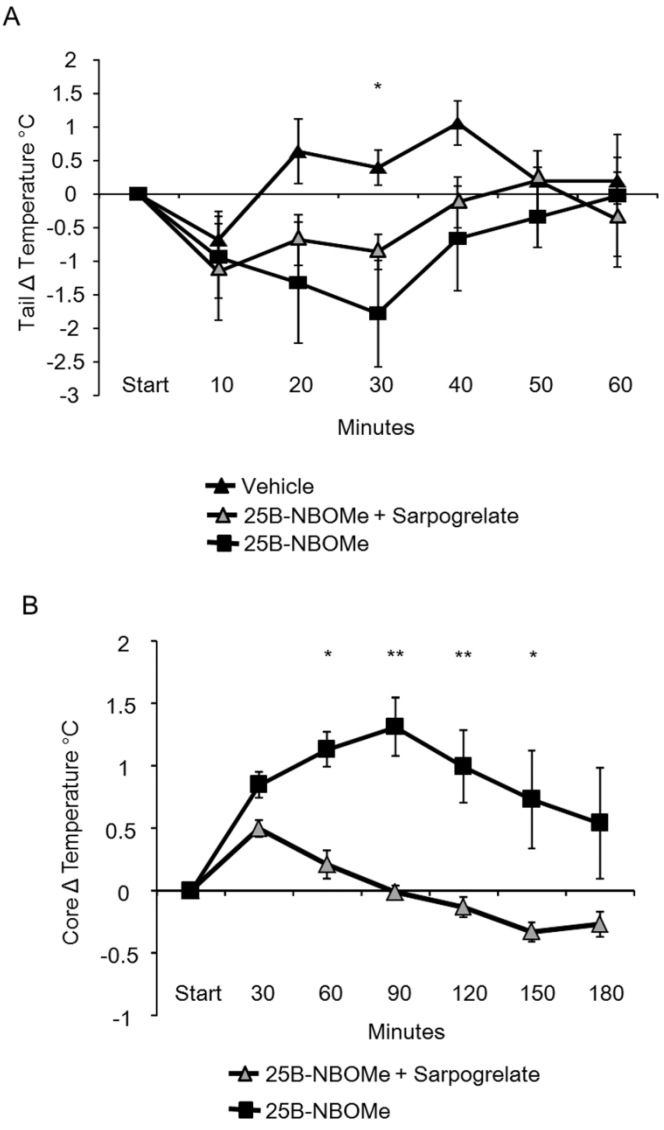

Fig 3.

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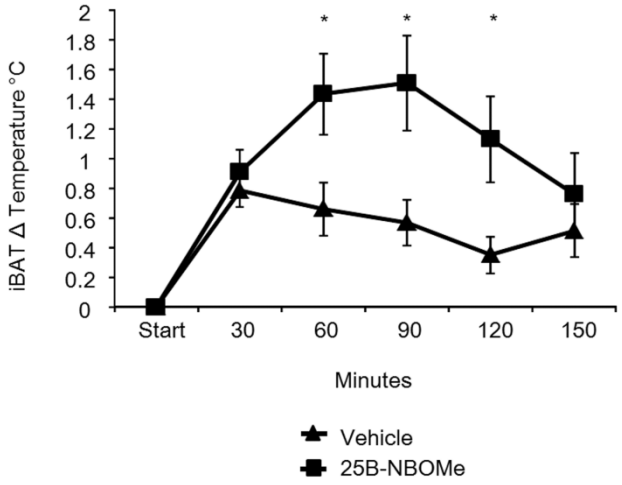

Fig 4.

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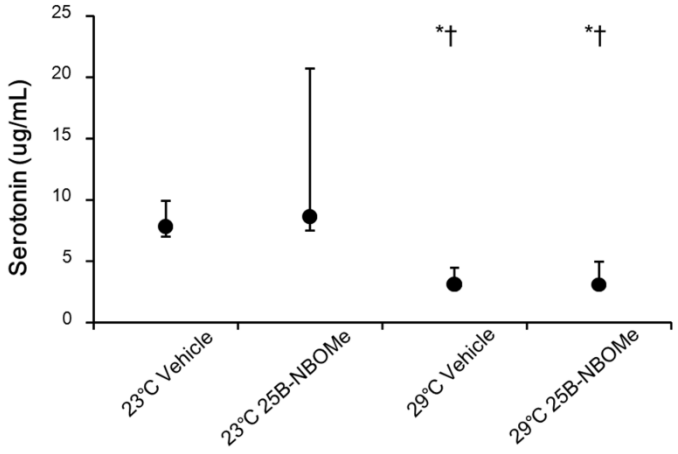

Fig 5.

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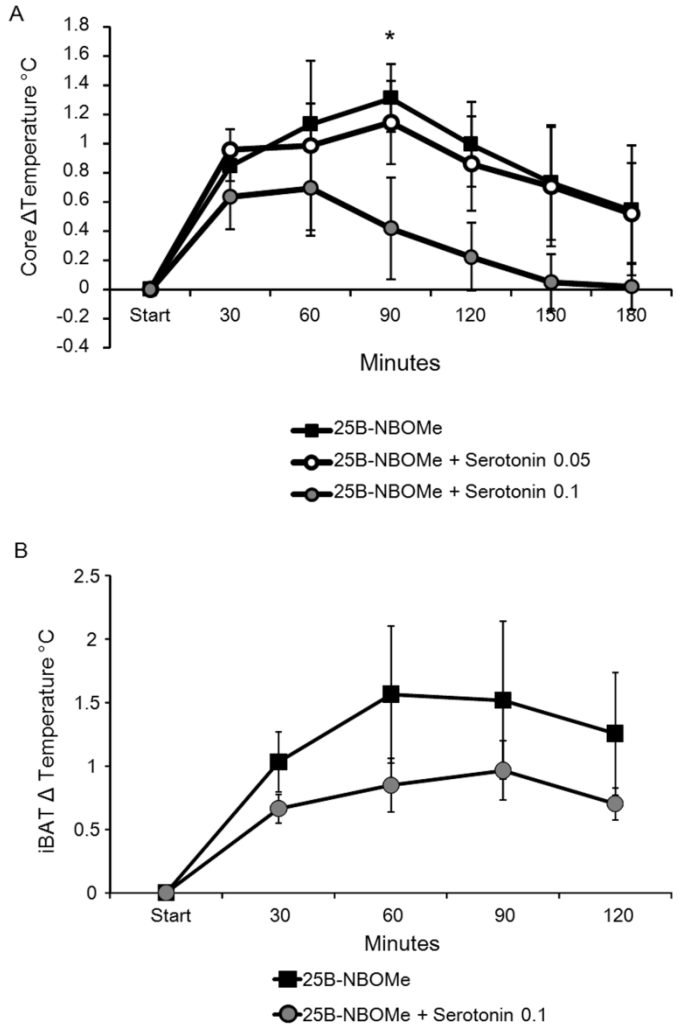

Fig 6.

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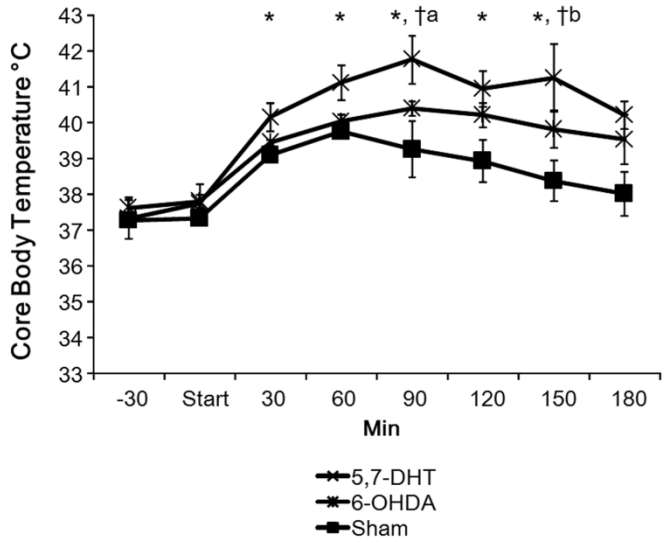

Fig 7.