REGULAR RESEARCH ARTICLE

Risperidone and 5-HT2A Receptor Antagonists Attenuate and Reverse Cocaine-Induced Hyperthermia in Rats

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Abstract

Background: Cocaine (benzoylmethylecgonine) is one of the most widely used illegal psychostimulant drugs worldwide, and mortality from acute intoxication is increasing. Suppressing hyperthermia is effective in reducing cocaine-related mortality, but a definitive therapy has not yet been found. In this study, we assessed the ability of risperidone to attenuate acute cocaine-induced hyperthermia and delineated the mechanism of its action.

Methods: Rats were injected i.p. with saline, risperidone, ketanserin, ritanserin, haloperidol, or SCH 23 390 before and after injection of cocaine (30 mg/kg) or with WAY-00 635, SB 206 553, or sulpiride before cocaine injection; thereafter, the rectal temperature was measured every 30 minutes for up to 4 hours. In vivo microdialysis was used to reveal the effect of risperidone on cocaine-induced elevation of dopamine (DA), serotonin (5-HT), and noradrenaline concentrations in the anterior hypothalamus. For post-administration experiments, saline or risperidone (0.5 mg/kg) were injected into rats, and cocaine (30 mg/kg) was injected 15 minutes later. For every 30 minutes thereafter, DA, 5-HT, and noradrenaline levels were measured for up to 240 minutes after cocaine administration.

Results: Risperidone, 5-HT2A receptor antagonists, and D1 receptor antagonistic drugs prevented and reversed cocaine-induced hyperthermia. In contrast, receptor antagonists for 5-HT1A, 5-HT2B/2C, and D2 did not alter cocaine-induced hyperthermia. Risperidone treatment further attenuated cocaine-induced elevation of DA.

Conclusions: Our results indicate that risperidone attenuates cocaine-induced hyperthermia primarily by blocking the activities of the 5-HT2A and D1 receptors and may be potentially useful for treating cocaine-induced acute hyperthermia in humans.

Key Words: Cocaine, hyperthermia, risperidone, 5-HT2A-receptor, DA1-receptor

Introduction

Cocaine (benzoylmethylecgonine) is a one of most widely used illegal psychostimulant drugs worldwide and is used by an estimated 15 to 21 million (0.37%–0.42%) of the population aged 15 to 64 years (United Nations Office of Drug and Crime, 2019). Cocaine use is particularly prevalent among younger populations (Zimmerman, 2012). As such, the abuse of cocaine represents a major health issue in much of the world, including in the United States, Latin America, and Europe. According to Sevigny and Caces (Sevigny and Caces, 2018), cocaine is the most common cause of illicit drug-related emergency department

Significance Statement

Mortality from acute cocaine intoxication is increasing worldwide. Hyperthermia is known to elevate the risk of mortality from acute cocaine intoxication, but no therapies exist for this symptom. In this study, risperidone, a commonly used atypical antipsychotic drug, attenuated and reversed cocaine-induced hyperthermia in a rat model, suggesting that it may offer effective treatment. Concurrent administration of risperidone and various antagonists attenuate and reverse cocaine-induced hyperthermia by 5-HT2A and D1 antagonism, but not 5-HT1A, 5-HT2B/2C, or D2 antagonism. This study is the first to our knowledge to demonstrate a clear receptor-related mechanism for cocaine-induced hyperthermia. We found that dopamine, serotonin, and noradrenaline levels increased in the anterior hypothalamus after cocaine administration, and on microdialysis study, risperidone suppressed the cocaine-induced elevation of dopamine. We believe that our findings have considerable implications in the clinical treatment of acute cocaine intoxication and elucidate the mechanism of hyperthermia induced by other drugs as well.

visits, accounting for an estimated 455 785–505 224 visits in the United States in 2011.

Cocaine abuse can induce life-threating adverse effects, including seizures, excited delirium, hypertensive crisis, tachycardia, ventricular arrhythmia, acute myocardial infarction, rhabdomyolysis, and hyperthermia (Welch et al., 1991; Wetli et al., 1996; Lange and Hillis, 2001; Crandall et al., 2002; Phillips et al., 2009). Multiple reports have also shown that cocaine can cause hyperthermia in humans and animals alike (Lomax and Daniel, 1990, 1993; Gonzalez, 1993; Marzuk et al., 1998; Crandall et al., 2002; Hamida et al., 2008). Hyperthermia is closely related to its lethality, and in cases of cocaine-related mortality, patients commonly present with hyperthermia (Loghmanee and Tobak, 1986; Campbell, 1988; Kosten and Kleber, 1988; Bauwens et al., 1989). While a typical fatal cocaine overdose occurs at a blood concentration of 6 mg/L, Kosten and Kleber have suggested that cocaine-related mortality can occur at low blood cocaine levels (0.14–0.9 mg/L) when hyperthermia is present (Kosten and Kleber, 1988). Previous animal studies have demonstrated that high ambient temperatures exacerbate cocaine-induced hyperthermia (Lomax and Daniel 1990, 1993; Gonzalez, 1993; Cappon et al., 1998). Moreover, recent human epidemiologic studies suggest that the rate of cocaine-related mortality increases in the presence of hot ambient temperatures (Marzuk et al., 1998; Bohnert et al., 2010; Auger et al., 2017). This psychiatric phenomenon is bolstered by animal experiments, which show that interventions preventing cocaine-induced hyperthermia also eliminate cocaine-induced lethality in dogs (Catravas and Waters, 1981). Therefore, hyperthermia suppression in cases of cocaine abuse represents a promising avenue for reducing cocaine mortality.

Mechanistically, cocaine is a monoamine transporter blocker, with approximately equal affinity for the dopamine (DA), serotonin (5-HT), and noradrenaline (NA) transporters (Ritz et al., 1990). In rat models, cocaine was shown to cause the release of DA, 5-HT, and NA in several brain lesions (Chen and Reith, 1994; Teneud et al., 1996; Reith et al., 1997; Andrews and Lucki, 2001; Müller et al., 2002; Bubar et al., 2003; Frank et al., 2008; Murnane et al., 2013), which, in turn, regulated body temperature (Kendrick et al., 1989; Lin et al., 1998; Hasegawa et al., 2000, 2011; Ishiwata et al., 2017). We previously reported that DA and 5-HT levels were increased in the rat brain by the psychostimulant drugs methamphetamine (METH) and 3.4-methylenedioxymethamphetamine (MDMA), and that administration of risperidone, D1, D2, and a 5-HT2A receptor antagonistic atypical antipsychotic drug prevented and reversed METH and MDMA-induced hyperthermia in rats (Shioda et al., 2008, 2010). Therefore, we hypothesized that risperidone could also be used to prevent and reverse cocaine-induced hyperthermia. Risperidone is an atypical antipsychotic drug that potently blocks the D2 and 5-HT2A receptors and weakly blocks D1 receptor activity; it also has a multitude of other 5-HT subtype receptor antagonistic effects (i.e., 5-HT1A, Ki=490 nM; 5-HT2A, Ki=0.6 nM; 5-HT2B, pKi=7.67; 5-HT2C, pKi=8.31; D1, Ki=75 nM; D2, Ki=3 nM) (Bymaster et al., 1996; Wood et al., 2006) (Table 1).

In the present study, we evaluated the ability of risperidone in suppressing cocaine-induced hyperthermia in rats. We attempted to delineate the specific DA and 5-HT receptors associated with cocaine-induced hyperthermia using various DA and 5-HT receptor antagonists. We subsequently used microdialysis to quantify cocaine-induced DA, 5-HT, and NA level changes in the rat anterior hypothalamus, the thermoregulation center of the brain.

Methods

All experimental procedures involving animals were approved by the Animal Investigation Committee of our institution and were performed in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animals, Drug Administration, and Experimental Protocol

Male Wistar rats (Clea Japan Inc., Tokyo, Japan) weighing 200–250 g were used in this study. We conducted microdialysis experiments and body temperature measurement experiments in separate groups of rats. Rats were housed in cages maintained at 26°C ±1°C under a 12-hour-light/-dark cycle and were provided free access to food and water. Risperidone, ketanserin, ritanserin, WAY-100 635, SCH 23 390, and SB 206 553 were purchased from Sigma-Aldrich Co. (St. Louis, MO). Haloperidol and sulpiride were obtained in injection ampoules from Astellas Pharma Inc., Tokyo, Japan, and cocaine was purchased from Shionogi & CO., LTD., Osaka, Japan. Risperidone was dissolved in HCl, and the pH was maintained between 6 and 7 using NaOH. Ritanserin was dissolved in 99.7% acetic acid, and the pH was maintained between 6 and 7 by using NaOH. All other drugs were dissolved in 0.9% saline. Rats were injected i.p. with 2 mL/ kg of the appropriate drug. On the day of the experiment, rats were placed in individual cages in a room maintained at an ambient temperature of 26°C ±1°C.

We conducted pre-administration experiments to evaluate the attenuating effect, and post-administration experiments to evaluate the reversing effect, of risperidone on cocaine-induced hyperthermia. The post-administration experiment was performed with the aim of using risperidone clinically to treat hyperthermia induced by cocaine. In the pre-administration

Table 1. Receptor Binding Affinity of Test Compounds Across Monoamine Receptors

Compounds	Ki (nM)						References
	5-HT1A	5-HT2A	5-HT2B	5-HT2C	D1	D2	
Risperidone	490	0.6	7.67	8.31	75	3	Bymaster (1996); Wood (2006)
Ketanserin	>10 000	1.6	3981	69	464	>10 000	Bonhaus (1995); Toll (1998)
Ritanserin	2919	4.7	5.01	11	933	84	Bonhaus (1995); Toll (1998)
Haloperidol	7930	78	1420	3085	25	1	Bymaster (1996); Wainscott (1998)
SCH 23 390	262.57	14	83.17	13.4	0.37	3200	Boess (1994); Bøgesø (1995); Gozlan (1986); Roth (1992)
WAY-100 635	0.24	1100	24	>10000	1000	79	Chemel (2006); Johansson (1997)
SB206553	>10000	1659.58	1.28825	12.02	ND	>10000	Kennett (1996)
Sulpiride	>10000	>10000	ND	>10000	>10000	23	Hall (1986); Neve (1990); Toll (1998)

experiments, the rectal temperature of the rats was monitored; when the temperature was observed to be stable for approximately 2 hours, the rats were injected i.p. with either saline, risperidone (0.5 mg/kg), ketanserin (5 mg/kg), ritanserin (3 mg/kg), WAY-100 635 (1 mg/kg), SB 206 553 (2.5 mg/kg), haloperidol (0.5 mg/kg), SCH 23 390 (0.5 mg/kg), or sulpiride (50 mg/kg). After waiting for 15 minutes, cocaine (30 mg/kg) was also injected i.p. Thereafter, we measured the rat's rectal temperature every 30 minutes for up to 4 hours from the time the cocaine was administered.

In the post-administration experiment, we first injected cocaine i.p. (30 mg/kg), and after waiting for 15 minutes, we i.p. injected risperidone (0.25 and 0.5 mg/kg), ketanserin (2.5 and 5 mg/kg), ritanserin (1.5 and 3.0 mg/kg), haloperidol (0.25 and 0.5 mg/kg), or SCH 23 390 (0.25 and 0.5 mg/kg). We subsequently measured the rat's rectal temperature every 30 minutes for up to 4 hours from the time of cocaine administration.

Our previous work has shown that the pre-administration of 0.5 mg/kg risperidone prevented MDMA, METH, and 5-HT syndrome model-induced hyperthermia in our previous studies (Nisijima et al., 2000; Shioda et al., 2008, 2010); thus, for this study we used 0.5 mg/kg risperidone. Since haloperidol blocks the D_2 receptor in a similar manner to risperidone (Schotte et al., 1996), we selected its dose to be 0.5 mg/kg. The doses of ketanserin (5 mg/kg), ritanserin (3 mg/kg), WAY-100 635 (1 mg/kg), SCH 23 390 (0.5 mg/kg), sulpiride (50 mg/kg), and SB 206 553 (2.5 mg/kg) were determined based on their success in our previous study (Nisijima et al., 2001; Shioda et al., 2008, 2010).

Measuring Rectal Temperature

Every 30 minutes, rats were gently restrained and their rectal temperature was measured by inserting a thermocouple probe connected to a digital thermometer (Shibaura Electronics Co., Tokyo, Japan) 3 cm into the rectum. A steady temperature readout was obtained within 10 seconds of probe insertion.

Surgery and Brain Dialysis

To perform in vivo microdialysis, rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and placed in a stereotaxic frame (David Kopf Instruments) with the nose bar positioned 3.5 mm below interaural zero. The skull was exposed during surgery, and a burr hole was drilled to accommodate the plantation. Straight-type cellulose dialysis tubing (length, 1.0 mm; internal diameter, 0.16 mm; molecular weight cutoff, 50 000) was steadily and carefully inserted into the anterior hypothalamus (AP, 1.1 mm; ML, 0.9 mm; DV, –9.2 mm from the bregma) and fixed with acrylic dental cement and 3 skull screws. The coordinates of the hypothalamus were determined using the Paxinos and Watson (1986) brain atlas.

Two days after the surgery, the probe was connected to a microinfusion pump and the brain was perfused with Ringer's solution (NaCl, 147 mmol; KCl, 4 mmol; $CaCl_2$, 1.9 mmol) at a flow rate of 1 μL/min. The rats were allowed to move freely during this period. The outflow from the hypothalamus was automatically injected into a high-performance liquid chromatography unit using an automatic injector (Eicom EAS-20S; Eicom, Kyoto, Japan). An ion-exchange column (Eicompack CAX, 200 mm × 2.0 mm, Eicom) was used for DA, 5-HT, and NA separation, and the results were obtained instantaneously. The concentrations of DA, 5-HT, and NA in the samples were determined by an electrochemical detector (Eicom HITEC-500, Eicom). The column and detector were maintained at 35°C using

temperature controllers. The mobile phase buffer used for DA, 5-HT, and NA analyses consisted of 0.1 M ammonium acetate buffer (pH 6.0), 30% methanol, 7.102 g/L sodium-sulfate, and 50 mg/L disodium ethylenediaminetetraacetic acid; the flow rate was set at 0.25 mL/min. Our method was capable of detecting NA, DA, and 5-HT at retention times of 5.15, 6.92, and 12.88 minutes, respectively.

After a 3-hour stabilization period, 2 consecutive dialysate samples were collected for measuring baseline DA, 5-HT, and NA levels. Saline or risperidone (0.5 mg/kg) were injected i.p. into rats, and cocaine (30 mg/kg) was injected i.p. 15 minutes thereafter. DA, 5-HT, and NA levels were subsequently measured every 30 minutes for up to 240 minutes after cocaine administration.

Statistical Analysis

Changes in rectal temperature from the baseline were measured every 30 minutes in each group and statistically analyzed using 2-way ANOVA with repeated measures. Between-group differences were assessed post-hoc with the Fisher's predicted least-square difference test. The average concentrations of DA, 5-HT, and NA before injecting cocaine were used as the baseline values (100%). Data from DA, 5-HT, and NA measurements were analyzed using 2-way ANOVA with repeated measures followed by post-hoc analysis with the Fisher's predicted least-square difference test. The data were expressed as percentages of the respective baseline values. Averaged data were presented as means ± SEM.

Results

Effect of Risperidone on Cocaine-Induced Hyperthermia

The rectal temperature of rats injected i.p. with 30 mg/kg cocaine increased rapidly, exceeding 39.5°C 60 minutes after cocaine administration, and subsequently decreased gradually. On comparing with the saline control group, the cocaine-induced rectal temperature increase was significantly suppressed in rats pre-injected with risperidone (0.25 mg or 0.5 mg/kg) at 30, 60, 90, and 120 minutes after cocaine administration (Figure 1A). Even when risperidone (0.25 and 0.5 mg/kg) was injected i.p. 15 minutes after cocaine administration, the increase in rectal temperature was significantly suppressed compared with temperature increase in the control group from 30 to 240 minutes (Figure 1B).

Effect of Various 5-HT Receptor Antagonists on Cocaine-Induced Hyperthermia

Pretreatment with ritanserin (3 mg/kg) or ketanserin (5 mg/kg), which have antagonistic effects on the 5-HT2A receptor (Table 1), significantly prevented cocaine-induced hyperthermia at 30, 60, and 90 minutes (Figure 2A). Likewise, posttreatment with either a high dose (3 mg/kg) or low dose of ritanserin (1.5 mg/kg) significantly reversed cocaine-induced hyperthermia (Figure 2B). Similarly, posttreatment with ketanserin (2.5 or 5 mg/kg) significantly reversed cocaine-induced hyperthermia (Figure 2C). In contrast, pretreatment with SB 206 553 (3.0 mg/kg), a 5-HT2B/2C receptor antagonist, and WAY-100 635 (1 mg/kg), a 5-HT1A receptor antagonist (Table 1), did not suppress cocaine-induced hyperthermia (Figures 3 and 4).

Effect of Different Types of DA Receptor Antagonists on Cocaine-Induced Hyperthermia

Pretreatment with haloperidol (0.5 mg/kg), which exerts potent antagonistic effects on the D_2 and D_1 receptors, significantly prevented cocaine-induced hyperthermia, as did pretreatment with SCH 23 390 (0.5 mg/kg), a selective D_1 receptor antagonist (Figure 5A) (Table 1). Posttreatment with either a high dose (0.5 mg/kg) or low dose of haloperidol (0.25 mg/kg) also significantly reversed cocaine-induced hyperthermia (Figure 5B). Posttreatment with SCH 23 390 (0.5 mg/kg), as well as a low dose of SCH 23 390 (0.25 mg/kg), significantly reversed cocaine-induced hyperthermia (Figure 5C). In contrast, pretreatment with sulpiride (50 mg/kg), which is a selective D_2 receptor antagonist (Table 1), did not prevent cocaine-induced hyperthermia (Figure 6).

Extracellular Levels of DA, 5-HT, and NA in the Anterior Hypothalamus After Cocaine Administration and the Effect of Risperidone Pretreatment on Those Levels

After i.p. injections of 30 mg/kg of cocaine, the DA levels in the anterior hypothalamus increased approximately 25-fold at 60 minutes post-injection (Figure 7A). Pretreatment with risperidone significantly suppressed the cocaine-induced increase of DA levels at 60 and 90 minutes (Figure 7A). Similarly, following cocaine administration, 5-HT levels increased immediately and exceeded sixfold that of baseline levels 60 minutes following cocaine administration (Figure 7B). Likewise, NA levels increased approximately 17-fold at 60 minutes following cocaine administration (Figure 7C). In contrast, pretreatment with risperidone did not significantly ablate cocaine-induced elevation of 5-HT and NA levels, which were similar to those in the saline control group.

Discussion

Occasionally, life-threatening hyperthermia occurs in persons who ingested cocaine (Loghmanee and Tobak, 1986; Campbell, 1988; Kosten and Kleber, 1988; Bauwens et al., 1989). However, no established pharmacological treatment for cocaine-induced hyperthermia is currently available. It has been reported that the effects of cocaine, including hyperthermia, are mainly caused by the release of DA (Kuhar et al., 1991; Rockhold et al., 1991). Recently, 5-HT, a well-known thermoregulation neurotransmitter (Lin et al., 1998; Kendrick et al., 1989; Ishiwata et al., 2017), was also reportedly released by cocaine administration (Chen and Reith 1994; Andrews and Lucki, 2001; Florin et al., 1994) and is thought to play an important role in mediating cocaine-induced hyperthermia. In this work, we aimed to identify pharmacological compounds for treating cocaine-induced hyperthermia. Previously, we reported that risperidone could be used to attenuate and reverse hyperthermia induced by MDMA or METH, compounds that likewise induce the intercellular release of DA and 5-HT in the rat brain (Shioda et al., 2008, 2010). Therefore, we hypothesized that risperidone, an atypical antipsychotic drug that blocks the DA and 5-HT receptors, would be effective against cocaine-induced hyperthermia. In the present study, we found that risperidone attenuated and reversed hyperthermia induced by cocaine.

Risperidone blocks multiple 5-HT receptor subtypes (Schmidt et al., 2001) (Table 1); we attempted to identify the 5-HT receptor subtype that plays the most important role in facilitating cocaine-induced hyperthermia. We found
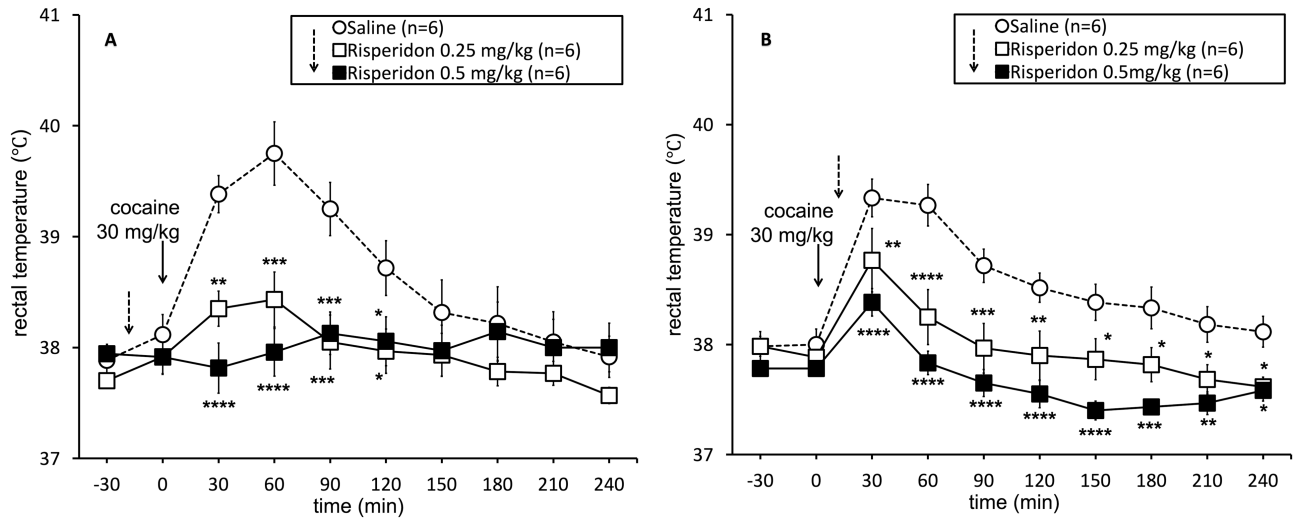
Figure 1. Effect of risperidone on cocaine-induced hyperthermia. When the rats were injected i.p. with 30 mg/kg cocaine, their rectal temperature increased rapidly, exceeding 39.5°C 60 minutes after cocaine administration and then decreasing gradually. (A) Injection 15 minutes before cocaine and (B) injection of risperidone (0.25 mg/kg) 15 minutes after cocaine significantly suppressed the increase of rectal temperature. Values are represented as means±SEMs. The statistical differences among the groups are indicated as follows: *P<.05 (vs saline), **P<.01 (vs saline), ***P<.001, ****P<.0001 (vs saline) (ANOVA followed by Fisher's predicted least-square difference [PLSD]).
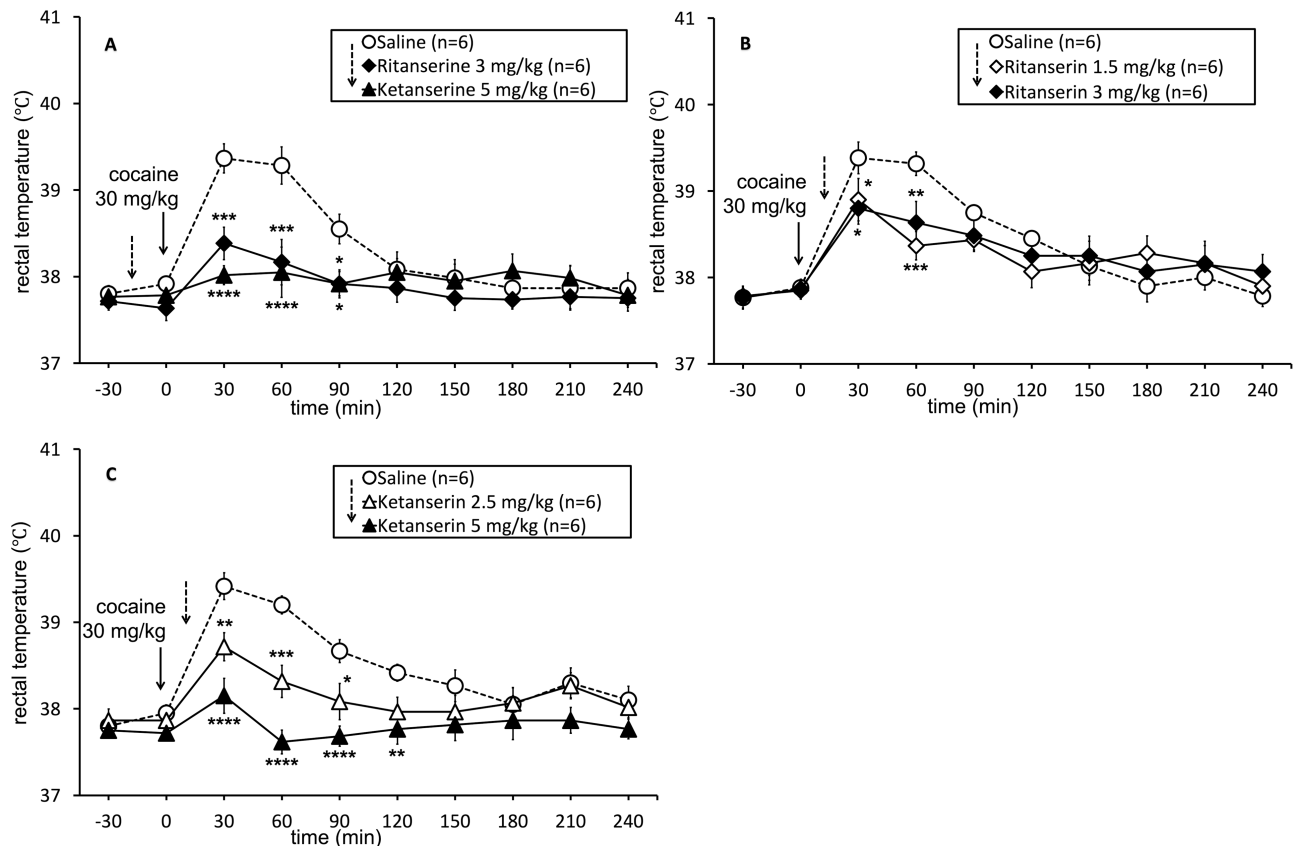
Figure 2. Effect of 5-HT2A antagonists on cocaine-induced hyperthermia. (A) Injection of ritanserin (3 mg/kg) and ketanserin (5 mg/kg) 15 minutes before cocaine significantly suppressed the increase of rectal temperature. (B) Injection of ritanserin (3 mg/kg and 1.5 mg/kg) and (C) ketanserin (5 mg/kg and 2.5 mg/kg) 15 minutes after cocaine significantly suppressed the increase of rectal temperature. Values are represented as means ± SEMs. The statistical differences among the groups are indicated as follows: *P<.05 (vs saline), **P<.01 (vs saline), ***P<.001, ****P<.0001 (ANOVA followed by Fisher's predicted least-square difference [PLSD]).

that cocaine-induced hyperthermia was prevented and reversed by the administration of 5-HT2A receptor antagonists, including ritanserin and ketanserin. The 5-HT2A receptor agonists 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane and 2-(4-Bromo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl) ethanamine are known to induce hyperthermia in human and animals (Mazzola-Pomietto et al., 1995; Gee et al., 2016; Nakamura et al., 2018). Therefore, 5-HT2A receptor antagonism
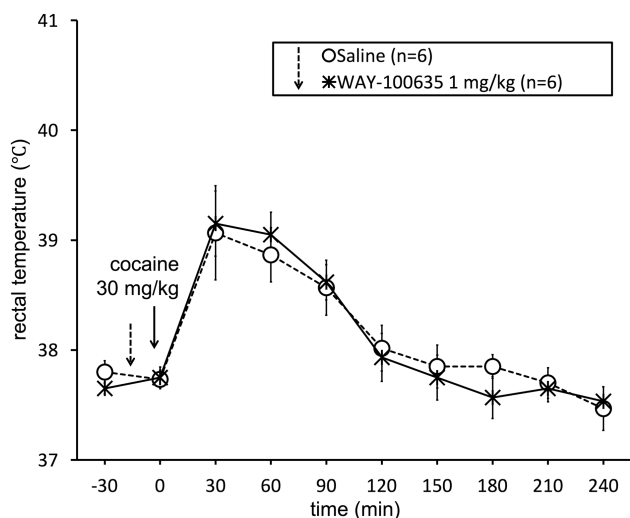
Figure 3. Effect of the 5-HT1A antagonist WAY-100 635 on cocaine-induced hyperthermia. Injection of WAY-100 635 (1 mg/kg) 15 minutes before cocaine did not suppress cocaine-induced hyperthermia.
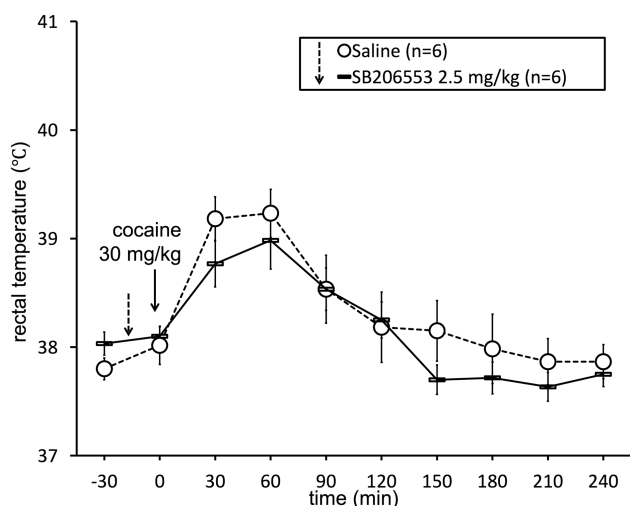

Figure 4. Effect of the 5-HT2B/2C antagonist SB 206 553 on cocaine-induced hyperthermia. Injection of SB 206 553 (2.5 mg/kg) 15 minutes before cocaine did not suppress cocaine-induced hyperthermia.

acts by reducing the central core temperature. Conversely, Nakamura et al. (2018) reported that administration of 5-HT2A agonists resulted in hyperthermia, with high temperatures comparable with those observed in our study; this is caused by peripheral 5-HT2A receptor-mediated vasoconstriction and brown adipose tissue thermogenesis. We cannot clearly distinguish between peripheral and central mechanisms in these experiments; 5-HT2A receptor antagonism may reduce hyperthermia via both peripheral and central mechanisms

In contrast to 5-HT2A receptor antagonists, SB 206 553, a 5-HT2B/2C receptor antagonist, did not prevent cocaine-induced hyperthermia (Figure 4). Moreover, WAY-100 635, a 5-HT1A receptor antagonist, did not prevent cocaine-induced hyperthermia (Figure 3). These findings suggest that neither the 5-HT2B/2C receptor nor the 5-HT1A receptor modulates cocaine-induced hyperthermia in rats. These results are consistent with those of our previous work, which showed that 5-HT2A, not but 5-HT2B/C or 5-HT1A receptor antagonists, attenuated

and reversed hyperthermia induced by psychostimulants (i.e., MDMA, METH) (Shioda et al., 2008, 2010). Thus, this suggests that the ability of risperidone in preventing cocaine-induced hyperthermia arises from its potent 5-HT2A receptor antagonism. This is the first report, to our knowledge, to demonstrate that 5-HT2A receptor activation is an important mediator of cocaine-induced hyperthermia. In addition, ritanserin appears to have a smaller post-administration reverse effect on cocaine-induced hyperthermia than do ketanserin and risperidone. This may be attributed to the weaker 5-HT2A receptor-binding affinity of ritanserin (5-HT2A: ritanserin Ki = 4.7 nM) than that of ketanserin and risperidone (5-HT2A: ketanserin Ki = 1.6 nM, risperidone Ki = 0.6 nM; Toll et al., 1998).

DA is involved in the central control of thermoregulation (Yamawaki et al., 1983; Yamada et al., 1988) and is known to play a role in cocaine-induced hyperthermia (Rockhold et al., 1991). In the present study, we investigated the effects of several DA antagonists on cocaine-induced hyperthermia. Haloperidol, a D1 and D2 receptor antagonist, and SCH 23 390, a selective D1 receptor antagonist, significantly attenuated and reversed cocaine-induced hyperthermia (Figure 5A–C). In contrast, sulpiride, a D2 receptor antagonist, had no effect on cocaine-induced hyperthermia (Figure 6). Rockhold et al. (Rockhold et al., 1991) reported similar results, showing that blocking D1 receptor activity blocked cocaine-induced hyperthermia, while blocking D2 receptor activity had no effect. Our results are also consistent with those of previous reports, which demonstrated that D1, but not D2 receptor antagonists, attenuated and reversed psychostimulant (MDMA and METH)-induced hyperthermia (Shioda et al., 2008, 2010). In post-administration experiments, haloperidol appeared to be less effective than SCH23390 at reversing hyperthermia. Post-administration SCH23390 (0.5 mg/kg) suppressed cocaine-induced hyperthermia for up to 120 minutes, while haloperidol (0.5 mg/kg) had a suppressive effect for up to 90 minutes. This may be due to the weaker D1 receptor-binding affinity of haloperidol compared with that of SCH23390 (D1: haloperidol Ki = 25 nM; SCH23390 Ki = 0.37 nM; Bymaster et al., 1996, Bøgesø et al., 1995) (Table 1). Although the D1-binding affinity of risperidone was weak at the dose used in this experiment (Bymaster et al., 1996; Wood et al., 2006) (Table 1), the D1 antagonist effect of risperidone may be involved in the attenuation and reversal of cocaine-induced hyperthermia.

Collectively, our results indicate that the induction of hyperthermia by cocaine may be related to the activities of both 5-HT2A and D1 receptors. Thus, risperidone primarily blocks and reverses cocaine-induced hyperthermia by 5HT2A receptor antagonism and partially by D1 receptor antagonism.

Previous in vivo microdialysis experiments have shown that cocaine increases the extracellular levels of DA, 5-HT, and NA in several regions of the rat brain. However, in many brain regions, including the striatum and nucleus accumbens, among others, the effects of cocaine on DA are greater than those on 5-HT or NA (Chen and Reith, 1994; Teneud et al., 1996; Reith et al., 1997; Andrews and Lucki, 2001; Bubar et al., 2003). In the present study, we found that the i.p. administration of cocaine elevated the levels of DA, 5-HT, and NA in the anterior hypothalamus by 25-, 6-, and 17-fold, respectively (Figure 7A–C). The peak in cocaine-induced hyperthermia is consistent with the peak of monoamine in microdialysis experiments. To the best of our knowledge, this is the first report to demonstrate that cocaine increases the levels of NA in the anterior hypothalamus, the central thermoregulation center. NA is also known to be involved in thermoregulation (Hardebo and Hindfelt, 1981; Lopachin and Rudy, 1982);
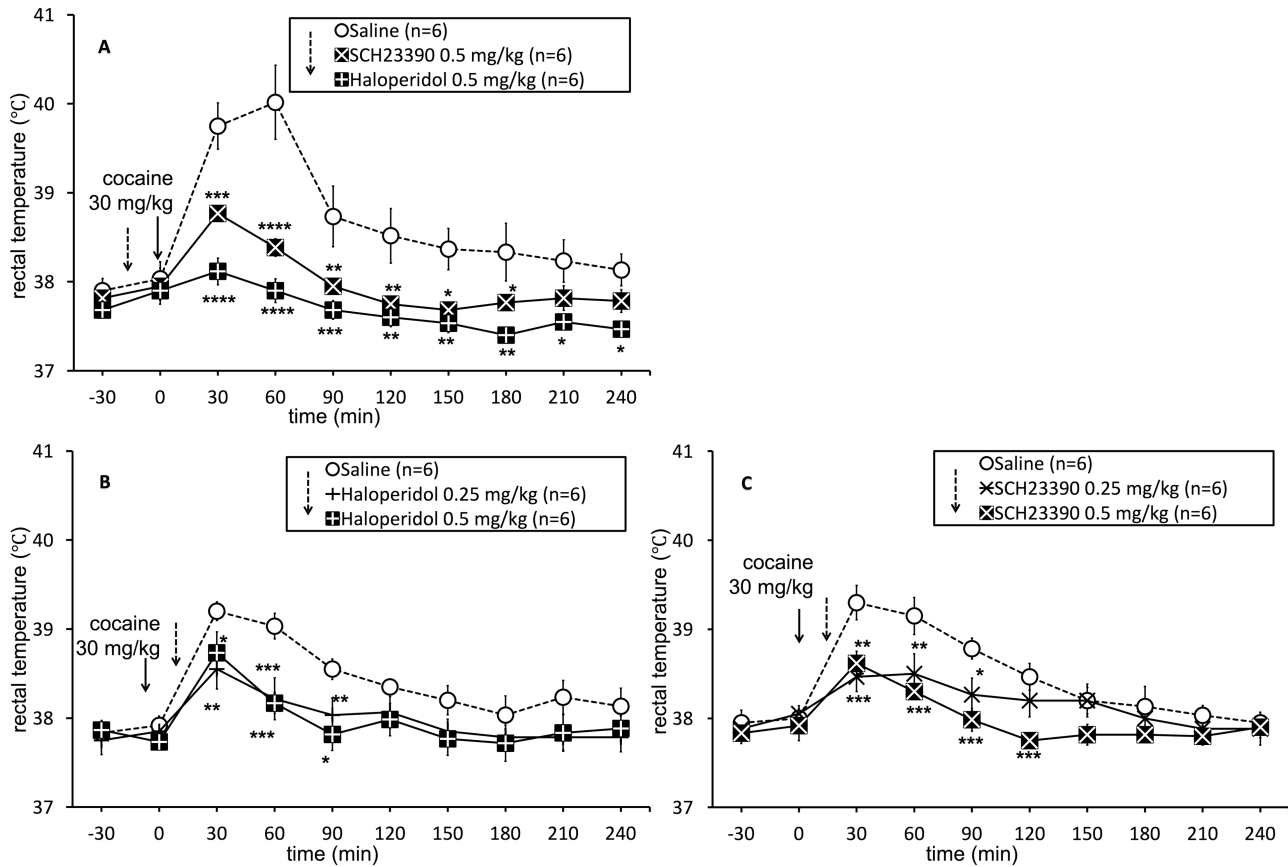
Figure 5. Effects of haloperidol and SCH 23 390 on cocaine-induced hyperthermia. (A) Injection of haloperidol (0.5 mg/kg) and SCH 23 390 (0.5 mg/kg) 15 minutes before cocaine significantly suppressed cocaine-induced hyperthermia. (B) Injection of haloperidol (0.5 mg/kg and 0.25 mg/kg) and (C) SCH 23 390 (0.5 mg/kg and 0.25 mg/kg) 15 minutes after cocaine significantly suppressed the increase of rectal temperature. Values are represented as means ± SEMs. The statistical differences among the groups are indicated as follows: *$P<.05$ (vs saline), **$P<.01$ (vs saline), ***$P<.001$, ****$P<.0001$ (ANOVA followed by Fisher's predicted least-square difference [PLSD]).
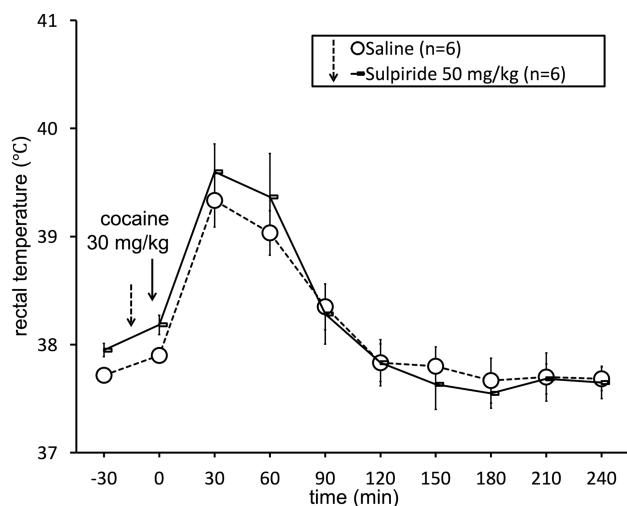

Figure 6. Effect of D2 receptor antagonist sulpiride on cocaine-induced hyperthermia. Injection of sulpiride (50 mg/kg) 15 minutes before cocaine did not suppress cocaine-induced hyperthermia.

thus, we anticipate that increased NA levels may be related to cocaine-induced hyperthermia.

Compared with the increased DA levels in the control group, pretreatment with risperidone significantly suppressed the cocaine-induced elevation of DA (Figure 7A). The time course of

increases of DA in the anterior hypothalamus and hyperthermia coincided remarkably. In addition, the effect of risperidone on cocaine-induced hyperthermia and increases in DA concentrations disappeared almost simultaneously. These results suggest that the suppression of hyperthermia by risperidone is related to direct or indirect suppression of DA increases in the anterior hypothalamus. However, risperidone did not alter the cocaine-induced elevations of 5-HT and NA levels. Steketee (1998) reported that injecting SCH23390, a D1 receptor antagonist, into the ventral tegmental area of rats abolished the cocaine-induced increase of DA in the nucleus accumbens. They proposed that D1 receptor activation may indirectly increase dopaminergic activity by enhancing glutamate release. This is because the D1 agonist SKF 82 958 increased extracellular glutamate levels, which is known to increase the release of DA (Kalivas and Duffy, 1995). Others have also reported that treatment with 5-HT2A receptor antagonists, including MDL100907 and ketanserin, prevented the elevation of DA following the administration of cocaine (Broderick et al., 2004; Zayara et al., 2011). In addition, previous studies have demonstrated that administration of the 5-HT2A receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, increased the levels of extracellular DA (Bowers et al., 2000; Yan, 2000). These reports are consistent with our findings. Thus, risperidone pretreatment may possibly suppress cocaine-induced elevation of DA levels due its antagonistic effect on the D1 and 5-HT2A receptors. In addition, the time of the peak DA increase in the anterior hypothalamus is later than that of the peak in the striatum, as reported by Chambers et al. (2010). Collectively, these results suggest that
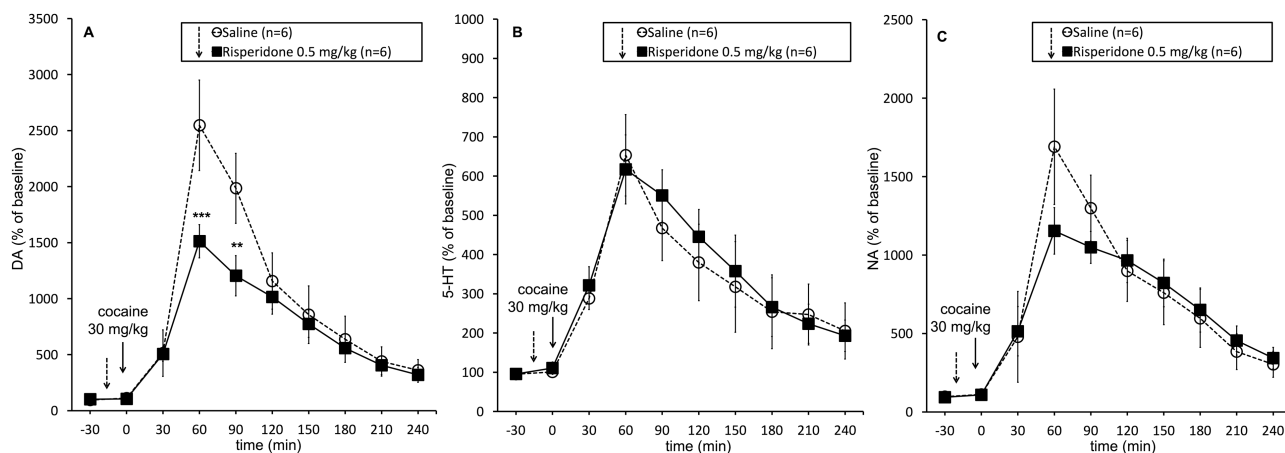
Figure 7. Extracellular levels of (A) DA, (B) 5-HT, and (C) NA in the anterior hypothalamus after cocaine administration and the effect of risperidone pretreatment on these levels. (A) After i.p. injection of 30 mg/kg of cocaine, the DA levels in the anterior hypothalamus increased approximately 25-fold at 60 minutes. Pretreatment of risperidone significantly suppressed DA level increases at 60 and 90 minutes. (B) The 5-HT levels immediately increased, exceeding sixfold at 60 minutes after cocaine administration. (C) The NA levels were increased approximately 17-fold at 60 minutes after cocaine administration. (B–C) There was no significant difference seen in 5-HT and NA increase between pretreatment risperidone and saline control groups. Values are represented as means±SEMs. The statistical differences among the groups are indicated as follows: *$P<.05$ (vs saline), **$P<.01$ (vs saline) (ANOVA followed by Fisher's predicted least-square difference [PLSD]). 5-HT, serotonin; DA, dopamine; NA, noradrenaline.

cocaine-induced increases in DA levels in the hypothalamus may be due to an indirect mechanism, and risperidone may inhibit cocaine-induced DA elevation by antagonizing various receptors at sites other than the hypothalamus. In conclusion, administering risperidone in rats attenuates and reverses cocaine-induced hyperthermia and attenuates the cocaine-induced elevation of DA levels in the anterior hypothalamus. Mechanistically, risperidone blocks the activities of the 5-HT2A and D1 receptors, and this may underlie the inhibition of cocaine-induced hyperthermia. Globally, risperidone is used as an atypical antipsychotic drug. Collectively, our results indicate that risperidone is a potentially useful drug for treating cocaine-induced acute hyperthermia in humans, particularly the finding that post-administration of risperidone reversed cocaine-induced hyperthermia.

Acknowledgments

Statement of Interest

None.

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