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Short communication

Involvement of serotonergic and dopaminergic mechanisms in hyperthermia induced by a serotonin-releasing drug, *p*-chloroamphetamine in mice

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Abstract

Serotonergic and dopaminergic involvement in hyperthermia induced by a serotonin (5-hydroxytryptamine, 5-HT)-releasing drug, *p*-chloroamphetamine, was investigated in mice. Neither the 5-HT transporter inhibitor fluoxetine nor the 5-HT depleter *p*-chlorophenylalanine affected *p*-chloroamphetamine-induced hyperthermia. The dopamine depleter α-methyl-*p*-tyrosine significantly reduced *p*-chloroamphetamine-induced hyperthermia. The dopamine D_1 receptor antagonist 7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH 23390) antagonized *p*-chloroamphetamine-induced hyperthermia, although the dopamine D_2 receptor antagonist sulpiride was without effect. These results indicate that *p*-chloroamphetamine-induced hyperthermia in mice is mediated by dopamine release followed by activation of the dopamine D_1 receptor.

Keywords: *p*-Chloroamphetamine; Hyperthermia; 5-HT (5-hydroxytryptamine, serotonin); Dopamine; Dopamine D_1 receptor; Dopamine D_2 receptor; (Mouse)

1. Introduction

p-Chloroamphetamine is a serotonin (5-hydroxytryptamine, 5-HT)-releasing drug and elicits several central effects, such as behavioral changes including 5-HT syndrome or activation of the hypothalamic–pituitary axis (Trulson and Jacobs, 1976; Fuller, 1992; Fuller and Snoddy, 1980; Hutson and Curzon, 1989). These responses to *p*-chloroamphetamine are mainly based on 5-HT-releasing effects, since these effects are antagonized by the depletion of 5-HT (Trulson and Jacobs, 1976; Fuller, 1992). We previously reported that *p*-chloroamphetamine elicited significant hyperglycemia in rats and that this effect was completely prevented by the 5-HT synthesis inhibitor, *p*-chlorophenylalanine (Yamada et al., 1998).

p-Chloroamphetamine induces hyperthermic responses in rats (Fuller, 1992; Colado et al., 1993). We recently demonstrated that *p*-chloroamphetamine induces hyperthermic responses in mice similar to those in rats (Sugimoto et al., 2000). *p*-Chloroamphetamine-induced hyperthermia in mice was mediated by activation of central 5-HT_{2A} receptors, since the 5-HT_{2A} receptor antagonist attenuated it (Sugimoto et al., 2000). We also found that blockade of the $5\text{-HT}_{2B/2C}$ receptor enhanced hyperthermia elicited by *p*-chloroamphetamine (Sugimoto et al., 2000).

p-Chloroamphetamine is taken up by the 5-HT transporter and releases 5-HT from nerve terminals, comparable to another 5-HT-releasing drug, 3,4-methylenedioxymethamphetmaine (MDMA, ecstasy). It is recognized that *p*-chloroamphetamine causes 5-HT neurotoxicity in the brain, inducing 5-HT depletion, destruction of 5-HT nerve terminals, and inactivation of tryptophan hydroxylase (Sanders-Bush et al., 1972; Fuller, 1992; Fuller and Perry, 1983; Fuller et al., 1973). Inhibitors of the 5-HT transporter can antagonize *p*-chloroamphetamine-induced pharmacological effects or neurotoxicity (Meek et al., 1971; Fuller, 1980, 1992). However, it remains unclear whether 5-HT transporter inhibitors affect the hyperthermia induced by *p*-chloroamphetamine. In this paper, we investigated the effects of a 5-HT depleter and a 5-HT transporter inhibitor on *p*-chloroamphetamine-induced hyperthermia in mice. Although *p*-chloroamphetamine preferentially facilitates 5-HT release, it can increase dopamine release (Sharp et al., 1986; Sugita et al., 1994). It has been reported that dopamine participates in thermoregulation (Yamawaki et al., 1983). As *p*-chloroamphetamine re-

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leases dopamine, dopamine may be related to hyperthermia induced by *p*-chloroamphetamine. Therefore, we further studied the involvement of dopamine in the hyperthermia elicited by *p*-chloroamphetamine.

2. Materials and methods

2.1. Animals

Male ddY mice weighing 28–32 g were obtained from SLC Japan (Japan). Mice were given free access to food and water and were housed under a controlled 12-h/12-h light–dark cycle (light from 7:00 a.m. to 7:00 p.m.), with room temperature at 23 ± 1 °C and humidity at 55 ± 5%. All experiments were performed under the same ambient conditions.

2.2. Drug treatment

p-Chloroamphetamine HCl was obtained from Sigma (USA). Fluoxetine HCl, *p*-chlorophenylalanine methyl-ester HCl, 7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine HCl (SCH 23390) and sulpiride were obtained from Research Biochemicals (USA). α-Methyl-*p*-tyrosine methylester HCl was purchased from Nacalai Tesque (Japan). Drugs were injected i.p except for SCH 23390, which was injected s.c. *p*-Chloroamphetamine, *p*-chlorophenylalanine, fluoxetine, α-methyl-*p*-tyrosine and SCH 23390 were dissolved in saline. Sulpiride was dissolved in a few drops of 0.1 N HCl and diluted in saline. *p*-Chlorophenylalanine at 400 mg/kg was injected i.p. 72, 48 and 24 h before *p*-chloroamphetamine. α-Methyl-*p*-tyrosine at 250 mg/kg was administered 4 h before *p*-chloroamphetamine. Fluoxetine at 10 mg/kg, SCH 23390 at 0.5 mg/kg and sulpiride at 10 mg/kg were given 30 min before *p*-chloroamphetamine.

Table 1
Effects of *p*-chlorophenylalanine (PCPA) and fluoxetine on *p*-chloroamphetamine (PCA)-induced hyperthermia in mice

Group	Rectal temperature (°C)		
	0 min	60 min	120 min
Saline + saline	37.6 ± 0.11	38.0 ± 0.11	38.0 ± 0.18
Saline + PCA	37.4 ± 0.05	39.5 ± 0.21[a]	39.8 ± 0.36[a]
PCPA + saline	37.5 ± 0.09	37.9 ± 0.13	37.9 ± 0.13
PCPA + PCA	37.5 ± 0.11	39.4 ± 0.24	39.3 ± 0.42
Saline + saline	37.8 ± 0.07	37.5 ± 0.12	37.7 ± 0.24
Saline + PCA	38.1 ± 0.12	39.6 ± 0.12[a]	40.0 ± 0.17[a]
Fluoxetine + saline	37.7 ± 0.09	38.0 ± 0.14	38.0 ± 0.19
Fluoxetine + PCA	38.0 ± 0.11	39.6 ± 0.24	39.4 ± 0.29

Results are shown as means ± S.E. ($N = 5–8$). PCA was injected i.p. at 20 mg/kg. PCPA at 400 mg/kg was injected i.p. 72, 48 and 24 h before PCA. Fluoxetine at 10 mg/kg was injected i.p. 30 min before PCA.
[a] $P < 0.001$ vs. saline + saline.

Table 2
Effects of α-methyl-*p*-tyrosine (α-MT), SCH 23390 and sulpiride on *p*-chloroamphetamine (PCA)-induced hyperthermia in mice

Group	Rectal temperature (°C)		
	0 min	60 min	120 min
Saline + saline	38.0 ± 0.14	37.7 ± 0.10	37.5 ± 0.13
Saline + PCA	37.9 ± 0.11	39.1 ± 0.14[a]	39.5 ± 0.15[a]
α-MT + saline	37.3 ± 0.22	37.2 ± 0.19	37.0 ± 0.15
α-MT + PCA	37.4 ± 0.23	38.1 ± 0.21[b]	37.8 ± 0.18[b]
Saline + saline	37.8 ± 0.15	37.3 ± 0.07	37.3 ± 0.08
Saline + PCA	37.7 ± 0.17	39.1 ± 0.16[a]	39.4 ± 0.14[a]
SCH 23390 + saline	37.8 ± 0.12	37.1 ± 0.08	37.1 ± 0.07
SCH 23390 + PCA	37.5 ± 0.14	38.1 ± 0.18[b]	37.9 ± 0.18[b]
Saline + saline	38.0 ± 0.09	37.6 ± 0.14	37.5 ± 0.17
Saline + PCA	38.0 ± 0.15	39.6 ± 0.20[a]	39.8 ± 0.40[a]
Sulpiride + saline	38.3 ± 0.10	37.8 ± 0.23	37.1 ± 0.14
Sulpiride + PCA	38.1 ± 0.07	39.6 ± 0.11	39.5 ± 0.19

Results are shown as means ± S.E. ($N = 5–7$). PCA was injected i.p. at 20 mg/kg. α-MT at 250 mg/kg was injected i.p. 4 h before PCA. SCH 23390 at 0.5 mg/kg was injected s.c. 30 min before PCA. Sulpiride at 10 mg/kg was injected i.p. 30 min before PCA.
[a] $P < 0.001$ vs. saline + saline.
[b] $P < 0.001$ vs. saline + PCA.

2.3. Body temperature measurement

Body temperature was monitored with a thermometer (Sensortek, USA) and a thermistor probe was inserted 2 cm into the rectum. Rectal temperature was measured immediately before the injection of test compounds and at 15-min intervals afterward for 120 min.

2.4. Statistics

Results were analyzed by two-way analysis of variance (ANOVA) followed by Tukey's test.

3. Results

3.1. Effects of p-chlorophenylalanine and fluoxetine on p-chloroamphetamine-induced hyperthermia in mice

Table 1 shows effects of the 5-HT depleter *p*-chlorophenylalanine and the 5-HT transporter inhibitor fluoxetine on *p*-chloroamphetamine-induced hyperthermia. *p*-Chloroamphetamine at a dose of 20 mg/kg elicited apparent hyperthermia. Pretreatment with *p*-chlorophenylalanine and fluoxetine did not affect *p*-chloroamphetamine-induced hyperthermia.

3.2. Effects of α-methyl-p-tyrosine, SCH 23390 and sulpiride on p-chloroamphetamine-induced hyperthermia in mice

Table 2 shows the effects of the dopamine depleter α-methyl-*p*-tyrosine and dopamine receptor antagonists

SCH 23390 and sulpiride on *p*-chloroamphetamine-induced hyperthermia in mice. As shown in the results, α-methyl-*p*-tyrosine reduced *p*-chloroamphetamine-induced hyperthermia. SCH 23390 significantly attenuated *p*-chloroamphetamine-induced hyperthermia. However, sulpiride did not affect hyperthermia elicited by *p*-chloroamphetamine.

4. Discussion

We previously found that *p*-chloroamphetamine elicited hyperthermia in mice similar to that in rats (Sugimoto et al., 2000). *p*-Chloroamphetamine-induced hyperthermia is mediated by the central 5-HT_{2A} receptor, because the 5-HT_{2A} receptor antagonist ketanserin reduced it (Sugimoto et al., 2000). As shown in the present study, *p*-chloroamphetamine at 20 mg/kg induced hyperthermia, which is in agreement with our previous report.

Previous findings reported that the pharmacological effects of *p*-chloroamphetamine are closely related to its ability to release 5-HT from nerve terminals. The depletion of 5-HT reduces several *p*-chloroamphetamine-induced effects. The 5-HT neurotoxin 5,7-dihydroxytryptamine or the 5-HT depleter *p*-chlorophenylalanine decreases 5-HT behavioral syndrome or ejaculation elicited by *p*-chloroamphetamine (Trulson and Jacobs, 1976; Renyi, 1985; Fuller, 1992). It is well known that *p*-chloroamphetamine is incorporated into nerve terminals via the 5-HT transporter, resulting in facilitation of 5-HT release (Fuller, 1992). Previous findings indicated that inhibitors of the 5-HT transporter can inhibit *p*-chloroamphetamine-induced neurotoxicity (Fuller, 1980). To clarify the involvement of the serotonergic system in hyperthermia, we examined the effects of the 5-HT depleter *p*-chlorophenylalanine and the 5-HT transporter inhibitor fluoxetine on *p*-chloroamphetamine-induced hyperthermia.

Pretreatment with the 5-HT depleter *p*-chlorophenylalanine did not affect *p*-chloroamphetamine-induced hyperthermia. The dose of *p*-chlorophenylalanine used in the present study decreases 5-HT levels in the brain by 75% (Yamada et al., 1999). Furthermore, the 5-HT transporter inhibitor fluoxetine did not attenuate the hyperthermia induced by *p*-chloroamphetamine, which differs from the results for the behavioral effects. It is reported that MDMA-induced hyperthermia is not affected by fluoxetine in rats, which is consistent with the present results (Malberg et al., 1996). Our results indicate that a mechanism other than the serotonergic system may participate in *p*-chloroamphetamine-induced hyperthermia.

Although *p*-chloroamphetamine releases 5-HT from nerve terminals, it can also release dopamine. Sharp et al. (1986) and Sugita et al. (1994), using microdialysis, demonstrated that *p*-chloroamphetamine increases dopamine release in rat brain. It was suggested that dopamine is involved in thermoregulation (Yamawaki et al., 1983). Since we found that *p*-chloroamphetamine-induced hyperthermia was not affected by *p*-chlorophenylalanine or fluoxetine, dopamine may be involved in hyperthermia. Recent evidence indicates that the hyperthermia induced by MDMA can be inhibited by dopamine depletion (Malberg et al., 1996). Thus, we examined the effects of the dopamine depleter on *p*-chloroamphetamine-induced hyperthermia.

The dopamine depleter α-methyl-*p*-tyrosine significantly attenuated *p*-chloroamphetamine-induced hyperthermia in mice. This result indicates that a reduction in dopamine levels suppresses *p*-chloroamphetamine-induced hyperthermia and that dopamine plays a role in hyperthermia. Dopamine D_1 and dopamine D_2 receptor subtypes have been reported to be involved in thermoregulation (Zarrindast and Tabatabai, 1992). Since α-methyl-*p*-tyrosine reduces *p*-chloroamphetamine-induced hyperthermia, effects of dopamine D_1 and dopamine D_2 receptor antagonists were examined. The dopamine D_1 receptor antagonist SCH 23390 significantly reduced *p*-chloroamphetamine-induced hyperthermia, while the dopamine D_2 receptor antagonist sulpiride did not affect it. This suggests that *p*-chloroamphetamine-induced hyperthermia is mediated by the dopamine D_1 receptor but not by the dopamine D_2 receptor. It was previously found that the dopamine D_1 and dopamine D_2 receptor agonist apomorphine elicited hypothermia in mice (Barnett et al., 1972). However, apomorphine-induced hypothermia is mediated by the dopamine D_2 receptor, because the dopamine D_2 receptor antagonist sulpiride attenuated it (Zarrindast and Tabatabai, 1992). Furthermore, the dopamine D_1 receptor agonist 7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SKF 38393) induces hyperthermia in mice (Zarrindast and Tabatabai, 1992). Thus, it is indicated that activation of dopamine D_1 and dopamine D_2 receptors elicits hyper- and hypothermia, respectively (Zarrindast and Tabatabai, 1992). Taken together, it is concluded that the hyperthermia induced by *p*-chloroamphetamine is elicited by an increased release of dopamine and that the released dopamine stimulates dopamine D_1 receptors.

α-Methyl-*p*-tyrosine reduces noradrenaline levels as well as dopamine levels. Since *p*-chloroamphetamine may increase noradrenaline release and the β adrenoceptor antagonist propranolol reduces methamphetamine-induced hyperthermia (Johnson et al., 1991; Albers and Sonsalla, 1995), the possibility that noradrenaline may be involved in hyperthermic responses to *p*-chloroamphetamine cannot be excluded.

Our previous report showed that *p*-chloroamphetamine-induced hyperthermia in mice was antagonized by the 5-HT_{2A} receptor antagonist ketanserin (Sugimoto et al., 2000). The role of 5-HT_{2A} receptors in dopaminergic transmission has been demonstrated. Thus, dopamine release in the rat brain elicited by another amphetamine derivative, MDMA, is enhanced by the coadministration of the 5-HT_{2A} receptor agonist, 1-(2,5-dimethoxy-4-iodophe-

nyl)-2-aminopropane (DOI) (Gudelsky et al., 1994), and ketanserin and 2,3-dimethoxyphenyl-1-[2-(4-piperidine)-methanol] (MDL 100,907), which block the 5-HT_{2A} receptor, reduce MDMA-induced dopamine release in the rat striatum (Nash, 1990; Schmidt et al., 1992). It has been reported that the activation of 5-HT_{2A} receptors increases dopamine synthesis in the brain (Huang and Nichols, 1993). Taken together with previous reports, the findings indicate that the 5-HT_{2A} receptor-mediated facilitation of dopamine release or synthesis is associated with *p*-chloroamphetamine-induced hyperthermia.

In summary, our results demonstrated that the hyperthermia induced by the 5-HT-releasing drug *p*-chloroamphetamine is mediated by the 5-HT_{2A} receptor linked with the dopaminergic system. *p*-Chloroamphetamine-induced hyperthermia is elicited by its facilitation of dopamine release, resulting in the activation of dopamine D_1 receptors.

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