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Involvement of the 5-HT_2 receptor in hyperthermia induced by *p*-chloroamphetamine, a serotonin-releasing drug in mice

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Abstract

The effects of a serotonin (5-hydroxytryptamine, 5-HT)-releasing drug, *p*-chloroamphetamine (PCA), on body temperature were investigated in mice. PCA induced hyperthermia in mice. PCA-induced hyperthermia was inhibited by the 5-$HT_{2A/2B/2C}$ receptor antagonist, 4-isopropyl-7-methyl-9-(2-hydroxy-1-methyl-propoxycarbonyl)-4,6A,7,8,9,10,10A-octahydro-indolo[4,3-FG]quinolone maleate (LY53857). The 5-HT_{2A} receptor antagonist, ketanserin, reduced the PCA-induced hyperthermia, while the 5-$HT_{2B/2C}$ receptor antagonist, *N*-3-pyridinyl-3,5-dihydro-5-methyl-benzo[1,2-b:4,5-b′]dipyrrole-1(2H)-carboxamide (SB 206553), enhanced it. LY 53857, ketanserin and SB 206553 did not affect hyperactivity in mice treated with PCA. These results suggest that PCA-induced hyperthermia in mice is mediated by 5-HT_{2A} receptors and is not related to changes in locomotor activity.

Keywords: *p*-Chloroamphetamine; Hyperthermia; 5-HT_{2A} receptor; 5-$HT_{2B/2C}$ receptor; (Mouse)

1. Introduction

It has been suggested that serotonin (5-hydroxytryptamine, 5-HT) participates in thermoregulation (Myers, 1981). The direct injection of 5-HT into the brain elicits hypothermia in mice and rats, an effect which may be mediated by postsynaptic 5-HT_{1A} receptors (Cox and Lee, 1975; Cox et al., 1983; Yamada et al., 1988). The 5-HT_{1A} receptor agonist, 8-hydroxy-2-di-*n*-(propylamino)tetralin (8-OH-DPAT), induces hypothermia in rats, which is an index of postsynaptic 5-HT_{1A} receptor activation (Gudelsky et al., 1986; Hutson et al., 1987). At present, the 5-HT_2 receptor subtypes are divided into 5-$HT_{2A,}$ 5-$HT_{2B,}$ 5-HT_{2C} receptors (Baxter et al., 1995). It has been reported that the stimulation of 5-HT_{2A} receptors elicits hyperthermia in heat-adapted rats, indicating that the 5-HT_{1A} and 5-HT_{2A} receptors play opposite roles in thermoregulation (Gudelsky et al., 1986).

p-Chloroamphetamine (PCA) is an amphetamine analog, reported to release 5-HT from nerve terminals, inhibit 5-HT uptake and deplete brain 5-HT levels for a prolonged period (Fuller, 1992). Administration of PCA acutely elicits several pharmacological effects in rodents by facilitating 5-HT release. PCA can induce a characteristic 5-HT behavioural syndrome such as head weaving, forepaw treading and hindlimb abduction in rats (Trulson and Jacobs, 1976; Hutson and Curzon, 1989). We previously reported that PCA can induce hyperglycemia in rats by facilitating 5-HT release, which is mediated by 5-HT_{1A} and 5-$HT_{2B/2C}$ receptors (Yamada et al., 1998). PCA also induces hyperthermic responses in rats and the hyperthermia may contribute to its 5-HT neurotoxicity (Fuller, 1992; Colado et al., 1993).

Although a single administration of PCA induces hyperthermia in rats, little is known about thermoregulatory responses after PCA administration in mice. We, therefore, investigated the effects of PCA on the body temperature of mice and the involvement of 5-HT_2 receptor subtypes in temperature responses to PCA.

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

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and water and were housed under a controlled 12-h/12-h light–dark cycle (light from 7:00 AM to 7:00 PM), with room temperature at 23 ± 1°C and humidity at 55 ± 5%. All experiments were performed under the same ambient conditions. The experimental procedure was approved by the Kobe Pharmaceutical University Animal Care and Use Committee.

2.2. Drug treatment

PCA HCl was obtained from Sigma (USA). The 4-isopropyl-7-methyl-9-(2-hydroxy-1-methyl-propoxycarbonyl)-4,6A,7,8,9,10,10A-octahydro-indolo[4,3-FG]quinolone maleate (LY53857), *N*-3-pyridinyl-3,5-dihydro-5-methyl-benzo[1,2-b:4,5-b′]dipyrrole-1(2H)-carboxamide HCl (SB 206553) and ketanserin tartrate were obtained from Research Biochemicals (USA). The 5-HT receptor antagonists were administered 30 min before the injection of 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 prior to injections of test compounds and at 15-min intervals afterward for 120 min.

2.4. Measurement of locomotor activity

Locomotor activity was measured by a digital counter with an infrared sensor (Neuroscience, Japan) for 120 min after the injection of *p*-chloroamphetamine. The apparatus detects and gives a digital count of horizonal movements of animals.

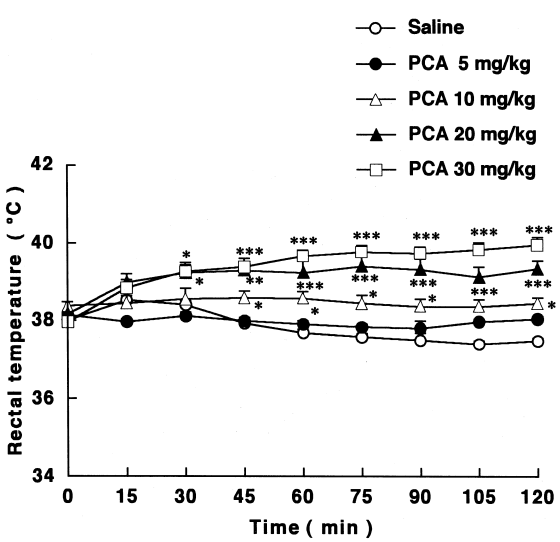

Fig. 1. Effects of PCA on rectal temperature of mice. PCA was given i.p. Results are shown as means ± S.E. Numbers of mice used: saline $N = 8$, PCA 5 mg/kg $N = 7$, 10 mg/kg $N = 7$, 20 mg/kg $N = 8$, 30 mg/kg $N = 5$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

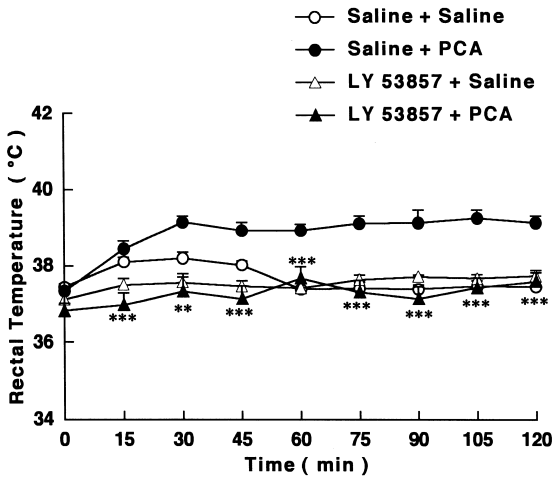

Fig. 2. Effects of LY 53857 on PCA-induced hyperthermia in mice. Results are shown as means ± S.E. ($N = 5$–7). PCA was injected i.p. at 20 mg/kg. LY 53857 at 5 mg/kg was injected i.p. before PCA. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. saline + PCA.

2.5. Statistics

Dose-related effects of PCA on body temperature were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test. Effects of 5-HT receptor antagonists on PCA-induced hyperthermia and increases in locomotor activity were analyzed by two-way ANOVA followed by Tukey's test.

3. Results

3.1. Effects of PCA on the body temperature of mice

Fig. 1 shows changes in the time course of body temperature following the injection of PCA. PCA elicited dose-dependent hyperthermia in mice.

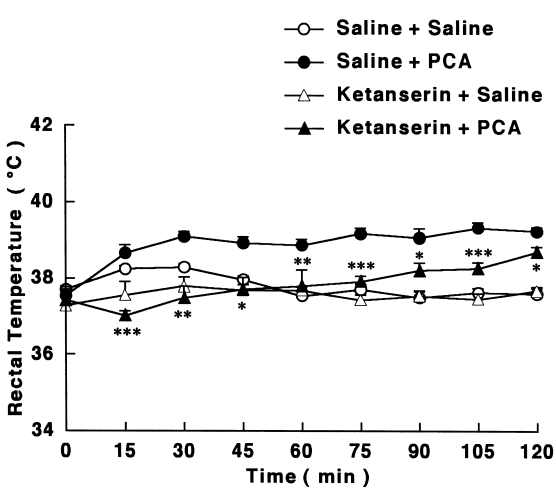

Fig. 3. Effects of ketanserin on PCA-induced hyperthermia in mice. Results are shown as means ± S.E. ($N = 5$–7). PCA was injected i.p. at 20 mg/kg. Ketanserin at 1 mg/kg was injected i.p. 30 min before PCA. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. saline + PCA.

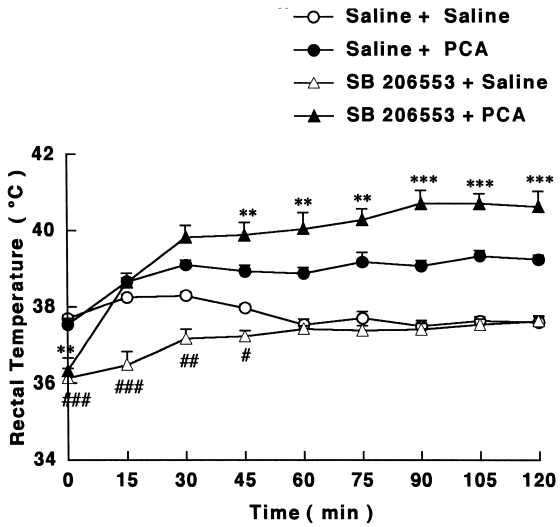

Fig. 4. Effects of SB 206553 on PCA-induced hyperthermia in mice. Results are shown as means ± S.E. ($N = 5–7$). PCA was injected i.p. at 20 mg/kg. SB 206553 at 5 mg/kg was injected i.p. 30 min before PCA. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. saline + PCA. $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$ vs. saline + saline.

3.2. Effects of 5-HT_2 receptor antagonists on PCA-induced hyperthermia in mice

Fig. 2 shows the effects of the 5-$HT_{2A/2B/2C}$ receptor antagonist, LY 53857, on PCA (20 mg/kg)-induced hyperthermia in mice. LY 53857 at a dose of 5 mg/kg significantly reduced PCA-induced hyperthermia, did not affect basal body temperature.

Effects of the 5-HT_{2A} receptor antagonist, ketanserin (1 mg/kg), on hyperthermia induced by PCA are shown in Fig. 3. Ketanserin inhibited hyperthermia as did LY 53857, and was without effect on basal rectal temperature of mice.

Fig. 4 shows the effects of the 5-$HT_{2B/2C}$ receptor antagonist, SB 206553 (5 mg/kg), on PCA-induced hyperthermia. Pretreatment with SB 206553 at 5 mg/kg significantly enhanced the hyperthermia. SB 206553 itself induced significant falls in body temperature for up to 45 min.

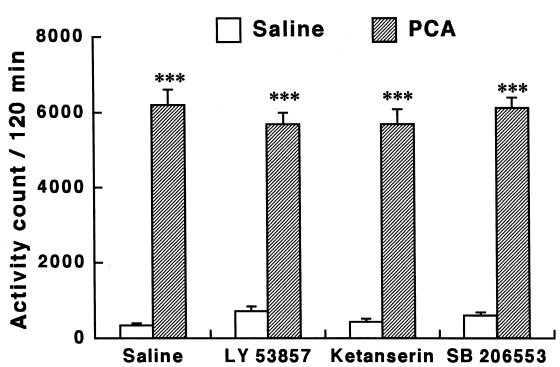

Fig. 5. Effects of LY 53857, ketanserin and SB 206553 on hyperactivity induced by PCA in mice. Results are shown as means ± S.E. ($N = 5–7$). PCA was injected i.p. at 20 mg/kg. LY 53857 at 5 mg/kg. Ketanserin 1 mg/kg and SB 206553 at 5 mg/kg was injected i.p. 30 min before PCA.

3.3. Effects of 5-HT_2 receptor antagonists on PCA-induced hyperactivity

Effects of LY 53857, ketanserin and SB 206553 on PCA-induced hyperactivity are shown in Fig. 5. PCA at 20 mg/kg induced increases in locomotor activity for 120 min. LY 53857 (5 mg/kg), ketanserin (1 mg/kg) and SB 206553 (5 mg/kg) were without effect on the hyperactivity elicited by PCA. Alone, these 5-HT_2 receptor antagonists did not influence locomotor activity.

4. Discussion

Our results demonstrated that PCA dose dependently induces hyperthermia in mice. PCA above the dosage of 10 mg/kg elicited a significant hyperthermia and its effects lasted for at least 2 h. Previous reports indicate that PCA can induce hyperthermia in rats at doses around 2–5 mg/kg (Colado et al., 1993; Murray et al., 1996). Thus, although hyperthermic responses to PCA in mice are similar to those in rats, doses higher than those used for rats are required to induce hyperthermia in mice. Hunskaar et al. (1986) reported that PCA, 25 mg/kg, elicits hyperthermia in mice, which is consistent with our finding. However, the 5-HT receptor subtypes involved in the hyperthermia elicited by PCA have not yet been described.

There is considerable evidence that 5-HT is involved in thermoregulation (Myers, 1981). Participation of 5-HT receptor subtypes in thermoregulation has been suggested for years. The 5-HT is related to both hypo- and hyperthermia, based on results using several 5-HT receptor agonists. The 5-HT_{1A} receptor agonist, 8-OH-DPAT, elicits hypothermia (Gudelsky et al., 1986; Hutson et al., 1987), while the 5-HT_{2A} receptor agonists, 1-(2,5-dimethoxy-4-iodophenyl)2-aminopropane (DOI) and 6-chloro-2-(1-piperazinyl)pyrazine (MK-212) elicit hyperthermic responses in rats (Gudelsky et al., 1986; Yamawaki et al., 1983; Salmi and Ahlenius, 1998). Hyperthermic responses induced by these agonists in rats are apparent at a higher ambient temperature, around 28°C (Yamawaki et al., 1983; Gudelsky et al., 1986). As shown in the present results, PCA elicited significant hyperthermia in mice at an ambient temperature of 23°C. Since 5-HT_{2A} receptor activation elicits hyperthermia in rats (Gudelsky et al., 1986; Salmi and Ahlenius, 1998), the effects of 5-HT_2 receptor antagonists on PCA-induced hyperthermia in mice were investigated.

The 5-$HT_{2A/2B/2C}$ receptor antagonist, LY 53857, and the 5-HT_{2A} receptor antagonist, ketanserin, significantly reduced the PCA-induced hyperthermia. The doses of LY 53857 and ketanserin used in this study are sufficient to block the central 5-HT_{2A} receptor-mediated head-twitch

responses (Kennett and Curzon, 1991). These results suggest that this hyperthermia is mediated by the 5-HT_{2A} receptor. SB 206553 is a 5-$HT_{2B/2C}$ receptor antagonist (Baxter et al., 1995) and can inhibit the 5-HT_{2C} receptor agonist 1-(3-chlorophenyl)piperazine (mCPP)-elicited hypophagia or hypolocomotion in rats (Kennett et al., 1996). Since LY 53857 blocks all 5-HT_2 receptor subtypes, we further determined the effects of the 5-$HT_{2B/2C}$ receptor antagonist, SB 206553, on PCA-induced hyperthermia. As shown in results, SB 206553 did not block the hyperthermia elicited by PCA but enhanced it. This result suggests that blockade of 5-HT_{2B} and/or 5-HT_{2C} receptors is not related to the inhibitory effects of LY 53857 on the hyperthermia elicited by PCA. These results indicate that the 5-HT_{2A} receptor mediates PCA-induced hyperthermia in mice. The finding that the 5-$HT_{2B/2C}$ receptor antagonist, SB 206553, enhanced hyperthermia suggests that 5-HT_{2B} and/or 5-HT_{2C} receptors may play a role in hypothermic responses. In heat-adapted rats, the 5-HT_{2C} receptor agonist, mCPP, elicits hyperthermia which is considered mediated by the 5-HT_{2C} receptor (Klodzinska and Chojnacka-Wójcik, 1992). It was reported that, on the contrary, mCPP induces hypothermia in mice and that hypothermia elicited by mCPP may be related to the 5-HT_{1B} receptor (Maj et al., 1988). Therefore, the role of the 5-HT_{2C} receptor in thermoregulation of mice has not been clarified and further studies are required. The systemic injection of LY 53857 and ketanserin can block both peripheral and central 5-HT_{2A} receptors. However, we had demonstrated that peripheral injection of 5-HT induces hypothermia mediated by the peripheral 5-HT_{2A} receptor (Sugimoto et al., 1991). Therefore, it is suggested that the peripheral 5-HT_{2A} receptor is not related to PCA-induced hyperthermia.

Since PCA increases locomotor activity (Hutson and Curzon, 1989; Fuller, 1992), this effect may be related to hyperthermic responses. However, our results indicate that the hyperactivity elicited by PCA was not changed by LY 53857, ketanserin or SB 206553, all of which modified the temperature responses. Therefore, the effects of these antagonists on PCA-induced hyperthermia are not related to hyperactivity.

In summary, our results demonstrate that PCA increases body temperature in mice at a normal temperature and that these hyperthermic responses are elicited by the activation of the central 5-HT_{2A} receptor. The 5-$HT_{2B/2C}$ receptors may have an opposite role to 5-HT_{2A} receptors in thermoregulation.

References

Baxter, G., Kennett, G.A., Blaney, F., Blackburn, T., 1995. 5-HT_2 receptor subtypes: a family re-united? Trends Pharmacol. Sci. 16, 105–110.

Colado, M.I., Murray, T.K., Green, A.R., 1993. 5-HT loss in rat brain following 3,4-methylenedioxymethamphetamine (MDMA), *p*-chloroamphetamine and fenfluramine administration and effects of chlormethiazole and dizocilpine. Br. J. Pharmacol. 108, 583–589.

Cox, B., Lee, T.F., 1975. Possible involvement of 5-hydroxytryptamine in dopamine-receptor-mediated hypothermia in the rat. J. Pharm. Pharmacol. 31, 352–354.

Cox, B., Davis, A., Valerier, J., Lee, T.F., 1983. A role for an indolamine other than 5-hydroxytryptamine in the hypothalamic thermoregulatory pathways of the rat. J. Physiol. 337, 441–450.

Fuller, R.W., 1992. Effects of *p*-chloroamphetamine on brain serotonin neurons. Neurochem. Res. 17, 449–456.

Gudelsky, G.A., Koenig, J.I., Meltzer, H.Y., 1986. Thermoregulatory responses to serotonin (5-HT) receptor stimulation in the rat: evidence for opposing roles of 5-HT_2 and 5-HT_{1A} receptors. Neuropharmacology 25, 1307–1313.

Hunskaar, S., Berge, O.G., Broch, O.J., Hole, K., 1986. Lesions of the ascending serotonergic pathways and antinociceptive effects after systemic administration of *p*-chloroamphetamine in mice. Pharmacol. Biochem. Behav. 24, 709–714.

Hutson, P.H., Curzon, G., 1989. Concurrent determination of effects of *p*-chloroamphetamine on central extracellular 5-hydroxytryptamine and behaviour. Br. J. Pharmacol. 96, 801–806.

Hutson, P.H., Donohoe, T.P., Curzon, G., 1987. Hypothermia induced by the putative 5-HT_{1A} receptor agonists LY 165163 and 8-OH-DPAT is not prevented by 5-HT depletion. Eur. J. Pharmacol. 143, 221–228.

Kennett, G.A., Curzon, G., 1991. Potencies of antagonists indicate that 5-HT_{1C} receptors mediate 1-3(chlorophenyl)piperazine-induced hypophagia. Br. J. Pharmacol. 103, 2016–2020.

Kennett, G.A., Wood, M.D., Bright, F., Cilia, J., Piper, D.C., Gager, T., Thomas, D., Baxter, G.S., Forbes, I.T., Ham, P., Blackburn, T.P., 1996. In vitro and in vivo profile of SB 206553, a potent 5-$HT_{2C/2B}$ receptor antagonist with anxiolytic-like properties. Br. J. Pharmacol. 117, 427–434.

Klodzinska, A., Chojnacka-Wójcik, E., 1992. Hyperthermia induced by *m*-trifluoromethylphenylpiperazine (TFMPP) or *m*-chlorophenylpiperazine (*m*-CPP) in heat-adapted rats. Psychopharmacology 109, 466–472.

Maj, J., Chojnacka-Wójcik, E., Klodzinska, A., Deren, A., Moryl, E., 1988. Hypothermia induced by *m*-trifluoromethylphenylpiperazine or *m*-chlorophenylpiperazine: an effect mediated by 5-HT_{1B} receptors. J. Neural Transm. 73, 43–55.

Murray, T.K., Williams, J.L., Mirsa, A., Colado, M.I., Green, A.R., 1996. The spin trap reagent PBN attenuates degeneration of 5-HT neurones in rat brain induced by *p*-chloroamphetamine but not fenfluramine. Neuropharmacology 35, 1615–1620.

Myers, R.D., 1981. Serotonin and thermoregulation: old and new views. J. Physiol. (Paris) 77, 505–513.

Salmi, P., Ahlenius, S., 1998. Evidence for functional interactions between 5-HT_{1A} and 5-HT_{2A} receptors in rat thermoregulatory mechanisms. Pharmacol. Toxicol. 82, l22–127.

Sugimoto, Y., Yamada, J., Horisaka, K., 1991. Activation of peripheral serotonin$_2$ receptors induces hypothermia in mice. Life Sci. 48, 419–423.

Trulson, M.E., Jacobs, B.L., 1976. Behavioral evidence for the rapid release of CNS serotonin by PCA and fenfluramine. Eur. J. Pharmacol. 36, 149–154.

Yamada, J., Sugimoto, Y., Wakita, H., Horisaka, K., 1988. The involvement of serotonergic and dopaminergic systems in hypothermia induced in mice by intracerebroventricular injection of serotonin. Jpn. J. Pharmacol. 48, 145–148.

Yamada, J., Sugimoto, Y., Yoshikawa, T., 1998. *p*-Chloroamphetamine, a serotonin-releasing drug, elicited in rats, a hyperglycemia mediated by the 5-HT_{1A} and 5-$HT_{2B/2C}$ receptors. Eur. J. Pharmacol. 359, 185–190.

Yamawaki, S., Lai, H., Horita, A., 1983. Dopaminergic and serotonergic mechanisms of thermoregulation: mediation of thermal effects of apomorphine and dopamine. J. Pharmacol. Exp. Ther. 227, 383–388.