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Evidence That 5-HT_{2A} Receptors Are Not Involved in 5-HT-Mediated Thermoregulation in Mice

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MORISHIMA, Y. AND T. SHIBANO. *Evidence that 5-HT_{2A} receptors are not involved in 5-HT-mediated thermoregulation in mice.* PHARMACOL BIOCHEM BEHAV **52**(4) 755–758, 1995.—To determine the role of 5-hydroxytryptamine$_{2A}$ (5-HT_{2A}) receptors in 5-HT-mediated thermoregulation in mice, we studied the effects of a 5-HT_{2A} receptor agonist and 5-HT_{2A} receptor antagonists on the body temperature, and the effects of selective 5-HT_{2A} receptor and nonselective 5-HT receptor antagonists on hypothermia induced by 5-hydroxytryptophan (5-HTP). (±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-HT_{2A} receptor agonist, did not change body temperature in mice at doses of 1 and 5 mg/kg, intraperitoneally (IP), which induced head twitch response. Three 5-HT_{2A} receptor antagonists, ketanserin (1 mg/kg, orally), ritanserin (1 and 10 mg/kg, orally), and DV-7028 (10 mg/kg, orally), also failed to alter body temperature, although these three 5-HT_{2A} receptor antagonists at ≥ 1 mg/kg, orally, inhibited head twitch response induced by 5-HTP (200 mg/kg, IP), a precursor of 5-HT. Ketanserin (1 mg/kg, orally), ritanserin (1 and 10 mg/kg, orally), and DV-7028 (10 mg/kg, orally) did not inhibit hypothermia induced by 5-HTP (200 mg/kg, IP). A nonselective 5-HT receptor antagonist, methysergide (1 mg/kg, subcutaneously), attenuated hypothermic response to 5-HTP. These results suggest that in mice, 5-HT_{2A} receptors are unlikely to be involved in 5-HT-mediated thermoregulation.

5-HT_{2A} receptors DOI Ketanserin Ritanserin DV-7028 Body temperature Mice

SEROTONERGIC neurons may contribute to the mechanism that underlies thermoregulation. Exposure of animals to cold or heat affects the metabolism of 5-hydroxytryptamine (5-HT) in the brain (13). Application of 5-HT into the hypothalamus, which is innervated by a rich supply of serotonergic neurons, or into the cerebral ventricles induces hypothermia in mice, rats, and rabbits, and hyperthermia in monkeys and cats (4). These opposite effects of 5-HT on body temperature may be explained by the stimulation of different 5-HT receptor subtypes, which initiate different intracellular signal transduction pathways.

In rats, 5-HT_{1A} receptors, which are negatively coupled to adenylate cyclase, are implicated in the hypothermic response, whereas 5-HT_{2A} receptors, which mediate the stimulation of phosphatidylinositol turnover, are involved in hyperthermia (7). In mice, intracerebroventricular injection of 5-HT or systemic administration of 5-hydroxytryptophan (5-HTP), a precursor of 5-HT, induces hypothermia, but not hyperthermia (1,3,19). Also, 8-hydroxy-2-(dipropylamino) tetralin (8-OH-DPAT), an agonist of 5-HT_{1A} receptors, decreases body temperature in mice (2,6). Thus, 5-HT_{1A} receptors are likely to be involved in hypothermia. In contrast, the role of 5-HT_{2A} receptors is unclear in thermoregulation in mice. Some evidence suggests that 5-HT_{2A} receptors are not involved in thermoregulation in mice; both an agonist [(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) at 0.1 mg/kg, subcutaneously (SC)] and an antagonist [ketanserin at 0.1 mg/kg, intraperitoneally (IP)] of 5-HT_{2A} receptors do not significantly change body temperature in mice (2,19). However, the earlier studies cannot exclude the following possibilities: a) The dose of DOI (0.1 mg/kg, SC) is insufficient to stimulate the central 5-HT_{2A} receptors, because DOI requires 0.63 mg/kg, IP, in mice to induce a head twitch response (5,9), which is related to the activation of the central 5-HT_{2A} receptors (15);

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or b) Ketanserin is not a highly selective antagonist for 5-HT_{2A} receptors, because it also blocks α_1-adrenergic receptors at higher doses (11).

The purpose of the present study was to examine whether 5-HT_{2A} receptors are involved in alterations in body temperature evoked by 5-HT in mice. In the present study, the effects of DOI at sufficient doses and highly selective 5-HT_{2A} receptor antagonists, ritanserin (12) and DV-7028 (16), were studied on thermoregulation in mice.

METHODS

Animals

Male *ddy* mice (Shizuoka Laboratory Animals Center, Shizuoka, Japan), weighing 25–50 g, were used. The animals were maintained in a temperature (24 ± 1°C) and light (lights on at 0700 h and off at 1900 h)-controlled room. All experiments were performed in the room between 1000 and 1600 h.

Body Temperature

Rectal temperature was measured with a probe connected to a digital thermometer (Termo, Tokyo, Japan). When body temperature was measured, the animals were restrained by hand for about 1 min and the probe was inserted 1 cm into the rectum.

DOI (1 and 5 mg/kg) was injected IP. Ketanserin (1 and 10 mg/kg), ritanserin (1 and 10 mg/kg), DV-7028 (10 mg/kg), and prazosin (10 mg/kg) were given orally with a stomach tube. The volume of solution was 1 ml/kg for parenteral administration and 10 ml/kg for oral administration. Body temperature was measured 30 min after DOI treatment and 1 h after ketanserin, ritanserin, DV-7028, and prazosin. When the effects of 5-HT receptor antagonists were studied on hypothermia induced by 5-HTP (200 mg/kg, IP), ketanserin (1 mg/kg), ritanserin (1 and 10 mg/kg), and DV-7028 (10 mg/kg) were administered 1 h before and methysergide (1 mg/kg, SC) was administered 30 min before the 5-HTP injection. Body temperature was measured 1 and 2 h after 5-HTP treatment. Control experiments were performed simultaneously in animals treated with vehicle solution (2% Tween 80 or saline).

Head Twitch Response

Head twitch response was induced by 5-HTP (200 mg/kg, IP). Then, 30 min after 5-HTP administration, we counted the total number of head twitch responses for 5 min. Ketanserin (0.1–10 mg/kg), ritanserin (0.3–10 mg/kg), and DV-7028 (0.1–10 mg/kg) were given orally 1 h before 5-HTP administration.

Statistical Analysis

All data are expressed as mean ± SEM. The statistical significance of results was evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's multiple range test. p values < 0.05 were considered to indicate statistically significant differences between groups.

Drugs

Ketanserin HCl, ritanserin HCl, and DV-7028 [3-(2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl)-6,7,8,9-tetrahydro-2H-pyrido(1,2-α)-1,3,5-triazine-2,4-(^{3}H)-dione] were synthesized at Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). DOI was purchased from Research Biochemicals (Natick, MA), 5-hydroxytryptophan (5-HTP) from Sigma (St. Louis, MO) and methysergide from USPC (Rockville, MD). Ketanserin, ritanserin, and DV-7028 were suspended or dissolved in 2% Tween 80. DOI, 5-HTP, and methysergide were dissolved in saline.

RESULTS

Effects of a 5-HT_{2A} Receptor Agonist, 5-HT_{2A} Receptor Antagonists, and an α_1-Adrenergic Receptor Antagonist on Body Temperature in Mice

There were no significant differences between the groups in baseline body temperature. Although DOI (1 and 5 mg/kg, IP), a 5-HT_{2A} receptor agonist, induced head twitch behavior (data not shown), the compound did not affect body temperature in mice (Table 1). Ritanserin (1 and 10 mg/kg, orally) and DV-7028 (10 mg/kg, orally) failed to alter body temperature. Ketanserin did not change body temperature at 1 mg/kg, but significantly lowered it at a higher dose (10 mg/kg, orally). Prazosin (10 mg/kg, orally) caused hypothermia.

Effects of 5-HT_{2A} Receptor Antagonists and a Nonselective 5-HT Receptor Antagonist on 5-HTP-Induced Hypothermia

5-HTP (200 mg/kg, IP) significantly decreased body temperature in mice. Ketanserin (1 mg/kg, orally), ritanserin (1 and 10 mg/kg, orally), and DV-7028 (10 mg/kg, orally) did not affect hypothermia induced by 5-HTP in mice (Table 2). In contrast, methysergide (1 mg/kg, SC) antagonized the hypothermic response to 5-HTP, although the compound did not significantly alter body temperature.

Effects of 5-HT_{2A} Antagonists on 5-HTP-Induced Head Twitch Response

The effects of 5-HT_{2A} receptor antagonists on head twitch response were studied to confirm that 5-HT_{2A} receptor antagonists actually acted on the central 5-HT_{2A} receptors.

The number of head twitch responses per 5 min reached its maximum 30 min after the administration of 5-HTP (200 mg/

TABLE 1

EFFECTS OF A 5-HT_{2A} RECEPTOR AGONIST AND ANTAGONISTS ON BODY TEMPERATURE IN MICE

Drug	Dose (mg/kg)	Change in Body Temperature (°C)	n
Agonist			
Control		−0.52 ± 0.16	5
DOI	1	−0.26 ± 0.14	5
DOI	5	−0.58 ± 0.44	5
Antagonist			
Control		−0.38 ± 0.16	12
Ketanserin	1	−0.50 ± 0.21	6
Ketanserin	10	−2.20 ± 0.49*	6
Ritanserin	1	−0.60 ± 0.26	5
Ritanserin	10	−1.22 ± 0.43	6
DV-7028	10	−0.50 ± 0.24	6
Prazosin	10	−3.24 ± 0.17*	5

All values are mean ± SEM of changes in body temperature. (±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI) was injected intraperitoneally. Ketanserin, ritanserin, DV-7028, and prazosin were given orally.

*Significant difference from control ($p < 0.01$).

TABLE 2

EFFECTS OF 5-HT ANTAGONISTS ON HYPOTHERMIA INDUCED BY 5-HTP IN MICE

Drug	Dose (mg/kg)	Change in Body Temperature (°C)	n
Experiment 1			
Control		−2.61 ± 0.26	15
Ketanserin	1	−2.54 ± 0.45	5
Ritanserin	1	−1.99 ± 0.36	5
	1	−1.30 ± 0.42*	5
Methysergide			
Experiment 2			
Control		−2.02 ± 0.28	5
Ritanserin	10	−2.22 ± 0.38	5
DV-7028	10	−2.46 ± 0.54	5

All values are mean ± SEM of changes in body temperature. 5-HTP (200 mg/kg) was injected intraperitoneally. Ketanserin, ritanserin, and DV-7028 were administered orally, and methysergide was given subcutaneously.

*Significant difference from control ($p < 0.05$).

kg, IP). Accordingly, the number of head twitch responses was measured from 30–35 minutes after 5-HTP administration. Ketanserin, ritanserin, and DV-7028 dose-dependently suppressed the head twitch response to 5-HTP. These three 5-HT_{2A} antagonists at ≥1 mg/kg inhibited head twitch by >80% (Fig. 1).

DISCUSSION

The results of the present study suggest that 5-HT_{2A} receptors are not involved in physiologic thermoregulation in mice. This suggestion is based on three observations.

First, DOI at 1 and 5 mg/kg, IP, doses that induced head twitch response, did not affect body temperature in mice. The doses of DOI were selected based on the reports by Heaton et al. (9) and Darmani et al. (5), in which DOI dose-dependently caused head twitches at 0.63–5 mg/kg, IP. Alternatively, DOI has an agonistic activity at 5-HT_{2C} (5-HT_{1C}) receptors, which share a high sequence homology with 5-HT_{2A} receptors (10). DOI may not affect body temperature because it activates both 5-HT_{2A} and 5-HT_{2C} receptors. However, this possibility is ruled out, because the activation of 5-HT_{2C} receptors by preferential 5-HT_{2C} receptor agonists 1-(meta-chlorophenyl)-piperazine (mCPP) and m-trifluoromethyl phenyl piperazine (TFMPP) does not change body temperature in mice (2).

Second, ketanserin at a low dose (1 mg/kg, orally) and highly selective 5-HT_{2A} antagonists ritanserin and DV-7028 had no effect on body temperature in mice. The affinity of DV-7028 is 25-fold higher for the 5-HT_{2A} receptors compared to the 5-HT_{2C} receptors, and the compound has no affinity for other 5-HT receptor subtypes (16). These 5-HT_{2A} receptor antagonists at ≥1 mg/kg, orally, suppressed head twitch responses induced by 5-HTP, indicating that the antagonists actually inhibit central 5-HT_{2A} receptors, in mice, 5-HT_{2A} receptors may not participate significantly in the regulation of body temperature. Ketanserin at 10 mg/kg, orally, caused hypothermia in mice, but the dose was higher than that required to block the central 5-HT_{2A} receptors. The hypothermic response by 10 mg/kg ketanserin, orally, may be related to the inhibition of α_1-adrenergic receptors (11). Indeed, a selective α_1-adrenergic antagonist, prazosin, induced hypothermia in the present and earlier studies (17).

Finally, ketanserin, ritanserin, and DV-7028 did not affect the hypothermic response to 5-HTP. In contrast, a nonselective 5-HT antagonist, methysergide, prevented a decrease in body temperature evoked by 5-HTP. These results are consistent with earlier reports that: a) central application of 5-HT to mice induces hypothermia (3,19), which is inhibited by methysergide and a 5-HT_1 receptor antagonist, pindolol (19); and b) a 5-HT_{1A} receptor agonist, 8-OH-DPAT, lowers body temperature in mice (2,6). Hence, hypothermia evoked by 5-HTP in mice is due mainly to the activation of 5-HT_1 (presumably 5-HT_{1A}) receptors but not 5-HT_{2A} receptors.

The results of this study differ from the report by Sugimoto et al. (18), who noted that the peripheral 5-HT_{2A} receptors are involved in hypothermia induced by injecting 5-HT IP in mice. The route of drug administration was different in these two studies. Ketanserin was injected IP in the study by Sugimoto et al. (18), and the 5-HT_{2A} receptor antagonists were given orally in our study. However, the difference in the method of drug administration may not be responsible for the discrepancy, because the 5-HT_{2A} receptor antagonists used in the present study were orally active. In fact, these antagonists significantly inhibited head twitch response with oral administration. Hence, the results of the present study suggest that the peripheral 5-HT_{2A} receptors are not involved in hypothermia induced by 5-HTP in mice, because the 5-HT_{2A} receptor antagonists act on both the central and peripheral 5-HT_{2A} receptors.

With regard to rats, activation of 5-HT_{2A} receptors by a 5-HT agonist, MK-212, and activation of 5-HT_{1A} receptors by 8-OH-DPAT elicit hyperthermia and hypothermia, respectively. Furthermore, blockade of 5-HT_{2A} receptors by ketanserin (0.1–3 mg/kg, IP) or pirenperone lowers body temperature, whereas blockade of 5-HT_{1A} receptors by pindolol results in an increase in body temperature (7). Therefore, in rats, 5-HT_{2A} and 5-HT_{1A} receptors appear to have opposing roles in thermoregulation. Taken together with these observations and the results of the present study, the role of 5-HT_{2A} receptors in thermoregulation in mice is less significant than that in rats.

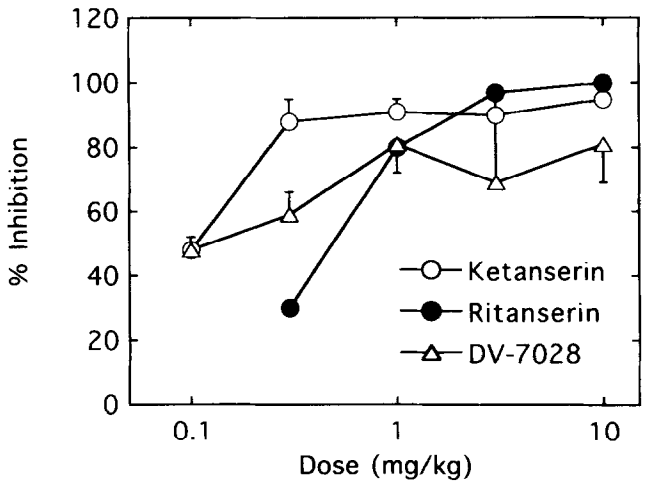

FIG. 1. Effects of ketanserin, ritanserin, and DV-7028 on the head twitch response to 5-HTP in mice. Thirty minutes after 5-HTP (200 mg/kg, IP) treatment, the number of head twitch responses was counted for 5 min. 5-HT_{2A} antagonists were administered 1 h before 5-HTP treatment. All values are mean ± SEM (n = 4 or 5).

The discrepancy between mice and rats in the role of 5-HT_{2A} receptors in thermoregulation is consistent with earlier studies showing that the content of 5-HT_{2A} receptors in the hypothalamus is higher in rats than in mice (8,14). The difference in the number of 5-HT_{2A} receptors in the hypothalamus may explain the different roles of 5-HT_{2A} receptors in thermoregulation. However, the present experiments do not permit further speculation concerning the differences between the two species.

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