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Hyperglycemia induced by the 5-HT receptor agonist, 5-methoxytryptamine, in rats: involvement of the peripheral 5-HT_{2A} receptor

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Abstract

The effects of the 5-HT receptor agonist, 5-methoxytryptamine, on plasma glucose levels were investigated in rats. 5-Methoxytryptamine induced a significant hyperglycemia above the dosage of 1 mg/kg. 5-Methoxytryptamine-induced hyperglycemia was antagonized by pretreatment with the 5-HT_1 and 5-HT_2 receptor antagonist, methysergide, or the 5-HT_{2A} receptor antagonist, ketanserin, whereas the 5-HT_3 and 5-HT_4 receptor antagonist, tropisetron, and the 5-HT_4 receptor antagonist, SDZ 205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino) ethyl ester), showed no effect. In addition, the peripheral 5-HT_2 receptor antagonist, xylamidine, reduced 5-methoxytryptamine-induced hyperglycemia. These results suggest that 5-methoxytryptamine-induced hyperglycemia is mediated by the peripheral 5-HT_{2A} receptor, although it has a high affinity for the 5-HT_4 receptor. Adrenodemedullation abolished the 5-methoxytryptamine-induced hyperglycemia. 5-Methoxytryptamine did not affect the blood levels of the pancreatic hormones, insulin and glucagon. The hyperglycemia induced by 5-methoxytryptamine was not affected by pretreatment with dexamethasone which inhibits corticosterone release. These results indicate that 5-methoxytryptamine-induced hyperglycemia is elicited by a facilitated adrenaline release from the adrenal gland. Therefore, it is suggested that the 5-HT_{2A} receptor may be partly involved in the pharmacological effects induced by the 5-HT_4 receptor agonist, 5-methoxytryptamine.

Keywords: 5-Methoxytryptamine; Hyperglycemia; 5-HT_{2A} receptor; 5-HT_4 receptor

1. Introduction

Considerable attention has been focused on the mechanism of glucose regulation related to 5-HT receptors. The central 5-HT_{1A} receptor agonist, 8-hydroxy-2-di-*n*-(propylamino)tetralin (8-OH-DPAT), or the 5-HT_{1A} partial agonists, buspirone or ipsapirone, can induce hyperglycemia in rats (Chaouloff et al., 1990a,b). We also had found that buspirone induces hyperglycemia and inhibits the hypoglycemia elicited by tolbutamide (Sugimoto et al., 1992, 1995). Hyperglycemia induced by 5-HT_{1A} receptor agonists has been suggested to be related to adrenaline release from the adrenal gland (Chaouloff et al., 1990a,b; Sugimoto et al., 1992, 1995). These results suggest that the 5-HT_{1A} receptor is involved in glucose regulation through adrenaline release. Recently it has been reported that the 5-HT_2 receptor also participates in glucose regulation in rats. Previous reports suggested that the central 5-$HT_{2A/2C}$ receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), elicits a hyperglycemia mediated by the centrally located 5-HT_{2A} receptor (Baudrie and Chaouloff, 1992; Sugimoto et al., 1996b). Furthermore, it was reported that the peripheral 5-HT_2 receptor agonist, α-methyl-5-HT, can cause a hyperglycemic response in rats (Baudrie and Chaouloff, 1992; Chaouloff et al., 1992; Sugimoto et al., 1996a). We previously found that peripherally administered 5-HT induces a hyperglycemia which is mediated by the peripheral 5-HT_{2A} receptor (Yamada et al., 1995). Both the central and peripheral 5-HT_{2A} receptor-related hyperglycemia is related to adrenaline release from the adrenal gland, since adrenalectomy or adrenodemedullation suppresses the hyperglycemia induced by these agonists (Baudrie and Chaouloff, 1992; Yamada et al., 1995). Furthermore, we recently reported that the 5-$HT_{2C/2B}$ receptor agonist, 1-(3-chlorophenyl)piperazine (mCPP), elicits hyperglycemia in rats and that these recep-

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tors may also be involved in glucose regulation (Sugimoto et al., 1996b).

5-Methoxytryptamine is a trace amine present in both brain and peripheral tissues (Prozialeck et al., 1978). 5-Methoxytryptamine has a high affinity for the 5-HT_4 receptor and is recognized as a 5-HT_4 receptor agonist, although it also shows significant affinities for other 5-HT receptor subtypes (Hoyer, 1989; Hoyer et al., 1994; Cushing and Cohen, 1992). The 5-HT_4 receptor is present in both the central nervous system and the peripheral tissues (Bockaert et al., 1992; Eglen et al., 1995). It has been suggested that the 5-HT_4 receptor is related to transmitter release from nerve terminals or the myenteric plexus (Bockaert et al., 1992). Moreover, the 5-HT_4 receptor agonists may enhance cognitive performance, facilitate gastrointestinal motility or induce analgesia (Eglen et al., 1995). However, the involvement of the 5-HT_4 receptor in glucose regulation remains unclear and the effects of 5-methoxytryptamine on blood glucose levels have not yet been examined. In the present work, therefore, we studied the effects of 5-methoxytryptamine on blood glucose levels of rats to resolve the involvement of the 5-HT_4 receptor in glucose regulation. In addition, we studied the involvement of 5-HT receptor subtypes in glycemic responses to 5-methoxytryptamine using 5-HT receptor antagonists, since 5-methoxytryptamine has an appropriate affinity for other 5-HT receptor subtypes.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats weighing 190–220 g were obtained from SLC Japan (Japan). They were maintained 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 24 ± 1°C and humidity 55 ± 5%. The rats were given food and water ad libitum.

2.2. Drug treatment

5-Methoxytryptamine hydrochloride and dexamethasone were purchased from Sigma (USA) and Wako (Japan), respectively. Methysergide hydrogen maleate, ketanserin tartrate, tropisetron and SDZ 205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino) ethyl ester) were obtained from Research Biochemicals Int. (USA). Xylamidine tosylate was kindly supplied by GlaxoWellcome (UK). All drugs were dissolved in saline. 5-Methoxytryptamine and 5-HT receptor antagonists were injected i.p.; 5-HT receptor antagonists, except for SDZ 205-557 and xylamidine, were given 30 min before the injection of 5-methoxytryptamine. SDZ 205-557 and xylamidine were injected i.p. 10 and 60 min before 5-methoxytryptamine, respectively. Dexamethasone was injected s.c. 2 h and 20 min before the injection of 5-methoxytryptamine.

2.3. Determination of plasma glucose levels

Blood samples were taken from the caudal vena cava under light ether anesthesia. Only one sample was taken from each rat. Plasma glucose levels were determined by previously described methods (Sugimoto et al., 1992).

2.4. Determination of blood insulin and glucagon levels

Pancreatic hormone insulin and glucagon were determined by radioimmunoassay as described earlier (Sugimoto et al., 1992). Serum insulin and glucagon levels were determined with commercially available kits, Phadeseph Insulin (Pharmacia, Sweden) and glucagon Daiichi (Daiichi Radio Isotope Center, Japan), respectively.

2.5. Operation of adrenodemedullation

Bilateral adrenodemedullation was performed under anesthesia with pentobarbital Na at 50 mg/kg. Experiments were carried out one week after the operation.

2.6. Statistics

Statistical significance was evaluated with Student's *t*-test for comparisons of two groups. Dose-related effects of 5-methoxytryptamine on plasma glucose levels were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test. Effects of 5-HT receptor antagonists on 5-methoxytryptamine-induced effects were analyzed by two-way ANOVA followed by Tukey's test.

3. Results

3.1. Effects of 5-methoxytryptamine on the plasma glucose and 5-HT receptor antagonists on 5-methoxytryptamine-induced hyperglycemia

Fig. 1 shows the effects of 5-methoxytryptamine on plasma glucose levels. 5-Methoxytryptamine induced a

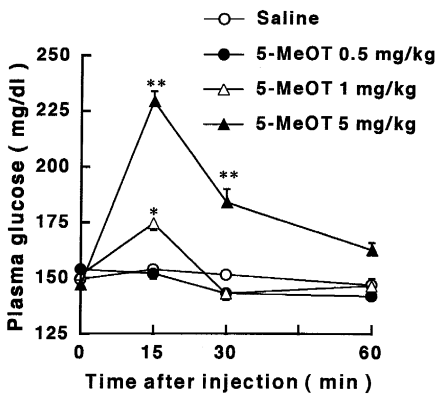

Fig. 1. Effects of 5-methoxytryptamine (5-MeOT) on plasma glucose levels of rats. Results are shown as means ± S.E. ($n = 6$–9). 5-MeOT was injected i.p. $^{*}P < 0.05$, $^{**}P < 0.01$.

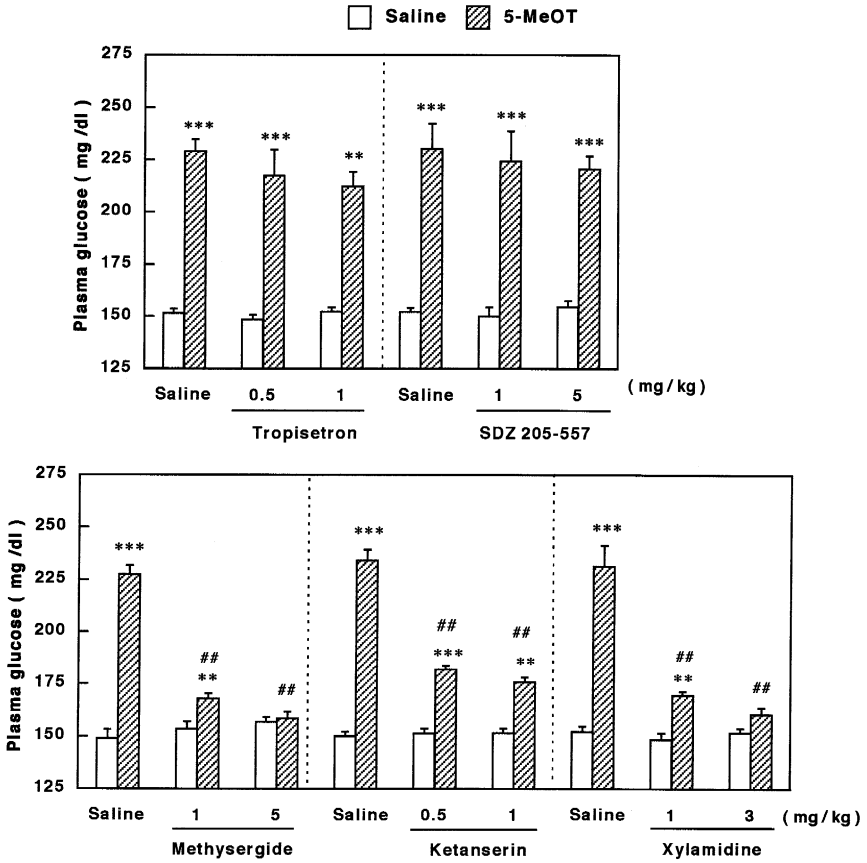

Fig. 2. Effects of 5-HT receptor antagonists on 5-methoxytryptamine (5-MeOT)-induced hyperglycemia. Results are shown as means ± S.E. ($n = 5–9$). 5-MeOT was injected i.p. at 5 mg/kg. Plasma glucose levels were determined 15 min after injection of 5-MeOT. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. saline control for respective group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. saline + 5-MeOT.

clear hyperglycemia at doses above 1 mg/kg. Effects of 5-HT receptor antagonists on 5-methoxytryptamine-induced hyperglycemia are shown in Fig. 2. Plasma glucose levels 15 min after treatment with 5-methoxytryptamine 5 mg/kg were evaluated. Neither the 5-HT_3 and 5-HT_4 receptor antagonist, tropisetron, nor the 5-HT_4 receptor antagonist, SDZ 205-557, affected the 5-methoxytryptamine-induced hyperglycemia. The 5-HT_1 and 5-HT_2 receptor antagonist, methysergide, and the 5-HT_{2A} receptor antagonist, ketanserin, strongly diminished the 5-methoxytryptamine-induced hyperglycemia. Lastly, the periph-

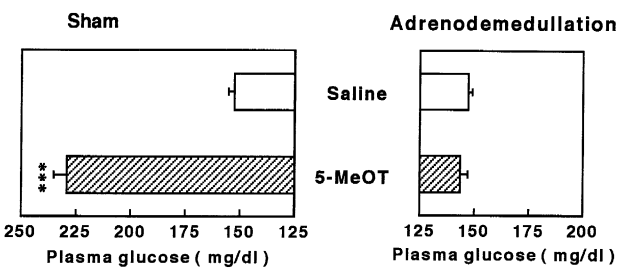

Fig. 3. Effects of adrenodemedullation on 5-methoxytryptamine (5-MeOT)-induced hyperglycemia in rats. Results are shown as means ± S.E. ($n = 7–9$). 5-MeOT was injected i.p. at 5 mg/kg. Plasma glucose levels were determined 15 min after injection of 5-MeOT. $^{***}P < 0.001$.

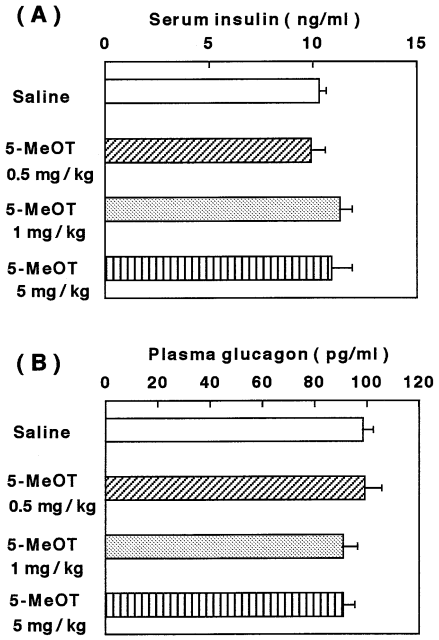

Fig. 4. Effects of 5-methoxytryptamine (5-MeOT) on the serum insulin and glucagon levels in rats. Results are shown as means ± S.E. ($n = 6–10$). 5-MeOT was injected i.p. Insulin and glucagon levels were determined 15 min after injection of 5-MeOT.

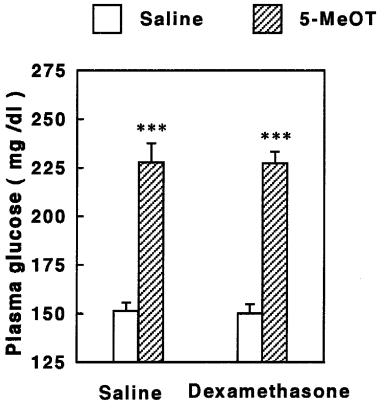

Fig. 5. Effects of dexamethasone on 5-methoxytryptamine (5-MeOT)-induced hyperglycemia. Results are shown as means ± S.E. ($n = 6–9$). 5-MeOT was injected i.p. at 5 mg/kg. Dexamethasone at 0.35 mg/kg was injected s.c. 2 h and 20 min before 5-MeOT. Plasma glucose levels were determined 15 min after injection of 5-MeOT. $^{***}P < 0.001$ vs. saline control for respective group.

eral 5-HT_2 receptor antagonist, xylamidine, also powerfully attenuated 5-methoxytryptamine-induced effects. 5-HT receptor antagonists did not affect the basal glucose levels.

3.2. Effects of 5-methoxytryptamine on plasma glucose levels in adrenodemedullated rats

The effects of 5-methoxytryptamine on plasma glucose levels in adrenodemedullated rats are shown in Fig. 3. Adrenodemedullation did not affect basal plasma glucose levels. In adrenodemedullated rats, 5-methoxytryptamine did not elicit hyperglycemia, although it induced hyperglycemia in sham-operated rats.

3.3. Effects of 5-methoxytryptamine on blood insulin and glucagon levels in rats

Fig. 4 shows the effects of 5-methoxytryptamine on serum insulin and plasma glucagon levels. 5-Methoxytryptamine did not change the levels of either pancreatic hormone.

3.4. Effects of 5-methoxytryptamine on plasma glucose levels in dexamethasone-pretreated rats

Fig. 5 demonstrates the effects of 5-methoxytryptamine on plasma glucose levels in dexamethasone (0.35 mg/kg × 2)-treated rats. 5-Methoxytryptamine induced hyperglycemic effects similar to those in vehicle-treated rats.

4. Discussion

Our results demonstrated that 5-methoxytryptamine induced hyperglycemia in rats. The glycemic response to 5-methoxytryptamine was dose-dependent and was observed at dosages above 1 mg/kg. Since 5-methoxytryptamine is recognized to be a 5-HT_4 receptor agonist, we examined the effects of tropisetron, which has marked 5-HT_3 and 5-HT_4 receptor blocking properties and of the 5-HT_4 receptor antagonist, SDZ 205-557, on 5-methoxytryptamine-induced hyperglycemia. Although tropisetron can block both the 5-HT_4 receptor and the 5-HT_3 receptor, pretreatment with tropisetron even at 1 mg/kg did not affect 5-methoxytryptamine-induced hyperglycemia. In addition, SDZ 205-557 at doses which can block 5-HTP-induced defecation mediated by 5-HT_4 receptor (Banner et al., 1996), did not affect 5-methoxytryptamine-induced hyperglycemia. These results suggest that 5-methoxytryptamine-induced hyperglycemia is not related to either the 5-HT_3 or 5-HT_4 receptor. Results of receptor binding studies indicated that 5-methoxytryptamine has significant affinity for other 5-HT receptor subtypes (Hoyer, 1989; Hoyer et al., 1994; Cushing and Cohen, 1992). Since 5-methoxytryptamine has an appropriate affinity for both the 5-HT_1 and 5-HT_2 receptor, we further examined the effects of the 5-HT_1 and 5-HT_2 receptor antagonist, methysergide, on 5-methoxytryptamine-induced hyperglycemia. As shown in Section 3, methysergide strongly suppressed the 5-methoxytryptamine-elicited hyperglycemia. Moreover, ketanserin, which has a high affinity for the 5-HT_{2A} receptor, also suppressed the hyperglycemia. These results suggest that 5-methoxytryptamine-induced hyperglycemia is closely related to the 5-HT_{2A} receptor. A previous report demonstrated that 5-methoxytryptamine administered i.c.v. in mice caused head twitch responses that are mediated by the 5-HT_{2A} receptor (Nakamura and Fukushima, 1978). Therefore, it is suggested that 5-methoxytryptamine induces hyperglycemia by acting as a 5-HT_{2A} receptor agonist.

However, it is not clear whether the 5-methoxytryptamine-induced hyperglycemia is mediated by centrally or peripherally located 5-HT_{2A} receptors, since previous reports demonstrated that activation of either central or peripheral 5-HT_{2A} receptors can elicit hyperglycemia in rats (Baudrie and Chaouloff, 1992; Sugimoto et al., 1996a; Yamada et al., 1995). Thus, we further studied the effects of the peripheral 5-HT_2 receptor antagonist, xylamidine, on 5-methoxytryptamine-induced hyperglycemia. As shown in Section 3, xylamidine attenuated the 5-methoxytryptamine-induced hyperglycemia just as methysergide or ketanserin did. This indicates that 5-methoxytryptamine-induced hyperglycemia is caused by stimulation of the peripheral 5-HT_{2A} receptor. 5-Methoxytryptamine, following peripheral injection, can cross the blood-brain barrier and induce central effects such as behavioural changes, that is, hyperactivity (Green et al., 1975). However, without a monoamine oxidase inhibitor, the behavioural effects are transient even at the high dose of 12.5 mg/kg (Green et al., 1975). As we had found, 5-methoxytryptamine induced hyperglycemia at doses

above 1 mg/kg. Therefore, the dosage of 5-methoxytryptamine used in the present study may have been insufficient to reach the brain. Our present findings further support the hypothesis that stimulation of peripheral 5-HT_{2A} receptor elicits hyperglycemia and that this receptor participates in glucose regulation. It is assumed that the 5-HT_4 receptor in peripheral tissues is not involved in glucose regulation, because 5-methoxytryptamine has a high affinity for the 5-HT_4 receptor.

Adrenaline released from the adrenal gland is a strong hyperglycemic factor and is closely related to the hyperglycemic effects of several 5-HT receptor agonists (Baudrie and Chaouloff, 1992; Chaouloff et al., 1990a,b, 1992; Sugimoto et al., 1992, 1996b). We had previously demonstrated that peripherally administered 5-HT induces hyperglycemia due to adrenaline release mediated by the peripheral 5-HT_{2A} receptor (Yamada et al., 1995). Since 5-methoxytryptamine-induced hyperglycemia is mediated by the 5-HT_{2A} receptor, we studied the effects of 5-methoxytryptamine on blood glucose levels in adrenodemedullated rats. Adrenodemedullation did not affect basal plasma glucose levels, which is consistent with results of Bouhelal and Mir (1992), because it may be a result of homeostasis of plasma glucose levels. In adrenodemedullated rats, 5-methoxytryptamine did not affect the plasma glucose levels. This result demonstrates that 5-methoxytryptamine-induced hyperglycemia is strongly related to adrenaline release from the adrenal gland. Therefore, it is suggested that 5-methoxytryptamine facilitates adrenaline release mediated by the peripheral 5-HT_{2A} receptor, which in turn causes hyperglycemia in rats. We demonstrated that 5-methoxytryptamine elicits hyperglycemia in ether-anesthetized rats. Since ether itself stimulates adrenaline release, synergistic effects of ether with 5-methoxytryptamine may be involved in the hyperglycemia.

The pancreatic hormones, insulin and glucagon, are known to regulate glucose homeostasis. Thus, we examined the effects of 5-methoxytryptamine on the levels of these hormones. As shown in Section 3, 5-methoxytryptamine did not affect the insulin levels. Although a clear hyperglycemia was elicited following 5-methoxytryptamine, insulin release was not observed, indicating that insulin release is inhibited. Since adrenaline is known to inhibit insulin release, the adrenaline elevated by 5-methoxytryptamine may suppress the insulin release triggered by hyperglycemia. 5-Methoxytryptamine was without effect on another pancreatic hormone, glucagon, suggesting that glucagon is not involved in 5-methoxytryptamine-induced hyperglycemia. It is suggested that the 5-HT receptor participates in corticosterone secretion from the adrenal cortex (Fuller, 1990). Although corticosterone affects glucose regulation, pretreatment with dexamethasone, which inhibits corticosterone release, did not affect the 5-methoxytryptamine-induced hyperglycemia. Therefore, it is unlikely that corticosterone contributes to hyperglycemic effects of 5-methoxytryptamine.

In summary, we have shown that the preferential 5-HT_4 receptor agonist, 5-methoxytryptamine, administered i.p. induced hyperglycemia in rats. Our results suggest that 5-methoxytryptamine-induced hyperglycemia is a result of the stimulation of the peripheral 5-HT_{2A} receptor mediated by adrenaline release. Our findings indicate that the peripheral 5-HT_4 receptor may not be related to glucose regulation in rats, although the participation of the central 5-HT_4 receptor in glucose homeostasis cannot be excluded at present. Our results suggest that involvement of the peripheral 5-HT_{2A} receptor should be considered part of the pharmacological effects elicited by 5-methoxytryptamine.

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