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# *p*-Chloroamphetamine, a serotonin-releasing drug, elicited in rats a hyperglycemia mediated by the 5-$HT_{1A}$ and 5-$HT_{2B/2C}$ receptors

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**Abstract**

The effects of a serotonin (5-HT) releasing drug, *p*-chloroamphetamine, on plasma glucose levels were investigated in rats. *p*-Chloroamphetamine elicited a significant hyperglycemia. The hyperglycemic effects of *p*-chloroamphetamine were completely prevented by the 5-HT synthesis inhibitor, *p*-chlorophenylalanine. Prior adrenodemedullation abolished the hyperglycemia elicited by *p*-chloroamphetamine. *p*-Chloroamphetamine-induced hyperglycemia was prevented by methysergide, which blocks the 5-$HT_1$ and 5-$HT_2$ receptor, the 5-$HT_{1A/1B/2C}$ receptor antagonist, (−)-propranolol, the selective 5-$HT_{1A}$ receptor antagonist, 4-(2′-methoxyphenyl-1-[2′-*n*-2″pyridinyl)-*p*-iodobenzamido]-ethyl-piperazine (*p*-MPPI), the 5-$HT_{2A/2B/2C}$ receptor antagonists, ritanserin and 4-isopropyl-7-methyl-9-(2-hydroxy-1-methyl-propoxycarbonyl)-4,6A,7,8,9,10,10A-octahydro-indolo[4,3-FG]quinolone maleate(LY 53857). However, the 5-$HT_3$ and 5-$HT_4$ receptor antagonist, tropisetron, the 5-$HT_4$ receptor antagonist, 2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino) ethyl ester (SDZ 205-557), and the 5-$HT_{2A}$ receptor antagonist, ketanserin, did not affect the *p*-chloroamphetamine-induced hyperglycemia. These results suggest that *p*-chloroamphetamine-induced hyperglycemia is elicited by an enhanced 5-HT release and facilitated adrenaline release. Moreover, our results indicate that *p*-chloroamphetamine-induced hyperglycemia is mediated by 5-$HT_{1A}$ and 5-$HT_{2B/2C}$ receptors. 

*Keywords:* *p*-Chloroamphetamine; Hyperglycemia; 5-$HT_{1A}$ receptor; 5-$HT_{2B/2C}$ receptor; Adrenaline

## 1. Introduction

It has been suggested that serotonin (5-HT) may play a role in glucose regulation. Activation of 5-HT receptor subtypes, including central 5-$HT_{1A}$, 5-$HT_{2A}$, 5-$HT_{2B/2C}$ receptors, induces hyperglycemic effects in rats, as found using 5-HT receptor agonists (Chaouloff and Jeanrenaud, 1987; Chaouloff et al., 1990a,b; Sugimoto et al., 1996). However, it remains unclear which 5-HT receptor subtype is significant in the physiological regulation of glucose by 5-HT.

*p*-Chloroamphetamine is known to release 5-HT from nerve terminals and to deplete brain 5-HT levels for a long time by inhibiting 5-HT re-uptake, and the activity of tryptophan hydroxylase which regulates 5-HT synthesis (Fuller, 1992). *p*-Chloroamphetamine elicits several pharmacological effects in rodents by facilitating 5-HT release following its acute injection. *p*-Chloroamphetamine stimulates the release of certain hormones such as prolactin or growth hormone in rats by increasing 5-HT release (Fuller, 1992). *p*-Chloroamphetamine also can induce hyperthermia or a behavioural syndrome characteristic of 5-HT syndromes such as head weaving, forepaw treading, hindlimb abduction in rats (Fuller, 1992).

Although single administration of *p*-chloroamphetamine induces several pharmacological effects by enhancing 5-HT release, the glycemic responses to *p*-chloroamphetamine are not yet clear. We, therefore, investigated the effects of *p*-chloroamphetamine on the plasma glucose levels of rats and the involvement of 5-HT receptor subtypes in glycemic responses to *p*-chloroamphetamine by using several 5-HT receptor antagonists.

## 2. Materials and methods

### 2.1. Animals

Male Sprague–Dawley rats weighing 190–220 g (SLC Japan, Japan) were used. They were housed under a con-

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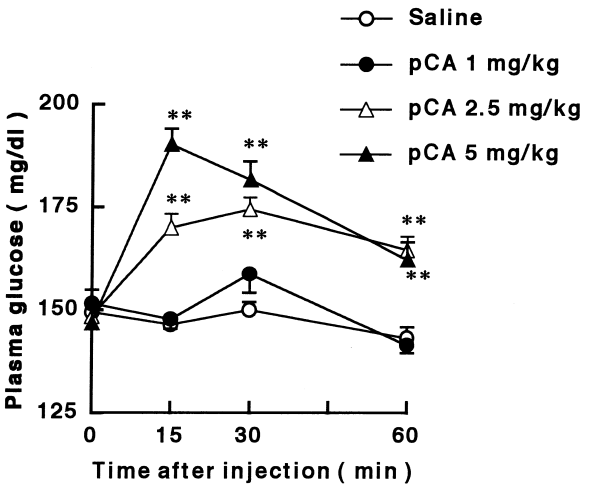


Fig. 1. Effects of *p*-chloroamphetamine (*p*CA) on plasma glucose of rats. Results are shown as means ± S.E. ($N = 5–8$). *p*CA was given i.p. $** P < 0.01$.

trolled 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 55 ± 5%. The rats were given food and water ad libitum.

### 2.2. Drug treatment

*p*-Chloroamphetamine hydrochloride and *p*-chlorophenylalanine methylester hydrochloride were obtained from Sigma (USA). Methysergide hydrogen maleate, tropisetron, 2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino) ethyl ester (SDZ 205-557), (−)-propranolol hydrochloride, 4-isopropyl-7-methyl-9-(2-hydroxy-1-methyl-propoxycarbonyl)-4,6A,7,8,9,10,10A-octahydro-indolo[4,3-F-G]quinolone maleate(LY53857), ritanserin and ketanserin tartrate were obtained from Research Biochemicals (USA). 4-(2′-Methoxyphenyl-1-[2′-*n*-2″ pyridinyl)-*p*-iodobenzamido]-ethyl-piperazine (*p*-MPPI) was obtained from Tocris (UK). The drugs, except ritanserin, tropisetron and *p*-MPPI, were dissolved in saline and injected i.p. Ritanserin was suspended in 1% carboxylmethylcellulose-Na. *p*-MPPI was dissolved in water. Tropisetron was dissolved in a few drops of 0.1 M HCl and diluted with saline. *p*-Chlorophenylalanine at a dose of 150 mg/kg was injected i.p. 3 days before the administration of *p*-chloroamphetamine. 5-HT receptor antagonists were administered 30 min before the injection of *p*-chloroamphetamine. The effects of *p*-chlorophenylalanine, adrenodemedulla-

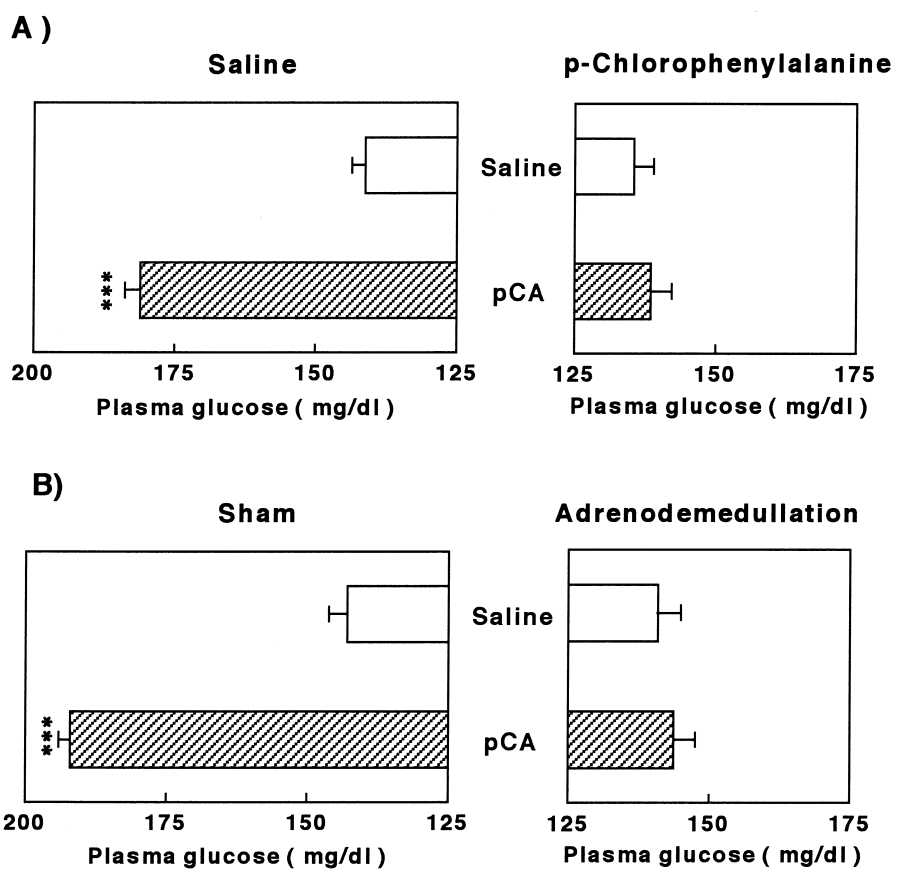


Fig. 2. Effects of *p*-chlorophenylalanine and adrenodemedullation on *p*-chloroamphetamine (*p*CA)-induced hyperglycemia in rats. Results are shown as means ± S.E. ($n = 6–8$). *p*CA was given i.p. at 5 mg/kg. Plasma glucose levels were determined 15 min after the injection of *p*CA. (A) Effects of *p*-chlrophenylalanine on *p*CA-induced hyperglycemia in rats. $*** P < 0.01$. (B) Effects of adrenodemedullation on *p*CA-induced hyperglycemia in rats. $*** P < 0.001$.

tion and 5-HT receptor antagonists on *p*-chloroamphetamine-induced hyperglycemia were evaluated 15 min after the injection of *p*-chloroamphetamine at 5 mg/kg.

### 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 and the rat was then killed. Plasma glucose levels were determined by previously described methods (Sugimoto et al., 1992).

### 2.4. Operation of adrenodemedullation

Bilateral adrenodemedullation was performed under anesthesia with pentobarbital Na at 50 mg/kg. A small incision was made along the apex of the cortex. Slight pressure applied, thus popping out the medulla. Experiments were carried out 1 week after the operation. After the experiments, the adrenodemedullated rats were killed and it was verified that the adrenal medulla was removed and that the adrenal cortex was preserved.

### 2.5. Statistics

Statistical significance was evaluated with Student's *t*-test for comparison of two groups. Dose-related effects of *p*-chloroamphetamine 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

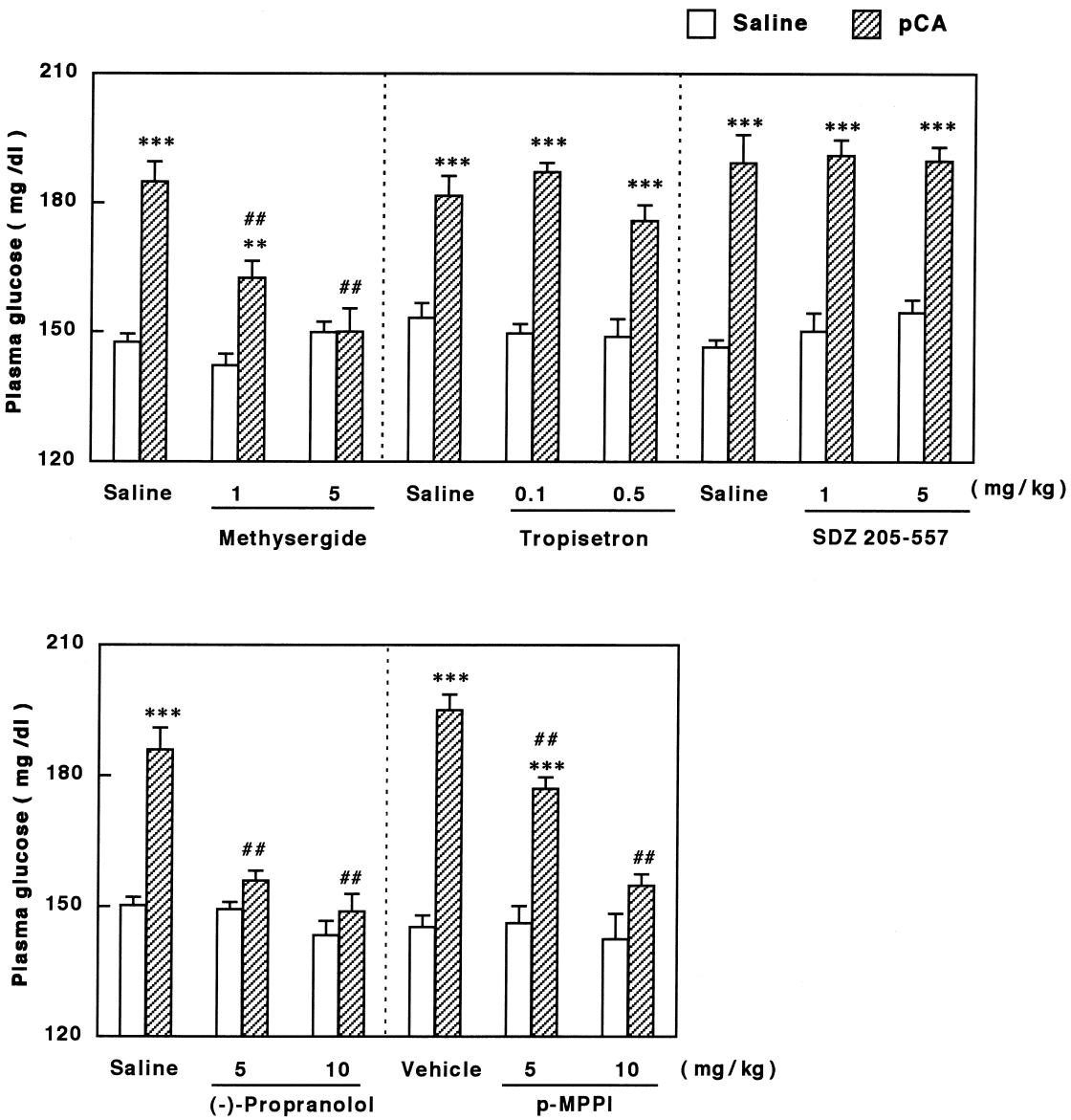


Fig. 3. Effects of methysergide, tropisetron, SDZ 205–557, (−)-propranolol and *p*-MPPI on *p*-chloroamphetamine (*p*CA)-induced hyperglycemia in rats. Results are shown as means ± S.E. ($n = 5$–7). *p*CA was injected i.p. at 5 mg/kg. Plasma glucose levels were determined 15 min after injection of *p*CA. $**P < 0.01$, $***P < 0.001$ vs. saline control for respective group. $\#\#P < 0.01$ vs. saline or vehicle + *p*CA.

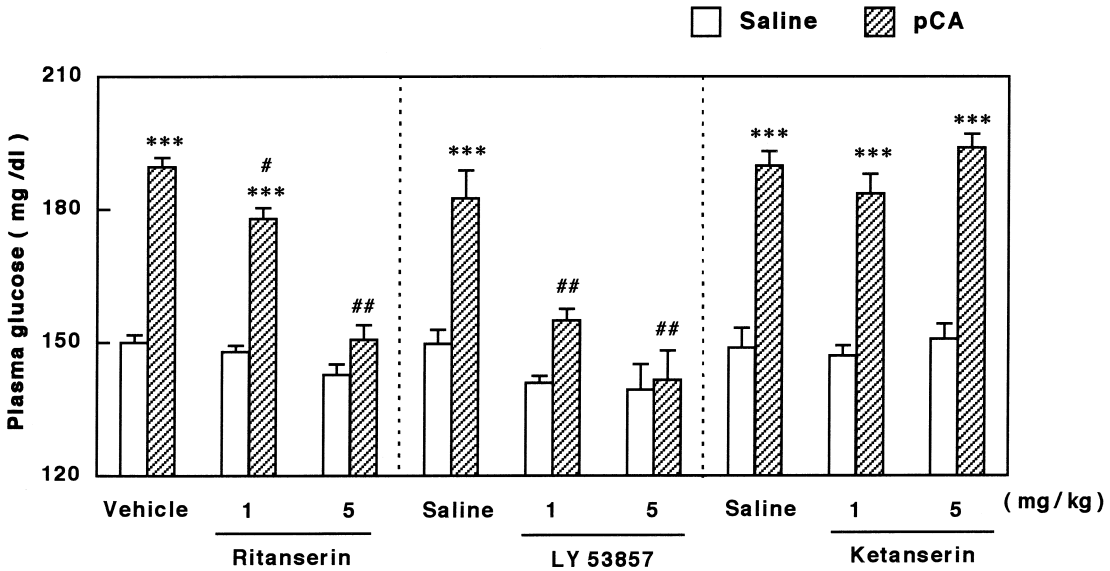


Fig. 4. Effects of 5-$HT_2$ receptor antagonists on *p*-chloroamphetamine (*p*CA)-induced hyperglycemia. Results are shown as means ± S.E. ($n = 5–8$). *p*CA was injected i.p. at 5 mg/kg. Plasma glucose levels were determined 15 min after injection of *p*CA. $***P < 0.001$ vs. saline control for respective group. $\#P < 0.05$, $\#\#P < 0.01$ vs. saline or vehicle + *p*CA.

*p*-chloroamphetamine-induced effects were analyzed by two-way ANOVA followed by Tukey's test.

## 3. Results

Fig. 1 shows the changes in time course of the plasma glucose levels following the injection of *p*-chloroamphetamine. *p*-Chloroamphetamine elicited a dose-dependent hyperglycemia.

Fig. 2A shows the effects of a 5-HT synthesis inhibitor, *p*-chlorophenylalanine, on *p*-chloroamphetamine-induced hyperglycemia. As shown, *p*-chlorophenylalanine abolished the *p*-chloroamphetamine-induced hyperglycemia. Effects of adrenodemedullation on *p*-chloroamphetamine-induced hyperglycemia are shown in Fig. 2B. *p*-Chloroamphetamine did not induce any effect on plasma glucose levels in adrenodemedullated rats, while it elicited apparent hyperglycemia in sham-operated rats.

Fig. 3 shows the effects of 5-HT receptor antagonists, methysergide, tropisetron, SDZ 205-557, (−)-propranolol, *p*-MPPI on *p*-chloroamphetamine-induced hyperglycemia. The plasma glucose levels were evaluated 15 min after treatment with *p*-chloroamphetamine 5 mg/kg. The 5-$HT_1$ and 5-$HT_2$ receptor antagonist, methysergide, and the 5-$HT_{1A/1B/2C}$ receptor antagonist, (−)-propranolol, the 5-$HT_{1A}$ receptor antagonist, *p*-MPPI, inhibited *p*-chloroamphetamine-induced hyperglycemia, while the 5-$HT_3$ and 5-$HT_4$ receptor antagonist, tropisetron, and the 5-$HT_4$ receptor antagonist, SDZ 205-557, did not affect *p*-chloroamphetamine-induced hyperglycemia.

As shown in Fig. 4, the 5-$HT_{2A/2B/2C}$ receptor antagonist ritanserin and LY 53857 strongly reduced the hyperglycemia elicited by *p*-chloroamphetamine, although the 5-$HT_{2A}$ receptor antagonist, ketanserin, did not show any effect.

## 4. Discussion

The present study demonstrated that the acute administration of the 5-HT releaser, *p*-chloroamphetamine, elicits apparent hyperglycemia in rats. The hyperglycemic effects of *p*-chloroamphetamine were dose-related and lasted for at least 60 min. Many *p*-chloroamphetamine-induced pharmacological effects are connected to the release of 5-HT, since 5,7-dihydroxytryptamine or *p*-chlorophenylalanine which deplete brain 5-HT, can strongly inhibit the effects of *p*-chloroamphetamine (Fuller, 1992). However, it was recently reported that hypophagia induced by another 5-HT releaser, fenfluramine, is resistant to 5-HT depletion and independent of 5-HT availability (Curzon et al., 1997). Thus, we investigated the effects of the 5-HT synthesis inhibitor, *p*-chlorophenylalanine, on *p*-chloroamphetamine-induced hyperglycemia. As shown in Section 3, pretreatment with *p*-chlorophenylalanine strongly reduced the hyperglycemic effects of *p*-chloroamphetamine. This suggests that *p*-chloroamphetamine-induced hyperglycemia is elicited by the release of 5-HT from nerve terminals.

It has been reported that 5-HT receptor agonist-elicited hyperglycemia is closely related to adrenaline release from the adrenal medulla, since these effects are abolished by adrenalectomy or adrenodemedullation (Chaouloff et al., 1990a,b; Baudrie and Chaouloff, 1992; Sugimoto et al., 1992, 1996). Thus, the effects of *p*-chloroamphetamine on

plasma glucose levels in adrenodemedullated rats were studied. In adrenodemedullated rats, *p*-chloroamphetamine did not affect the plasma glucose levels, although it elicited hyperglycemia in sham-operated rats. This indicates that *p*-chloroamphetamine-induced hyperglycemia is closely related to adrenaline release.

The involvement of 5-HT receptor subtypes in glucose regulation has been suggested for years. To date, it has been suggested that central 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2B/2C}$ receptors participate in glucose regulation (Chaouloff and Jeanrenaud, 1987; Chaouloff et al., 1990a,b; Sugimoto et al., 1996). Thus, the effects of several 5-HT receptor antagonists on *p*-chloroamphetamine-induced hyperglycemia were examined.

The 5-$HT_1$ and 5-$HT_2$ receptor antagonist, methysergide, significantly prevented *p*-chloroamphetamine-induced hyperglycemia, although the 5-$HT_3$ and 5-$HT_4$ receptor antagonist, tropisetron, and the 5-$HT_4$ receptor antagonist, SDZ 205-557, at doses sufficient for blocking these receptors-mediated responses (Wilson et al., 1990; Banner et al., 1996) did not show any effect. The 5-$HT_{1A/1B/2C}$ receptor antagonist, (−)-propranolol (Hoyer, 1991), also blocked *p*-chloroamphetamine-induced hyperglycemia. Furthermore, the new selective 5-$HT_{1A}$ receptor antagonist, *p*-MPPI, at a dose which prevents postsynaptic 5-$HT_{1A}$ receptor-mediated hypothermia in rats (Allen et al., 1997), reduced the hyperglycemia elicited by *p*-chloroamphetamine. This indicates that *p*-chloroamphetamine-induced hyperglycemia is mediated by the 5-$HT_{1A}$ receptor but not by the 5-$HT_3$ or 5-$HT_4$ receptor.

Moreover, the 5-$HT_{2A/2B/2C}$ receptor antagonists, ritanserin, and LY 53857 (Baxter et al., 1995) apparently reduced the hyperglycemic effects of *p*-chloroamphetamine. It is unlikely that the 5-$HT_{2A}$ receptor is involved in *p*-chloroamphetamine-induced hyperglycemia, since the 5-$HT_{2A}$ receptor antagonist, ketanserin (Baxter et al., 1995), even at the high dose of 5 mg/kg did not affect it. Thus, it is suggested that the 5-$HT_{2B/2C}$ receptor is also related to *p*-chloroamphetamine-induced hyperglycemia.

Together, these findings allow one to conclude that *p*-chloroamphetamine induces hyperglycemia by enhancing 5-HT release, which may stimulate both 5-$HT_{1A}$ and 5-$HT_{2B/2C}$ receptors. It has been reported that the 5-$HT_{1A}$ receptor agonist, 8-hydroxy-2-di-*n*-(propylamino)tetralin (8-OH-DPAT), and the 5-$HT_{2B/2C}$ receptor agonist, 1-3(chlorophenyl)piperazine(mCPP), elicit in rats a hyperglycemia which is mediated by 5-$HT_{1A}$ and 5-$HT_{2B/2C}$ receptors, respectively (Chaouloff and Jeanrenaud, 1987; Sugimoto et al., 1996). This agonist-induced hyperglycemia is elicited by adrenaline release from the adrenal medulla. Thus, *p*-chloroamphetamine-induced hyperglycemia involves adrenaline release, probably derived from activation of the 5-$HT_{1A}$ and 5-$HT_{2B/2C}$ receptors.

Chaouloff et al. (1992) previously reported that the another 5-HT releasing drug, fenfluramine, can induce in rats a hyperglycemia which is elicited by stimulation of adrenaline release. This is consistent with our findings with *p*-chloroamphetamine. However, the latter authors showed that fenfluramine-induced hyperglycemia is blocked by the 5-$HT_{2A}$ receptor antagonist, ketanserin (0.3 mg/kg), which differs from the present results (Chaouloff et al., 1992). In our study, ketanserin at a dose of 5 mg/kg, which is a higher dosage that blocks the 5-$HT_{2A}$ receptor-mediated glycemic responses or head shakes in rats (Kennett and Curzon, 1991; Sugimoto et al., 1996), did not alter *p*-chloroamphetamine-induced hyperglycemia. The reason for the discrepancy between the effects of ketanserin on the *p*-chloroamphetamine and the fenfluramine-induced glycemic effects is not clear at present. The effects of 5-HT depletion on fenfluramine-induced hyperglycemia have not been studied earlier and remain unclear. A recent report suggests that the hypophagic effect of fenfluramine is not related to 5-HT availability but to a direct action on 5-HT receptors (Curzon et al., 1997). Thus, the hyperglycemia induced by fenfluramine may be mediated by its direct action on 5-HT receptors.

In conclusion, our results demonstrate that *p*-chloroamphetamine induces hyperglycemia in rats and that this is elicited by enhancing 5-HT release. *p*-Chloroamphetamine-induced hyperglycemia is mediated by 5-$HT_{1A}$ and 5-$HT_{2B/2C}$ receptors and by facilitation of adrenaline release. These findings suggest that 5-$HT_{1A}$ and 5-$HT_{2B/2C}$ receptors may have a role in glucose regulation by 5-HT.

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