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European Journal of Pharmacology 406 (2000) 153–157

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The 5-$HT_{2C/2B}$ receptor agonist, *m*-chlorophenylpiperazine, increases plasma glucagon levels in rats

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Received 19 July 2000; received in revised form 15 August 2000; accepted 18 August 2000

Abstract

Effects of the 5-$HT_{2C/2B}$ receptor agonist *m*-chlorophenylpiperazine (mCPP) on plasma glucagon levels were investigated in rats. mCPP dose dependently increased plasma glucagon levels. Hyperglucagonemia elicited by mCPP was prevented by the 5-$HT_{2A/2B/2C}$ receptor antagonist, ritanserin, while the 5-HT_{2A} receptor antagonist, ketanserin, did not show any effect. Increases in glucagon levels induced by mCPP were inhibited by prior adrenodemedullation. These results indicate that increases in plasma glucagon levels induced by mCPP are mediated by the 5-$HT_{2C/2B}$ receptor which in turn facilitates adrenaline release. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: mCPP (*m*-chlorophenylpiperazine); Glucagon; 5-HT (5-hydroxytryptamine, serotonin); 5-$HT_{2C/2B}$ receptor; Adrenaline

1. Introduction

The 5-HT_2 receptor family is divided into 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors (Hoyer et al., 1994; Baxter et al., 1995; Hoyer and Martin, 1997). The 5-HT_{2C} receptor is widely distributed in the brain (Hoyer et al., 1994; Pompeiano et al., 1994; Sharma et al., 1997) and this receptor plays a significant role in several central functions (Kennett, 1992a). *m*-Chlorophenylpiperazine (mCPP) has a high affinity for the 5-HT_{2C} receptor (Kennett, 1992a; Baxter et al., 1995) and has been extensively used as a probe of the 5-HT_{2C} receptor in brain function. The administration of mCPP in animals elicits hypolocomotion, anxiogenesis, decreased food intake resulting from the activation of the central 5-HT_{2C} receptor (Curzon, 1990; Curzon and Kennett, 1990; Kennett, 1992a,b; Kennett and Curzon, 1988, 1991). mCPP has an affinity with the 5-HT_{2B} receptor and pharmacological effects of mCPP may be partly mediated by 5-HT_{2B} receptors (Baxter et al., 1995). mCPP also elicits neuroendocrinological responses, inducing adrenocorticotropic hormone (ACTH), prolactin or corticosterone release in rats (Bagdy et al.,1992; Bagdy, 1996).

It has been suggested that 5-HT is involved in glucose regulation, since stimulation of the central 5-HT_{1A} and 5-HT_{2A} receptors using these receptor agonists induces hyperglycemia in rats (Chaouloff and Jeanrenaud, 1987; Chaouloff et al., 1990a,b; Sugimoto et al., 1992, 1996). Moreover, we found that mCPP causes hyperglycemia in rats (Sugimoto et al., 1996). Hyperglycemic responses to mCPP are mediated by the 5-$HT_{2C/2B}$ receptor, since there was antagonism by the 5-$HT_{2A/2B/2C}$ receptor antagonist, ritanserin, but not by the 5-HT_{2A} receptor antagonist, ketanserin (Sugimoto et al., 1996). The pancreatic hormone, glucagon regulates glucose homeostasis and it is well recognized that enhancement of glucagon release elicits hyperglycemia. Although mCPP elicits hyperglycemia, little is known as to whether it modifies circulating glucagon levels. Furthermore, it was reported that mCPP elevates blood adrenaline and noradrenaline levels (Bagdy et al., 1989), which can facilitate glucagon release. The aim of the present work was to elucidate the effects of the 5-$HT_{2C/2B}$ receptor agonist mCPP on glucagon secretion in rats.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (190–220 g) were purchased from SLC Japan (Japan). They 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 23 ± 1°C and

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PII: S0014-2999(00)00644-0

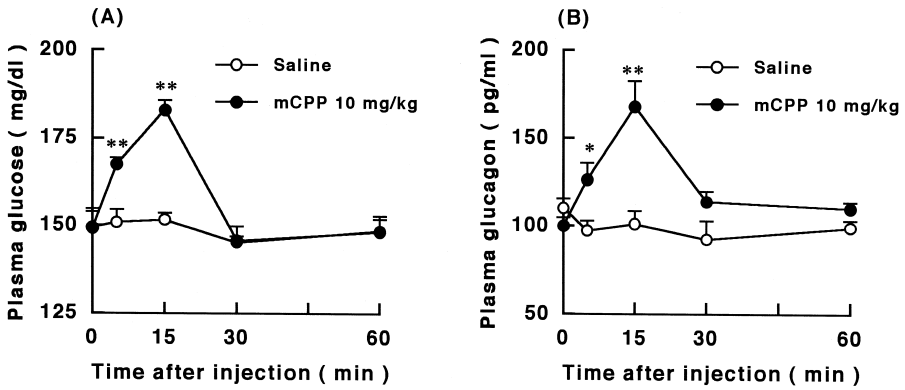

Fig. 1. Effects of mCPP on plasma glucose and glucagon levels of rats. (A) Plasma glucose. (B) Plasma glucagon. Results are shown as the means ± S.E. ($N = 5–7$). mCPP, 10 mg/kg was injected i.p. $^{*}P < 0.05$. $^{**}P < 0.01$.

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

2.2. Drug treatment

mCPP, ritanserin and ketanserin tartrate were obtained from Research Biochemicals (USA). mCPP and ketanserin were dissolved in saline. Ritanserin was suspended in 1% carboxymethylcellulose–Na. All drugs were injected i.p. 5-HT receptor antagonists were given 30 min before the injection of mCPP.

2.3. Determination of blood glucose and glucagon levels

Blood samples were taken from the caudal vena cava under ether anesthesia. Only one sample was taken from each rat. Plasma glucose levels were determined with methods described in a previous report (Sugimoto et al., 1992). Glucagon levels were measured by radioimmunoassay using commercially available Daiichi kits for glucagon (Daiichi Radioisotope Center, Japan).

2.4. Adrenodemedullation

Bilateral adrenodemedullation was performed under anesthesia with pentobarbital Na 50 mg/kg. Experiments were carried out 1 week after the operation.

2.5. Statistics

Statistical significance was evaluated by Student's *t*-test for comparisons of two groups. Dose-related effects of mCPP 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 mCPP-induced effects were analyzed by two-way ANOVA followed by Tukey's test.

3. Results

3.1. Effects of mCPP on plasma glucose and glucagon levels of rats

Fig. 1A and B shows time course changes in plasma glucose and glucagon levels following the treatment with

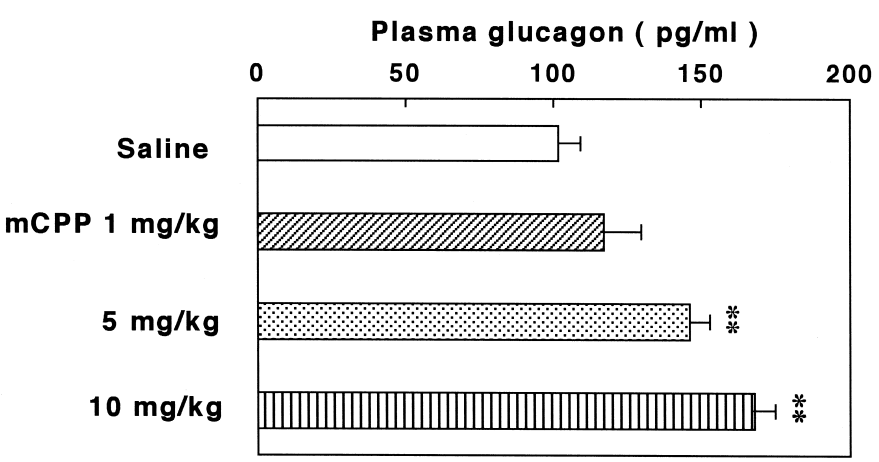

Fig. 2. Dose–response studies of hyperglucagonemic effects of mCPP. Results are shown as the means ± S.E. ($N = 5–7$). mCPP was injected i.p. Plasma glucagon levels were determined 15 min after the injection of mCPP. $^{**}P < 0.01$.

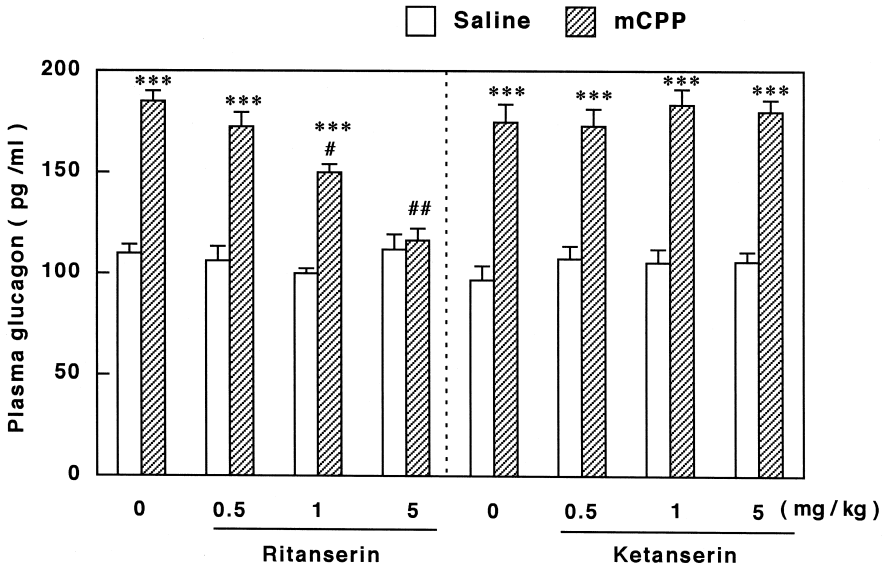

Fig. 3. Effects of ritanserin and ketanserin on mCPP-induced hyperglucagonemia in rats. Results are shown as the means ± S.E. ($N = 5–7$). Ritanserin and ketanserin were given i.p. 30 min before mCPP. mCPP at 10 mg/kg was injected i.p. $^{***}P < 0.001$ vs. saline of respective groups. $\#P < 0.05$, $\#\#P < 0.01$ vs. saline + mCPP.

mCPP 10 mg/kg. mCPP significantly increased plasma glucose and glucagon levels and these responses reached a maximum 15 min after the injection. Fig. 2 shows the dose–responses for hyperglucagonemic effects of mCPP. mCPP increased plasma glucagon levels dose dependently.

3.2. Effects of ritanserin and ketanserin on mCPP-induced hyperglucagonemia in rats

Fig. 3 shows the effects of the 5-$HT_{2A/2B/2C}$ receptor antagonist, ritanserin, and the 5-HT_{2A} receptor antagonist, ketanserin, on the elevation of glucagon levels induced by mCPP. Ritanserin apparently reduced the hyperglucagonemia elicited by mCPP while ketanserin did not affect it.

3.3. Effects of mCPP on plasma glucagon levels in adrenodemedullated rats

Fig. 4 shows the effects of adrenodemedullation on mCPP-induced hyperglucagonemia in rats. In sham operated rats, mCPP increased plasma glucagon levels. Prior adrenodemedullation completely abolished this increase.

4. Discussion

The present results demonstrated that mCPP increases plasma glucagon levels in rats dose dependently. The increases in plasma glucagon levels reached a maximum 15 min after the injection of mCPP, 10 mg/kg. Endocrine responses to mCPP reveal that it facilitates the release of several hormones including corticosterone, ACTH, oxytocin, and prolactin (Bagdy et al., 1992; Bagdy, 1996). Our results show that mCPP is also involved in glucagon secretion. Our previous data demonstrated that mCPP induces hyperglycemic responses in rats (Sugimoto et al., 1996). As shown in Results, the time course changes in glucagon levels following treatment with mCPP are paral-

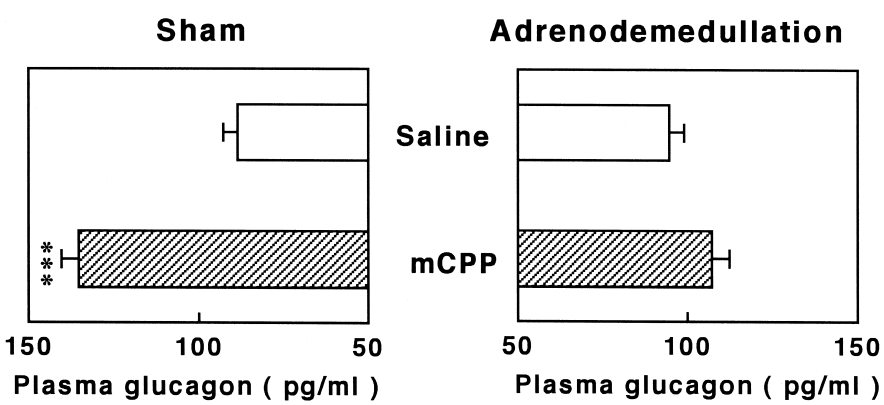

Fig. 4. Effects of mCPP on plasma glucagon levels in adrenodemedullated rats. Results are shown as the means ± S.E. ($N = 5–7$). mCPP at 10 mg/kg was injected i.p. Plasma glucagon levels were determined 15 min after the injection of mCPP. $^{***}P < 0.001$.

lel with its hyperglycemic effects. This suggests that hyperglucagonemia may be related to mCPP-induced hyperglycemia.

Pharmacological effects induced by mCPP, such as hypophagia or penile erection, are mediated by the 5-HT_{2C} receptor, based on the finding that the 5-HT_{2C} receptor antagonists prevented them (Curzon, 1990; Kennett and Curzon, 1991; Kennett, 1992a). As demonstrated in Results, the hyperglucagonemia elicited by mCPP was prevented by the 5-$HT_{2A/2B/2C}$ receptor antagonist, ritanserin, although it was not affected by the 5-HT_{2A} receptor antagonist, ketanserin, at the same doses as ritanserin. Ketanserin has a higher affinity for 5-HT_{2A} receptors than for other 5-HT_2 receptor subtypes (Baxter et al., 1995). The doses of ketanserin used in this study were those inhibiting the 5-HT_{2A} receptor-mediated hyperglycemia induced by 1-(2,5-dimethoxy-4-iodophenyl)2-aminopropane (DOI) or head shake responses elicited by 5-hydroxytryptophan (Sugimoto et al., 1996; Kennett and Curzon, 1991). This suggests that the mCPP-induced hyperglucagonemia is not related to the 5-HT_{2A} receptor. In contrast, pretreatment with the 5-$HT_{2A/2B/2C}$ receptor antagonist, ritanserin, inhibited mCPP-induced hyperglucagonemia. Although ritanserin can block all 5-HT_2 receptor subtypes, higher doses are required to inhibit the 5-HT_{2C} receptor-mediated responses (Kennett and Curzon, 1991). The doses of ritanserin in the present study were those inhibiting the hypophagia induced by mCPP which is mediated by the 5-HT_{2C} receptor (Kennett and Curzon, 1991). Since mCPP and ritanserin show affinity with 5-HT_{2B} receptors, the involvement of the 5-HT_{2B} receptor in mCPP-induced hyperglucagonemia cannot be excluded at present. Therefore, these results suggest that mCPP-elicited hyperglucagonemia is mediated by the 5-$HT_{2C/2B}$ receptor.

We previously reported that the hyperglycemia induced by mCPP was inhibited by ritanserin but not by ketanserin, suggesting that mCPP-induced hyperglycemia is mediated by the 5-$HT_{2C/2B}$ receptor, which is similar to hyperglucagonemic effects (Sugimoto et al., 1996). Therefore, hyperglucagonemic effects of mCPP may contribute to its hyperglycemic effects. Ketanserin has an affinity with α_1 receptors and blocks these receptors (Leysen et al., 1981). It was reported that activation of α_1 receptors leads to an enhancement of glucagon release (Skoglund et al., 1987). Since ketanserin did not affect basal glucagon levels and mCPP-induced glucagon release, any blocking effects of ketanserin on α_1 receptors are probably slight at the doses used in the present study.

Adrenaline is known to elevate circulatory glucose levels by facilitation of glycogenolysis, inhibition of glucose uptake and insulin release. Adrenaline amplifies pancreatic glucagon release, which also elevates blood glucose levels. Therefore, we investigated the effects of mCPP on plasma glucagon levels in adrenodemedullated rats. Adrenodemedullation completely abolished the mCPP-induced hyperglucagonemia. This indicates that increases in plasma glucagon levels induced by mCPP are closely related to adrenaline release from the adrenal gland. This effect is also consistent with that of adorenodemedullation on mCPP-induced hyperglycemia (Sugimoto et al., 1996). Bagdy et al. (1988, 1989) reported that mCPP produced an increase in plasma adrenaline levels and that it stimulates the sympathoadrenomedullary system. Thus, increases in glucagon release induced by mCPP are mediated by its adrenaline releasing effects. Since it was reported that the adrenaline release elicited by mCPP was prevented by ritanserin but not by ketanserin (Bagdy, 1996), adrenaline-releasing effects are considered to be elicited by stimulation of 5-$HT_{2C/2B}$ receptors. Therefore, it is concluded that mCPP stimulates the 5-$HT_{2C/2B}$ receptor, resulting in facilitation of adrenaline release and, in turn, inducing hyperglucagonemia. This suggests that release of adrenaline and adrenaline-stimulated glucagon release leads to hyperglycemic effects of mCPP. We demonstrated that mCPP elicits hyperglucagonemia in ether-anesthetized rats. Since ether itself stimulates adrenaline release, synergistic effects of ether with mCPP may enhance hyperglucagonemia.

Bagdy et al. (1988) reported that the adrenaline release elicited by mCPP was strongly reduced in pithed and splanchnic denervated rats, suggesting that mCPP-induced adrenaline release is centrally mediated. Therefore, it is suggested that the activation of the 5-$HT_{2C/2B}$ receptor in the central nervous system may be also related to hyperglucagonemia. mCPP elicited hyperglucagonemia at doses higher than those that had produced effects such as hypolocomotion, which appeared below 1 mg/kg (Kennett and Curzon, 1988). It was reported that mCPP increases adrenaline release at doses of 2.5 and 10 mg/kg in rats (Bagdy et al., 1988, 1989). Therefore, higher doses of mCPP are required for inducing adrenaline release and increasing plasma glucagon levels.

Numerous studies have shown that stimulation of 5-HT receptors modifies glucose regulation. The stimulation of the 5-HT_{1A} receptor elevates plasma glucose levels in rats by facilitating adrenaline release (Chaouloff et al., 1990a,b,c; Sugimoto et al., 1992). The 5-HT_{1A} partial receptor agonist, buspirone, produces hyperglycemia and hyperglucagonemia, which are induced by an enhanced adrenaline release, since these effects were prevented by adrenodemedullation (Sugimoto et al., 1992). Thus, both 5-HT_{1A} and 5-$HT_{2C/2B}$ receptors may partly influence plasma glucose levels via glucagon released by adrenaline.

In summary, the results indicate that mCPP increases glucagon secretion in rats by stimulation of the 5-$HT_{2C/2B}$ receptor. These responses are mediated by adrenaline release, since they were abolished by adrenodemedullation. These results are comparable to those for hyperglycemic effects of mCPP. Our results suggest that the 5-$HT_{2C/2B}$ receptor participates in glucagon release through adrenaline release and that hyperglucagonemia induced by mCPP plays a role in its hyperglycemic effects.

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