The 5-HT_{2C/2B} Receptor Agonist *m*-Chlorophenylpiperazine (mCPP) Inhibits 2-Deoxy-D-glucose (2-DG)-Induced Hyperphagia in Rats

Yumi SUGIMOTO*, Tomoko YOSHIKAWA, Toshiko NOMA, and Jun YAMADA

Department of Pharmacology, Kobe Pharmaceutical University, Motoyamakita-machi, Higashinada-ku, Kobe 658–8558, Japan. Received July 5, 2001; accepted September 28, 2001

Effects of the 5-HT_{2C/2B} receptor agonist *m*-chlorophenylpiperazine (mCPP) on hyperphagia elicited by 2-deoxy-D-glucose (2-DG) were investigated in rats. mCPP apparently reduced 2-DG-induced hyperphagia. Suppressive effects of mCPP on hyperphagia induced by 2-DG were inhibited by the 5-HT_{2A/2B/2C} receptor antagonist, ritanserin, although the 5-HT_{2A} receptor antagonist ketanserin was without effect. Thus, inhibitory effects of mCPP on 2-DG-induced hyperphagia are mediated by the 5-HT_{2C/2B} receptor. Our results demonstrate that mCPP can inhibit the bulimia model, 2-DG-induced hyperphagia.

Key words 5-HT_{2C/2B} receptor; *m*-chlorophenylpiperazine (mCPP); 2-deoxy-D-glucose (2-DG); hyperphagia

m-Chlorophenylpiperazine (mCPP) is a serotonergic ligand and has a high affinity with the 5-HT_{2C} receptor.^{1,2)} mCPP also has an affinity with the 5-HT_{2B} receptor.^{1,2)} mCPP is known as a metabolite of an antidepressant, trazodone.³⁾ mCPP elicits several pharmacological effects in rodents including hypolocomotion, anxiety and hormone release including prolactin.^{2,4–7)} Recently, we found that mCPP induces hyperglycemia and hyperglucagonemia in rats mediated by the 5-HT_{2C/2B} receptor.^{8,9)}

Serotonin (5-HT) in the central nervous system is closely connected to food intake and it is well recognized that 5-HT receptor agonists inhibit feeding.^{4,10,11)} mCPP elicits hypophagia in both food-deprived and satiated rats by the activation of the 5-HT_{2C} receptor.^{4,12)} Moreover, the administration with mCPP can evoke hypophagia in human.¹³⁾ Thus, previous findings that mCPP decreases eating, revealed that mCPP or the other 5-HT_{2C} receptor agonists may be effective in eating disorder like bulimia.

2-Deoxy-D-glucose (2-DG) is a glucose analogue and inhibits glucose utilization in the brain, thereby inducing remarkable hyperphagia in animals.¹⁴⁾ We previously reported that the peripheral 5-HT_{2A} receptor agonist reduces 2-DG-induced hyperphagia, while the peripheral 5-HT₃ receptor agonist was without effect.¹⁵⁾ Although 2-DG-induced hyperphagia is a bulimia model, the effects of mCPP on 2-DG-induced hyperphagia are not yet investigated. Therefore, in the present study, we studied the effects of mCPP on 2-DG-induced hyperphagia in rats.

MATERIALS AND METHODS

Male Sprague–Dawley rats (200–240 g, SLC Japan, Shizuoka, Japan) were housed in individual stainless-steel wire cages. They were maintained under a controlled 12 h/12 h light/dark cycle (light from 7:00 a.m. to 7:00 p.m.), at a room temperature of 24±1 °C and humidity 55±5% for at least 7 d prior to the experiments.

2-DG was purchased from Wako Pure Chemical Co. (Japan). mCPP, ritanserin and ketanserin tartrate were obtained from Research Biochemicals (U.S.A.). 2-DG, mCPP and ketanserin were dissolved in saline. Ritanserin was suspended in 1% carboxymethylcellulose-Na. All drugs were injected i.p. mCPP and 2-DG were injected i.p. simultaneously at opposite sites. 5-HT receptor antagonists were given 30 min before the injection of mCPP.

Prewighed food was placed in the cage and the amount of food remaining was weighed 2 and 4 h after the injection of 2-DG. Drugs were injected between 13.00 and 14.00 h.

Results were analyzed by two-way analysis of variance (ANOVA) followed by Tukey's test.

RESULTS

The effects of mCPP on 2-DG-induced hyperphagia are demonstrated in Fig. 1. In saline-treated rats, mCPP at 5 mg/kg significantly decreased food intake. 2-DG at a dose of 750 mg/kg induced marked hyperphagia in non-food-de-

Fig. 1. Effects of mCPP on 2-DG-Induced Hyperphagia in Rats

Results are shown as means±S.E. ($n=5-6$). 2-DG at 750 mg/kg was given i.p. 2-DG and mCPP were injected i.p. simultaneously. * $p<0.05$, ** $p<0.01$ vs. saline of respective group. ### $p<0.001$ vs. saline+saline.

Fig. 2. Effects of Ritanserin on mCPP-Induced Inhibition of Hyperphagia Elicited by 2-DG in Rats

Results are shown as means $\pm$ S.E. ($n=5-6$). 2-DG at 750 mg/kg and mCPP at 5 mg/kg were injected i.p. Ritanserin at 5 mg/kg was injected i.p. 30 min before 2-DG and mCPP. ** $p < 0.01$ vs. saline + 2-DG of respective group.

Fig. 3. Effects of Ketanserin on mCPP-Induced Inhibition of Hyperphagia Elicited by 2-DG in Rats

Results are shown as means $\pm$ S.E. ($n=5$). 2-DG at 750 mg/kg and mCPP at 5 mg/kg were injected i.p. Ketanserin at 1 mg/kg was injected i.p. 30 min before 2-DG and mCPP. ** $p < 0.01$ vs. saline + 2-DG of respective group.

prived rats. mCPP at 2.5 and 5 mg/kg significantly reduced 2-DG-induced hyperphagia for 2 and 4 h.

Effects of the 5-HT_{2A/2B/2C} receptor antagonist ritanserin on the hypophagic effects of mCPP are shown in Fig. 2. Pretreatment with ritanserin (5 mg/kg) attenuated the suppressive effects of mCPP on 2-DG-induced hyperphagia.

Figure 3 shows effects of the 5-HT_{2A} receptor antagonist ketanserin on the inhibitory effects of mCPP on 2-DG-elicited hyperphagia. However, ketanserin (1 mg/kg) did not have any effect.

DISCUSSION

It has been suggested that stimulation of serotonergic transmission or the activation of the post-synaptic 5-HT receptor elicits hypophagia.^{4,10} A selective serotonin reuptake inhibitor (SSRI), such as fluoxetine or fluvoxamine and the 5-HT releaser fenfluramine induce anorexia in animals.^{10,11,16} The involvement of 5-HT_{2C} or 5-HT_{1B} receptor subtypes in SSRI- or fenfluramine-induced hypophagia has been reported.^{10,16} Thus, it is postulated that these 5-HT receptor subtypes play a significant role in the control of eating. Since mCPP has a high affinity with the 5-HT_{2C} receptor,^{1,2} effects of mCPP on food intake have been examined to clarify the involvement of these receptors. Previous studies reported that mCPP elicits anorexia in non-food deprived rats.^{4,12,17} In the present study, we also found that mCPP at 5 mg/kg elicits significant anorexia in satiated rats, which is consistent with the results of previous studies.

It has been suggested that the 5-HT_{2C/2B} receptor agonist mCPP induces hypophagia in humans as well as in rats.^{10,13}

In humans, treatment for bulimia has not been fully established. It has been suggested that mCPP also decreases feeding in food-deprived rats, that is, enhanced feeding.^{4,12,17,18} Treatment with 2-DG increases food intake in animals elicited by inhibiting glucose utilization and uptake in the hypothalamus controlling food intake.¹⁴ Therefore, we studied the effects of mCPP on 2-DG-induced hyperphagia as a bulimia model. The present study indicates that mCPP inhibited 2-DG-induced hyperphagia in rats dose-dependently. Hypophagic effects of mCPP on 2-DG-elicited feeding appeared at a dose of 2.5 mg/kg, which had no effect on food intake of satiated rats. Thus, mCPP inhibits 2-DG-induced hyperphagia in rats and these effects were more sensitive than those in non-food deprived rats. As described above, as mCPP elicited apparent effects on hyperphagia in food deprived rats, its anorectic effects are effective for enhanced feeding.

mCPP is known as a ligand of the 5-HT_{2C} receptor and its pharmacological effects have been discussed regarding the involvement of this receptor.^{2,19} Recently, it was found that the 5-HT_{2B} receptor may be connected to the pharmacological effects of mCPP, since mCPP is a weak agonist of the 5-HT_{2B} receptor.²⁰ Therefore, we studied effects of the 5-HT₂ receptor antagonists on mCPP-induced anorectic effects in 2-DG-induced hyperphagia. Ritanserin is the non-selective 5-HT₂ receptor antagonist and has an affinity with all 5-HT₂ receptor subtypes. pK_i values of ritanserin for 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} receptors are 8.8, 8.3 and 8.9, respectively.¹ Although ritanserin can block 5-HT_{2A} and the 5-HT_{2C} receptor-mediated effects, higher doses are required for inhibiting responses induced by the 5-HT_{2C} receptor than the 5-HT_{2A} receptor.¹² As shown in the results, the 5-HT_{2A/2B/2C} receptor antagonist, ritanserin significantly reduced hypophagic effects of 2-DG-induced hyperphagia. It was reported that ritanserin at a dose of 5 mg/kg used in the present study is those inhibiting both the 5-HT_{2A} and the 5-HT_{2C} receptor-mediated effects *in vivo*.¹² However, the 5-HT_{2A} receptor antagonist ketanserin did not modify anorectic effects of mCPP. Ketanserin has a higher affinity for 5-HT_{2A} receptors than for other 5-HT₂ receptor subtypes: pK_i values for 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} receptors are 8.9, 5.4 and 7.0, respectively.¹ The dose of ketanserin used in the present study was those inhibiting the 5-HT_{2A} receptor-mediated head shake responses elicited by 5-hydroxytryptophan.¹² These results indicate that the inhibitory effects of mCPP on 2-DG-elicited hyperphagia is mediated by the 5-HT_{2B} and/or 5-HT_{2C} receptor, but not by the 5-HT_{2A} receptor. Recently, Kennett *et al.* reported that the 5-HT_{2B} receptor agonist BW 723 C86 elicits hyperphagia in rats, which is related to the 5-HT_{2B} receptor.²¹ Thus, it is unlikely that the hypophagic effects of mCPP in 2-DG-treated rats are related to the 5-HT_{2B} receptor. Kennett and Curzon demonstrated that mCPP-induced hypophagia in food-deprived rats is mediated by the 5-HT_{2C} receptor based on results using several 5-HT receptor antagonists.¹² Therefore, taken together, it is considered that the inhibitory effects of mCPP in 2-DG-induced hyperphagia may be also related to the 5-HT_{2C} receptor.

The present results demonstrate that the mCPP inhibits the bulimia model, 2-DG-induced hyperphagia in rats and that this effects are mediated by the 5-HT_{2C/2B} receptor. Recent evidence suggests that the 5-HT_{2C} receptor is significant in eating behavior, since mice lacking the 5-HT_{2C} receptor in

the brain show remarkable hyperphagia.²²⁾ Our data that mCPP, which acts as the 5-HT_{2C} receptor agonist, inhibits 2-DG-induced hyperphagia in rats, further implies the significance of these receptors in controlling food intake. Since mCPP also decreases food intake in humans,^{10,13)} the possibility of clinically applying the 5-HT_{2C} receptor agonist for eating disorder is postulated.^{2,5)}

Acknowledgement This work was supported in part by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES

- 1) Baxter G., Kennett G. A., Blaney F., Blackburn T., *Trends Pharmacol. Sci.*, **16**, 105—110 (1995).
- 2) Curzon G., Kennett G. A., *Trends. Pharmacol. Sci.*, **11**, 181—182 (1990).
- 3) Smith T. M., Suckow R. F., *Neuropharmacology*, **24**, 1067—1071 (1985).
- 4) Curzon G., *Ann. N.Y. Acad. Sci.*, **600**, 521—531 (1990).
- 5) Kennett G. A., *Psychopharmacology*, **107**, 379—384 (1992).
- 6) Kennett G. A., Curzon G., *Br. J. Pharmacol.*, **94**, 137—147 (1988).
- 7) Bagdy G., *Behav. Brain Res.*, **73**, 277—280 (1996).
- 8) Sugimoto Y., Yamada J., Yoshikawa T., Horisaka K., *Eur. J. Pharmacol.* **307**, 75—80 (1996).
- 9) Yamada J., Sugimoto Y., *Eur. J. Pharmacol.*, **406**, 153—157 (2000).
- 10) Dourish C. T., *Obesity Res.*, **3**, 449S—462S (1995).
- 11) Samanin R., Garattini S., *Pharmacol. Toxicol.*, **73**, 63—78 (1993).
- 12) Kennett G. A., Curzon G., *Brit. J. Pharmacol.* **103**, 2016—2020 (1991).
- 13) Walsh A. E. S., Smith K. A., Oldman A. D., Williams C., Goodall E. M., Cowen P. J., *Psychopharmacology*, **116**, 120—122 (1994).
- 14) Smith G. P., Epstein A. N., *Am. J. Physiol.*, **217**, 1083—1087 (1969).
- 15) Sugimoto Y., Yamada J., Yoshikawa T., Noma T., Horisaka K., *Eur. J. Pharmacol.*, **316**, 15—21 (1996).
- 16) Halford J. C. G., Blundell J. E., *Pharmacol. Biochem. Behav.*, **54**, 745—751 (1996).
- 17) Kennett G. A., Dourish C. T., Curzon G., *Eur. J. Pharmacol.*, **141**, 429—435 (1987).
- 18) Yamada J., Sugimoto Y., Yoshikawa T., Horisaka K., *Neurosci. Lett.*, **209**, 113—116 (1996).
- 19) Kennett G. A., *Curr. Opin. Invest. Drugs.*, **2**, 317—362 (1992).
- 20) Clineschmidt B. V., Reiss D. R., Pettibone D. J., Robinson J. L., *J. Pharmacol. Exp. Ther.*, **235**, 696—708 (1985).
- 21) Kennett G. A., Ainsworth K., Trail B., Blackburn T. P., *Neuropharmacology*, **36**, 233—239 (1997).
- 22) Tecott L. H., Sun L. M., Akana S. F., Strack A. M., Lowenstein D. H., Dallman M. F., Julius D., *Nature (London)*, **374**, 542—546 (1995).