The Effects of Peripheral Serotonin₂ (5-HT₂) and Serotonin₃ (5-HT₃) Receptor Agonists on Blood Glucose Levels in Rats

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The involvement of the peripheral serotonin_{2A} (5-HT_{2A}) receptor in α -methyl-5-HT-induced hyperglycemia was examined in rats. The 5-HT_{2A} receptor antagonist, ketanserin, significantly inhibited α -methyl-5-HT-elicited hyperglycemia. Taken together with a previous report that 5-HT-induced hyperglycemia was prevented by ketanserin, it is suggested that the peripheral 5-HT_{2A} receptor participates in glucose regulation. As α -methyl-5-HT increased serum insulin but did not affect glucagon levels, it is indicated that these pancreatic hormones are probably not related to α -methyl-5-HT-induced hyperglycemia. Moreover, the peripheral 5-HT₃ receptor agonist, 2-methyl-5-HT, did not affect blood glucose, insulin or glucagon levels. Our results therefore suggest that the peripheral 5-HT₃ receptor is not involved in glucose regulation.

Key words peripheral 5-HT₂ receptor; peripheral 5-HT₃ receptor; blood glucose; insulin; glucagon

Previous studies have accumulated evidence that the serotonin (5-HT) receptor participates in glucose regulation. The central 5-HT_{1A} receptor is involved in glucose regulation, since the central 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) elicits hyperglycemia in rats.^{1,2)} In addition, 5-HT_{1A} partial agonists buspirone or ipsapirone, elevate the blood glucose levels of rats.³⁾ We also found that buspirone induces hyperglycemia and that it reduces tolbutamide-induced hypoglycemia.^{4,5)} It is considered that the glycemic effects of 5-HT_{1A} receptor agonists are caused by facilitating adrenaline release from the adrenal gland.^{1–5)} Furthermore, as shown in previous studies, the central 5-HT_{2A} and 5-HT_{2C} receptor agonists, DOI and mCPP, cause hyperglycemia in rats which is mediated by adrenaline release.^{6,7)} Thus, the central 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors participate in the control of glucose homeostasis.

Recent reports indicate that the peripheral 5-HT receptor is also involved in glucose regulation.^{8,9)} Chaouloff *et al.* demonstrated that the peripheral 5-HT₂ receptor agonist, α -methyl-5-HT, elicits hyperglycemia in rats, and that α -methyl-5-HT-induced hyperglycemia was prevented by the 5-HT₂ receptor antagonist LY 53857.⁸⁾ In a recent review, the 5-HT₂ receptor was subdivided into 5-HT_{2A,2B,2C} receptors.¹⁰⁾ LY 53857 is a non-selective 5-HT₂ receptor antagonist.¹⁰⁾ Thus, it is not yet clear which 5-HT₂ receptor subtype is involved in α -methyl-5-HT-induced hyperglycemia. We previously reported that peripherally administered 5-HT that cannot cross the blood brain barrier induces hyperglycemia in rats mediated by the peripheral 5-HT_{2A} receptor.⁹⁾ Therefore, in the present paper, to clarify the involvement of the peripheral 5-HT_{2A} receptor, we studied the effects of the 5-HT_{2A} receptor antagonist ketanserin on α -methyl-5-HT-induced hyperglycemia. Moreover, the effects of α -methyl-5-HT on the pancreatic hormones insulin and glucagon, which are important factors in glucose regulation, were examined.

The physiological significance of the peripheral 5-HT₃ receptor is relevant. The peripheral 5-HT₃ receptor is

associated with cisplatin-induced emesis, the contraction of intense, hypotensive effects, and transmitter release in the ganglion and autonomic system.¹¹⁾ However, the involvement of the peripheral 5-HT₃ receptor in glucose regulation remains unclear. Thus, we investigated the effects of the peripheral 5-HT₃ receptor agonist 2-methyl-5-HT on blood glucose and pancreatic hormone levels in rats.

MATERIALS AND METHODS

Animals Male Sprague-Dawley rats (180–230 g) were obtained from SLC Japan, Inc. They were maintained under a controlled 12 h/12 h light/dark cycle (light from 7 a.m. to 7 p.m.), with a room temperature at $24 \pm 1^\circ\text{C}$ and humidity at $55 \pm 5\%$. Rats were given free access to food and water.

Drugs and Treatment α -Methyl-5-HT, ketanserin tartrate and 2-methyl-5-HT maleate were purchased from Research Biochemicals, Inc. (U.S.A.). Drugs were dissolved in saline and injected i.p. These drugs were injected i.p. at a volume 0.2 ml/100 g.

Determination of Plasma Glucose and Pancreatic Hormone Levels Blood samples were taken from the caudal *vena cava* under light ether anesthesia. Only one sample was removed from each rat. Plasma glucose levels were measured by a previously described method.⁴⁾ Pancreatic hormone insulin and glucagon were determined by radioimmunoassay following a previous method.⁴⁾ Serum insulin and glucagon levels were determined by commercially available kits, Phadeseph Insulin (Pharmacia, Sweden) and Glucagon Daiichi (Daiichi Radio Isotope Center, Japan), respectively.

Statistics Statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test.

RESULTS AND DISCUSSION

Figure 1 shows the time course changes of the effect of α -methyl-5-HT on plasma glucose levels. α -Methyl-5-HT

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Fig. 1. Effects of α -Methyl-5-HT on Blood Glucose Levels of Rats

Results are shown as the mean $\pm$ S.E. ($n=5-7$). α -Methyl-5-HT was injected i.p. * $p < 0.05$, ** $p < 0.01$.

Fig. 2. Effects of Ketanserin on α -Methyl-5-HT-Induced Hyperglycemia in Rats

Results are shown as the mean $\pm$ S.E. ($n=5-7$). α -Methyl-5-HT was injected i.p. Ketanserin was given i.p. 30 min before the injection of α -methyl-5-HT at 5 mg/kg. Plasma glucose levels were determined 15 min after the injection of α -methyl-5-HT. *** $p < 0.001$ vs. saline of respective group. * $p < 0.05$, ** $p < 0.01$ vs. saline + α -methyl-5-HT.

induced significant hyperglycemia above the dosage of 1 mg/kg. The effects of ketanserin on α -methyl-5-HT-induced hyperglycemia are demonstrated in Fig. 2. As shown in the results, ketanserin significantly reduced the hyperglycemia elicited by α -methyl-5-HT. The effects of α -methyl-5-HT on the pancreatic hormones, insulin and glucagon, are demonstrated in Fig. 3. α -Methyl-5-HT at a dose of only 5 mg/kg increased serum insulin levels, while it did not affect glucagon levels. Figure 4 shows the effects of 2-methyl-5-HT on plasma glucose and on blood insulin and glucagon levels. However, 2-methyl-5-HT at 1 and 5 mg/kg did not change the glucose, insulin or glucagon levels.

The peripheral 5-HT₂ receptor agonist, α -methyl-5-HT, induced hyperglycemia in rats, which is in agreement with

Fig. 3. Effects of α -methyl-5-HT on Blood Insulin and Glucagon Levels of Rats

Results are shown as the mean $\pm$ S.E. ($n=5-7$). A, serum insulin; B, plasma glucagon. α -Methyl-5-HT was injected i.p. Insulin and glucagon levels were determined 15 min after the injection of α -methyl-5-HT. ** $p < 0.01$.

a previous report.⁸⁾ A previous finding demonstrated that α -methyl-5-HT-induced hyperglycemia was inhibited by the 5-HT₂ receptor antagonist LY 53857.⁸⁾ The 5-HT₂ receptor has now been reclassified into 5-HT_{2A}, 2B, 2C receptors.¹⁰⁾ Since LY 53857 has a high affinity for all 5-HT₂ receptor subtypes,¹⁰⁾ the involvement of the 5-HT₂ receptor subtypes in α -methyl-5-HT-induced hyperglycemia remains unclear. As shown by our results, α -methyl-5-HT-induced hyperglycemia was dose-dependently inhibited by ketanserin. Since ketanserin has a higher affinity for 5-HT_{2A} receptors,¹⁰⁾ it is indicated that α -methyl-5-HT-induced hyperglycemia is mediated by the peripheral 5-HT_{2A} receptor. We previously reported that peripherally administered 5-HT elicits hyperglycemia in rats.⁹⁾ Hyperglycemia elicited by 5-HT is mediated by the peripheral 5-HT_{2A} receptor, since ketanserin strongly reduced it.⁹⁾ The present results obtained with α -methyl-5-HT further support the idea that the peripheral 5-HT_{2A} receptor participates in glucose regulation. Insulin and glucagon are known to regulate blood glucose levels. Therefore, we studied the effects of α -methyl-5-HT on these hormone levels. As shown in the results, α -methyl-5-HT only at 5 mg/kg significantly increased the serum insulin levels in rats, which was in accord with the results of Chaouloff *et al.*⁸⁾ Insulin is recognized to be a hypoglycemic hormone, so it is likely that insulin is not related to the hyperglycemic effects of α -methyl-5-HT. Although the reason for the elevation in insulin levels elicited by α -methyl-5-HT remains unsolved, α -methyl-5-HT may directly stimulate insulin release or the hyperglycemia may trigger insulin release. Moreover, α -methyl-5-HT did

Fig. 4. Effects of 2-Methyl-5-HT on Blood Glucose, Insulin and Glucagon Levels of Rats

Results are shown as the mean $\pm$ S.E. ($n=5-7$). A, plasma glucose; B, serum insulin; C, plasma glucagon. 2-Methyl-5-HT was given i.p. Insulin and glucagon levels were determined 15 min after the injection of 2-methyl-5-HT.

not affect plasma glucagon levels. This suggests that α -methyl-5-HT-induced hyperglycemia is not connected to glucagon-inducing hyperglycemia. We previously found that 5-HT elevates plasma adrenaline levels and that adrenaline is strongly related to 5-HT-induced hyperglycemia.⁹⁾ Chaouloff *et al.* also reported that α -methyl-5-HT can increase plasma adrenaline levels.¹²⁾ Thus, taken together with previous reports, the peripheral 5-HT_{2A}

receptor may be associated with glucose regulation via adrenaline release.

To date, the involvement of the 5-HT₃ receptor in glucose regulation has not been investigated. To clarify the involvement of the peripheral 5-HT₃ receptor in glucose regulation, we further examined the effects of the peripheral 5-HT₃ receptor agonist 2-methyl-5-HT on plasma glucose levels. However, as shown in the results, 2-methyl-5-HT did not change plasma glucose levels. Furthermore, 2-methyl-5-HT was without effect on pancreatic hormones, serum insulin or plasma glucagon levels. Although 5-HT i.p. induces hyperglycemia in rats, the 5-HT₃ receptor antagonist ICS 205—930 did not affect it.⁹⁾ Therefore, these findings suggest that the peripheral 5-HT₃ receptor is unrelated to glucose homeostasis.

In summary, our results demonstrate that the peripheral 5-HT_{2A} receptor is closely associated with glucose regulation and that the peripheral 5-HT₃ receptor is not involved in glucose homeostasis. Recently the 5-HT receptor has been further divided into subtypes.¹³⁾ The effects of other the 5-HT receptor subtypes, such as 5-HT_{4,5,6,7}, remain unclear, and further studies on the role of 5-HT receptors in glucose homeostasis are required.

REFERENCES

- 1) Chaouloff F., Jeanrenaud B., *J. Pharmacol. Exp. Ther.*, **243**, 1159—1166 (1987).
- 2) Chaouloff F., Laude D., Baudrie V., *Eur. J. Pharmacol.*, **185**, 11—18 (1990).
- 3) Chaouloff F., Baudrie V., Laude D., *Eur. J. Pharmacol.*, **177**, 107—110 (1990).
- 4) Sugimoto Y., Yamada J., Kimura I., Watanabe Y., Horisaka K., *Jpn. J. Pharmacol.*, **60**, 145—148 (1992).
- 5) Sugimoto Y., Yamada J., Kimura I., Horisaka K., *Biol. Pharm. Bull.*, **18**, 1296—1298 (1995).
- 6) Baudrie V., Chaouloff F., *Eur. J. Pharmacol.*, **213**, 41—46 (1992).
- 7) Sugimoto Y., Yamada J., Yoshikawa T., Horisaka K., *Eur. J. Pharmacol.*, **307**, 75—80 (1996).
- 8) Chaouloff F., Laude D., Baudrie V., *Eur. J. Pharmacol.*, **187**, 435—443 (1990).
- 9) Yamada J., Sugimoto Y., Yoshikawa T., Kimura I., Horisaka K., *Life Sci.*, **57**, 819—825 (1995).
- 10) Baxter G. S., Kennett G. A., Blackburn T. P., Blaney F., *Trends Pharmacol. Sci.*, **16**, 105—110 (1995).
- 11) Zifa E., Fillion G., *Pharmacol. Rev.*, **44**, 401—458 (1992).
- 12) Chaouloff F., Gunn S. H., Young J. B., *J. Pharmacol. Exp. Ther.*, **260**, 1008—1016 (1992).
- 13) Hoyer D., Clarke D. E., Fozard J. R., Hartig P. R., Martin G. R., Mykecharane E. J., Saxena P. R., Humphrey P. P. A., *Pharmacol. Rev.*, **46**, 157—203 (1994).