The Effects of the Serotonin_{1A} Receptor Agonist Buspirone on Tolbutamide-Induced Hypoglycemia in Rats

Yumi SUGIMOTO,* Jun YAMADA, Ikuko KIMURA, and Kazuyoshi HORISAKA

Department of Pharmacology, Kobe Pharmaceutical University, Motoyamakita-machi, Higashinada-ku, Kobe 658, Japan. Received May 10, 1995; accepted June 27, 1995

The effects of the serotonin_{1A}(5-HT_{1A}) receptor agonist buspirone on hypoglycemia elicited by tolbutamide were investigated in rats. Buspirone, at doses not affecting plasma glucose levels, inhibited the hypoglycemic effects of tolbutamide. The inhibitory effects of buspirone on tolbutamide-induced hypoglycemia were antagonized by the 5-HT_{1A} receptor antagonist pindolol. As tolbutamide is known to induce hypoglycemia by facilitating insulin release, the effects of buspirone on a tolbutamide-induced increase in serum insulin levels were also studied. However, buspirone did not affect tolbutamide-induced insulin release. Adrenodemedullation inhibited the effects of buspirone. These results suggest that buspirone inhibits tolbutamide-induced hypoglycemia mediated by the 5-HT_{1A} receptor, and adrenaline release may be involved in the effects of buspirone.

Key words 5-HT_{1A} receptor; buspirone; plasma glucose; tolbutamide

It has been suggested that the serotonin_{1A}(5-HT_{1A}) receptor may be involved in the regulation of blood glucose levels.¹⁻³⁾ Previous reports demonstrated that the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) elicits hyperglycemia in rats.¹⁻³⁾ Buspirone, an azapirone derivative, is known to be an agonist of the 5-HT_{1A} receptor.⁴⁾ In our previous study, we also demonstrated that buspirone induces hyperglycemia and hyperglucagonemia in rats.⁵⁾ These findings suggest that the 5-HT_{1A} receptor agonists may affect glucose homeostasis.

Previous evidence suggests that the central 5-HT_{1A} receptor is related to anxiety.⁴⁾ Buspirone has a high affinity with the 5-HT_{1A} receptor in the brain, like other azapirone derivatives including ipsapirone or gepirone, and has a potent anxiolytic activity in both man and animals.^{4,6-9)} Unlike benzodiazepines, buspirone is devoid of sedative or muscle relaxant properties.⁶⁻⁹⁾ A recent finding also indicates that buspirone may be useful as an antidepressant.⁴⁾ Therefore, buspirone is considered to be a useful drug for psychiatric disorders. In the treatment with buspirone, various drugs may be used together with it. This raises the possibility that buspirone may interact with other drugs affecting blood glucose levels. However, the interaction between the 5-HT_{1A} receptor agonists and hypo- or hyperglycemic drugs has not been investigated. Tolbutamide is a drug for diabetes and induces hypoglycemia. In this paper, we studied the effects of buspirone on hypoglycemia induced by tolbutamide in rats.

MATERIALS AND METHODS

Animals Male SD rats weighing 180–240 g (SLC Japan, Inc., Japan) were used, and the rats were housed in a room with a controlled 12 h/12 h light/dark cycle, with lights on between 7:00 a.m. and 7:00 p.m., a temperature of 24±1 °C and at a humidity of 55±5%. Experiments were performed between 1:00 and 4:00 p.m.

Drugs and Treatment Buspirone was obtained from Research Biochemicals, Inc. (U.S.A.) and dissolved in saline. Tolbutamide and pindolol were purchased from

Sigma (U.S.A.). Tolbutamide was dissolved in 1 N NaOH and adjusted to pH 7.5 with 1 N HCl. Pindolol was dissolved in a few drops of 0.1 N HCl and diluted with saline. Buspirone was given s.c. and tolbutamide was injected i.p. at 50 mg/kg. Buspirone and tolbutamide were administered simultaneously. Pindolol was injected i.p. 30 min before buspirone.

Determination of Blood Glucose and Insulin Levels Blood samples were taken from the caudal vena cava under light ether anesthesia. Only one sample was removed from each rat. Plasma glucose and serum insulin levels were determined by the method previously described.⁵⁾ Rat insulin (Novo, Denmark) was used as a standard for the determination of insulin.

The Surgery of Adrenodemedullation The surgery of bilateral adrenodemedullation was performed under anesthesia with pentobarbital-Na. Rats were used for experiments 1 week after the surgery. After 1 week, the body weight of adrenodemedullated rats nearly returned to that of the sham operated rats. After the experiments, the rats were dissected and we confirmed that the adrenal medulla was removed and atrophy of the adrenal cortex did not occur.

Statistical Analysis Statistical significance was evaluated by the Student's *t*-test between two groups and by the Dunnett's test among three or more groups.

RESULTS

Figure 1 shows the effects of buspirone on tolbutamide-induced hypoglycemia. Since buspirone induced a maximum hyperglycemia 15 min after the injection in our previous report,⁵⁾ the effects of buspirone were evaluated at that time. Buspirone itself induced significant hyperglycemia at a dose of 10 mg/kg as described in our previous study⁵⁾ (Saline: 151.4±3.64 mg/dl, Buspirone 10 mg/kg: 180.1±7.95 mg/dl, ** *p*<0.01). However, below 5 mg/kg, buspirone did not affect basal plasma glucose levels (Buspirone 1 mg/kg: 148.2±3.22 mg/dl, 2.5 mg/kg: 154.3±5.24 mg/dl, 5 mg/kg: 158.4±4.82 mg/dl). Tolbutamide at 50 mg/kg induced apparent hypoglycemia in rats. As shown in Fig. 1, buspirone above the dosage of

* To whom correspondence should be addressed.

Fig. 1. Effects of Buspirone on Tolbutamide-Induced Hypoglycemia in Rats

Results are shown as mean $\pm$ S.E. ($n=6-9$). Buspirone was given s.c. and tolbutamide at 50 mg/kg was injected i.p. Buspirone and tolbutamide were administered simultaneously. ** $p < 0.01$.

Table 1. Effects of Pindolol on Inhibitory Effects of Buspirone on Tolbutamide-Induced Hypoglycemia in Rats

Group	Pretreatment	
	Saline	Pindolol 5 mg/kg
Saline + saline	140.5 $\pm$ 2.87	145.2 $\pm$ 1.88
Tolbutamide + saline	75.9 $\pm$ 1.96 ^{a)}	76.5 $\pm$ 3.92 ^{a)}
Tolbutamide + buspirone	100.8 $\pm$ 1.91 ^{a,b)}	76.0 $\pm$ 2.05 ^{a)}

Results are shown as mean plasma glucose (mg/dl) $\pm$ S.E. ($n=6-9$). Buspirone was given s.c. at 5 mg/kg. Tolbutamide at 50 mg/kg was injected i.p. Buspirone and tolbutamide were administered simultaneously. Pindolol was injected i.p. 30 min before the injection of tolbutamide + buspirone. Plasma glucose levels were determined 15 min after the treatment with tolbutamide + buspirone. a) $p < 0.01$ vs. saline + saline of respective group. b) $p < 0.01$ vs. tolbutamide + saline of respective group.

Table 2. Effects of Buspirone on Tolbutamide-Induced Hyperinsulinemia in Rats

Group	Serum insulin (ng/ml)
Saline + saline	13.0 $\pm$ 0.90
Tolbutamide + saline	26.3 $\pm$ 0.97 ^{a)}
Tolbutamide + buspirone	24.7 $\pm$ 1.13 ^{a)}

Results are shown as mean $\pm$ S.E. ($n=7-10$). Buspirone was given s.c. at 5 mg/kg. Tolbutamide at 50 mg/kg was injected i.p. Buspirone and tolbutamide were administered simultaneously. Serum insulin levels were determined 15 min after the treatment with tolbutamide + buspirone. a) $p < 0.01$ vs. saline + saline-treated group.

Table 3. Effects of Buspirone on Tolbutamide-Induced Hypoglycemia in Adrenomedullated Rats

Group	Sham	Adrenomedullation
Saline + saline	142.7 $\pm$ 4.49	138.0 $\pm$ 3.18
Tolbutamide + saline	75.8 $\pm$ 1.97 ^{a)}	70.5 $\pm$ 4.11 ^{a)}
Tolbutamide + buspirone	103.4 $\pm$ 2.11 ^{a,b)}	75.9 $\pm$ 1.32 ^{a)}

Results are shown as mean plasma glucose (mg/dl) $\pm$ S.E. ($n=5-7$). Buspirone was given s.c. at 5 mg/kg. Tolbutamide at 50 mg/kg was injected i.p. Buspirone and tolbutamide were administered simultaneously. Plasma glucose levels were determined 15 min after the treatment with tolbutamide + buspirone. a) $p < 0.01$ vs. saline + saline of respective group. b) $p < 0.01$ vs. saline + tolbutamide of respective group.

2.5 mg/kg significantly reduced tolbutamide-induced hypoglycemia.

The effects of pindolol on the inhibitory effects of buspirone on tolbutamide-induced hypoglycemia are shown in Table 1. Pindolol did not affect the basal plasma glucose levels or tolbutamide-induced hypoglycemia. Pretreatment with pindolol suppressed the inhibitory effects of buspirone on tolbutamide-induced hypoglycemia.

Table 2 demonstrates the serum insulin levels of tolbutamide and buspirone in 5 mg/kg-treated rats. Buspirone alone did not affect serum insulin levels. Although tolbutamide elicits hyperinsulinemia, buspirone did not affect it.

Table 3 demonstrates the effects of buspirone in adrenomedullated rats. Buspirone inhibited tolbutamide-induced hypoglycemia in sham operated rats. However, in adrenomedullated rats, buspirone did not affect tolbutamide-induced hypoglycemia.

DISCUSSION

In the present study, we demonstrated that buspirone significantly reduced tolbutamide-induced hypoglycemia. As shown in our results, the inhibitory effects of buspirone on hypoglycemia were observed at low doses which did not affect basal glucose levels. Our results indicate that buspirone may diminish the effects of hypoglycemic agents. As buspirone has a high affinity to the 5-HT_{1A} receptor, we examined the effects of the 5-HT_{1A} receptor antagonist pindolol on the effects of buspirone. The inhibitory effects of buspirone on tolbutamide-induced hypoglycemia were antagonized by pretreatment with pindolol. This indicates that buspirone reduces tolbutamide-induced hypoglycemia mediated by the 5-HT_{1A} receptor.

Since tolbutamide is known to induce hypoglycemia by stimulating insulin release from the pancreatic islet, the inhibitory effects of buspirone on tolbutamide-induced hypoglycemia may be related to tolbutamide-induced insulin release. However, there has been no report examining whether buspirone affects stimulant-induced insulin release. Thus, we determined the serum insulin levels after the treatment with tolbutamide and buspirone. However, buspirone did not affect tolbutamide-induced increases in insulin levels. These results suggest that the antagonistic effects of buspirone on tolbutamide-induced hypoglycemia are not related to the insulin releasing effects of tolbutamide. Other hyperglycemic factors probably contribute to the inhibitory effects of buspirone on hypoglycemia elicited by tolbutamide.

It has been suggested that the 5-HT_{1A} receptor agonist 8-OH-DPAT induces hyperglycemia, and its hyperglycemic effects are elicited by adrenaline release from the adrenal gland.¹⁻³⁾ We have demonstrated that buspirone elevates both plasma glucose and adrenaline levels in ether anesthetized rats,⁵⁾ which is consistent with the results of another report using conscious rats.¹⁰⁾ Furthermore, we have shown that hyperglycemia induced by a single administration of buspirone at 20 mg/kg is antagonized by adrenomedullation.⁵⁾ These findings indicate that buspirone-induced hyperglycemia is related to adrenaline release. Therefore, it is supposed that the inhibitory effects

of buspirone on tolbutamide-induced hypoglycemia may also be connected to adrenaline. In fact, as shown in the results, adrenalectomy abolished the antagonistic effects of buspirone on tolbutamide-induced hypoglycemia. This suggests that buspirone reduces hypoglycemia by facilitating adrenaline release from the adrenal medulla. In a hypoglycemic state, it is considered that adrenaline release is potentiated by physiological mechanisms. Under such a condition, buspirone, even at low doses, may induce hyperglycemic effects. 5-HT_{1A} receptor agonists, including 8-OH-DPAT or buspirone, have been suggested to increase corticosterone release from the adrenal cortex.¹¹⁾ However, it is unlikely that corticosterone is related to the inhibitory effects of buspirone on tolbutamide-induced hypoglycemia, as adrenalectomy strongly inhibited the effects of buspirone.

As described above, the inhibitory effects of buspirone on tolbutamide-induced hypoglycemia were inhibited by both pindolol and adrenalectomy. Therefore, it is suggested that buspirone facilitates adrenaline release mediated by the 5-HT_{1A} receptor, which in turn resulted in the inhibition of hypoglycemia elicited by tolbutamide. In our previous report, we demonstrated that buspirone elevates plasma glucagon, which is a hyperglycemic factor.⁵⁾ We have also shown that the increase in plasma glucagon levels elicited by buspirone is caused by adrenaline, since adrenalectomy abolished buspirone-induced hyperglucagonemia.⁵⁾ It seems that treatment with pindolol or adrenalectomy also inhibited the hyperglucagonemic effects of buspirone following the facilitation of adrenaline release. Therefore, it is indicated that adrenaline plays a major role in the inhibitory effects of buspirone on tolbutamide-induced hypoglycemia.

As shown in the results, buspirone at a dose of 5 mg/kg did not affect tolbutamide-induced insulin release, although adrenaline is known to inhibit insulin release. Furthermore, we examined a higher dose of buspirone, at 10 mg/kg on tolbutamide-induced hyperinsulinemia. However, coadministration with buspirone 10 mg/kg did not

affect tolbutamide-induced insulin release (tolbutamide + buspirone 10 mg/kg: 24.9 ± 0.71 ng/ml, $N=6$), although more potent inhibitory effects on tolbutamide-induced hypoglycemia than those at 5 mg/kg were observed. It is suggested that the amount of elevated adrenaline induced by buspirone did not affect insulin release. Released adrenaline induced by buspirone may inhibit tolbutamide-induced hypoglycemia by glycolysis or the inhibition of glucose utilization.

In summary, the present results demonstrate that buspirone at doses not affecting basal plasma glucose levels can reduce the effects of hypoglycemic drugs. The effects of buspirone are mediated by the 5-HT_{1A} receptor and are connected to adrenaline release. Our findings indicate the possibility that drugs which have an affinity to the 5-HT_{1A} receptor may modify the effects of hypoglycemic drugs. It suggests that attention to the control of blood glucose levels may be required in the coadministration of the 5-HT_{1A} receptor agonists and hypoglycemic drugs.

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