Inhibitory Effects of the 5-HT_{1A} Receptor Agonist Buspirone on Stress-Induced Hyperglycemia in Mice: Involvement of Insulin and a Buspirone Metabolite, 1-(2-Pyrimidinyl)piperazine (1-PP)

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Effects of serotonergic anxiolytic buspirone on immobilization-induced hyperglycemia were studied in mice. Stress elicited hyperglycemia in mice. Pretreatment with buspirone significantly reduced immobilization-induced hyperglycemia. Buspirone increased serum insulin levels in both non- and stressed mice. The major metabolite of buspirone, 1-(2-pyrimidinyl)piperazine (1-PP) also increased and this further inhibited immobilization-induced hyperglycemia, since 1-PP increased serum insulin levels in both non-stressed and stressed mice, similar to the increases induced by buspirone. These results suggest that buspirone can reduce stress-induced hyperglycemia by facilitating insulin release. Moreover, 1-PP, a metabolite of buspirone may participate in the effects of buspirone. Since 1-PP is an antagonist of \( \alpha_2 \) receptors, \( \alpha_2 \) receptors may be related to effects of 1-PP.

Key words buspirone; 1-(2-pyrimidinyl)piperazine; anxiolytic; glucose; insulin; 5-HT_{1A} receptor

Benzodiazepine derivatives are widely used for anxiety, by the facilitation of GABA neurotransmission. It is well known that serotonin (5-HT) is involved in several neurological functions.\(^2\) The 5-HT_{1A} receptor is related to emotion and the 5-HT_{1A} receptor agonists induce anxiolytic and antidepresant effects in humans and animals.\(^2,\) Buspirone is known to be a 5-HT_{1A} receptor agonist and is used to treat anxiety.\(^2\) Stress induces several neuroendocrinological effects such as activation of the hypothalamus-pituitary-adrenal (HPA) axis and eilicits activation of sympathetic tone.\(^4,\) Hyperglycemic responses to stress are recognized as an index of the sympathetic nervous system.\(^4\) Stress induces changes in emotion and mood.\(^5\) Recently, it was reported that the 5-HT_{1A} receptor agonist might be effective for stress-induced behavioral depressant symptoms.\(^2\)

Previous reports demonstrated that 5-HT receptor is involved in glucose regulation. The 5-HT_{1A} receptor agonist 8-OH-DPAT elicits hyperglycemia in rats and mice.\(^6,\) The 5-HT_{1A} receptor partial agonist buspirone and ipsapirone elevate the glucose levels of rats.\(^8,\) These effects of 5-HT_{1A} receptor agonists are considered due to facilitating adrenaline release from the adrenal medulla.\(^10,11\) However, there may be species differences in the effects of buspirone on glucose levels between rats and mice. We previously reported that buspirone did not elevate glucose levels of mice and it increases serum insulin levels.\(^12\) In addition, buspirone reduces glucose-induced hyperglycemia in mice by increasing insulin levels.\(^12\)

It is well known that stress elicits hyperglycemia and stress-induced hyperglycemia may be a factor in contributing to diabetes.\(^4,13\) Since buspirone is available in stressed-patients with accompanying with anxiety, the possibility that buspirone may also suppress stress-induced hyperglycemia is postulated. However, it is not clear whether buspirone modifies stress-elevated hyperglycemia. In this paper, we examined the effects of buspirone on stress-induced hyperglycemia and involvement of insulin in the effects of buspirone.

MATERIALS AND METHODS

Animals Male ddY mice weighing 28—32 g were obtained from SLC Japan Inc (Japan). Mice were fed with free access to food and water and 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 at 23±1 °C and humidity at 55±5%. For experiments with glucose, mice were fasted for 20 h. The experimental procedure was approved by the Kobe Pharmaceutical University Animal Care and Use Committee.

Drug Treatment Buspirone HCl, 1-(2-pyrimidinyl)piperazine (1-PP) HCl were obtained from Sigma (U.S.A.). Drugs were dissolved in saline. Buspirone and 1-PP were injected s.c. 30 min before immobilization stress.

Immobilization and Determination of Plasma Glucose and Insulin Levels Mice were restrained by placing in individual wire cages (3.5 cm×8.3 cm×2.3 cm). After immobilization for 15, 30, 60 and 120 min, mice were immediately decapitated and blood was collected in plastic tubes. For plasma glucose determination, blood was collected in plastic tubes containing NaF (5 mg/ml blood) to avoid glycolysis in blood. Plasma glucose was measured following the method described in our previous study.\(^9\) Serum insulin was measured using a commercially available ELISA kit (Morinaga insulin kit, Japan). Mice treated with buspirone or 1-PP were decapitated 30 min after immobilization stress.

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

RESULTS

Effects of Buspirone on Immobilization-Induced Hyperglycemia Stress elicited significant hyperglycemia in mice and hyperglycemia reached a maximum 30 min after immobilization (Fig. 1). Effects of buspirone on immobilization stress (30 min)-induced hyperglycemia in mice are shown in Fig. 2. Buspirone at 10 mg/kg apparently reduced stress-induced hyperglycemia. Buspirone increased the serum insulin levels of non-stressed and stressed mice.

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Effects of 1-PP on Immobilization-Induced Hyperglycemia Figure 3 shows effects of 1-PP on hyperglycemia induced by immobilization stress for 30 min. As shown in results, 1-PP at 1 mg/kg inhibited immobilization stress-induced hyperglycemia and also increased serum insulin levels of stressed mice.

DISCUSSION

Stress induces several neuroendocrinological responses, such as activation of the HPA axis, resulting the elevation of glucocorticoid levels or increases catecholaminergic activity.3,5) Stress induces emotional changes like anxiety or depression.5) It is well known that stress induces hyperglycemic responses in humans and rodents.9) It is suggested that stress-induced increases in plasma glucose levels may trigger diabetes or enhance hyperglycemia in diabetes. We previously reported that immobilization stress elevates plasma glucose levels of mice.14) In the present study, we further confirmed that immobilization induces a significant hyperglycemia in mice. Anxiolytics can suppress stress-induced emotional changes.2) However, it remains unclear whether buspirone modifies stress-induced hyperglycemia.

Although buspirone induces significant hyperglycemia in rats, buspirone itself did not affect basal plasma glucose levels of mice, which is in agreement with our previous study.12) Flesinoxan, another 5-HT1A receptor agonist did not affect glucose levels in mice either, although it was reported that it induced hyperglycemia in rats.15,16) Thus, there is a difference in glycemic responses to 5-HT1A receptor agonists between mice and rats.

We previously reported that in fasted mice, buspirone reduces glucose-induced hyperglycemia.12) Our present results demonstrate that buspirone reduces immobilization stress-elevated hyperglycemia in mice. These results suggest that buspirone could reduce hyperglycemia, although it did not affect basal plasma glucose levels in mice.

Our previous findings indicate that buspirone increases the serum insulin levels of mice and also facilitates glucose-induced insulin secretion.12) This suggests that inhibitory effects of buspirone on stress-induced hyperglycemia may be mediated by insulin. Thus, we examined the effects of buspirone on serum insulin levels in stressed mice. As shown in the results, stress slightly reduced serum insulin levels. In stressed mice treated with buspirone, serum insulin levels apparently increased compared to these in the control group. Therefore, it is suggested that buspirone inhibits immobiliza-
tion-induced hyperglycemia by amplifying insulin release.

Azapirone derivatives including buspirone are metabolized to the major metabolite, 1-PP.\(^1\) It was reported that 1-PP may be involved in the anxiolytic effects of buspirone.\(^2\) It was previously reported that buspirone is rapidly metabolized to 1-PP and 1-PP levels in the brain and plasma increased 10 and 15 fold higher than those of buspirone 15 min after the injection of buspirone.\(^3\) We demonstrated that 1-PP increased serum insulin levels and facilitates glucose-induced insulin release.\(^4\) In the present study, 1-PP increased serum insulin levels in both non-stressed and stressed mice, similar to the results with buspirone. Therefore, it is suggested that 1-PP formed from buspirone plays a role in inhibitory effects of stress-induced hyperglycemia following the administration of buspirone.

The buspirone metabolite, 1-PP is recognized as an antagonist of \(\alpha_2\) receptors with very low affinity with 5-HT\(_{1A}\) receptors.\(^5\) The \(\alpha_2\) receptors express in \(\beta\) cells of islet and inhibit insulin release.\(^6\) Under stress, increased catecholamines, epinephrine and norepinephrine act on the \(\alpha_2\) receptor expressed in \(\beta\) cells and inhibit insulin release, resulting in hyperglycemia. Since 1-PP is an \(\alpha_2\) receptor antagonist and increases blood insulin levels of rats,\(^7\) it may antagonize the effect of catecholamines on the \(\alpha_2\) receptor, leading to inhibition of stress-induced hyperglycemia.

As mentioned above, there is a species differences between rats and mice in glycemic responses to buspirone. As 1-PP can increase insulin secretion, there may be differences of metabolism from buspirone to 1-PP in rats and mice. Although the buspirone increases serum insulin levels in non-stressed mice, hypoglycemia was not shown. It was reported that activation of the 5-HT\(_{1A}\) receptor induces hyperglycemia.\(^8\) Therefore, it is considered that the hyperglycemic effects based on activation of the 5-HT\(_{1A}\) receptor by buspirone may suppress hypoglycemia elicited by increased insulin in mice. Effects of buspirone on hyperglycemia elicited by immobilization-induced stress in rats may be also different from in mice and studies using rats are further required.

Anxiolytic effects of buspirone are suggested to be mediated by the central 5-HT\(_{1A}\) receptor.\(^9\) As shown in the results, 1-PP formed from buspirone may also be related to hyperinsulinemic effects of mice treated with buspirone. Experiments using 5-HT\(_{1A}\) receptor antagonists may be helpful for discriminating the involvement of the 5-HT\(_{1A}\) receptor and 1-PP in hyperinsulinemic effects of buspirone.

In conclusion, our results have shown that serotonergic anxiolytic buspirone reduces stress-induced hyperglycemia in mice by facilitating insulin release. The buspirone metabolite 1-PP may participate in inhibitory effects of stress-induced hyperglycemia after the injection of buspirone. We reported that administration of an another anxiolytic benzodiazepine diazepam induces hyperglycemia in mice.\(^10\) On the contrary, our results suggest that buspirone may be effective in hyperglycemia in addition to anxiety.

REFERENCES