The Tricyclic Antidepressant Clomipramine Increases Plasma Glucose Levels of Mice

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Abstract. Effects of the tricyclic antidepressant clomipramine on plasma glucose levels in mice were studied. Clomipramine at doses ranging 5 – 20 mg/kg elicited significant hyperglycemia in mice. Hyperglycemia elicited by clomipramine was not reduced by pretreatment with the 5-hydroxytryptamine (5-HT) depleter p-chlorophenylalanine. The 5-HT1/2/5/7-receptor antagonist methysergide and the 5-HT2A/2B/2C-receptor antagonist LY 53857 enhanced clomipramine-induced hyperglycemia, while the 5-HT1A/1B-receptor antagonist (−)-propranolol and the 5-HT3/4-receptor antagonist tropisetron did not affect it. The 5-HT2B/2C-receptor antagonist SB 206553 facilitated hyperglycemia induced by clomipramine, although the 5-HT2A-receptor antagonist ketanserin was without effect. Clomipramine-induced hyperglycemia was reduced by prior adrenalectomy. These results suggest that clomipramine induces hyperglycemia in mice by blocking the 5-HT2B and/or 5-HT2C receptors, which results in facilitation of adrenaline release.

Keywords: clomipramine, 5-hydroxytryptamine (5-HT), hyperglycemia, 5-HT2B/2C receptor, antidepressant

Introduction

A number of observations suggest that enhancement of neurotransmitters such as noradrenaline and 5-hydroxytryptamine (5-HT) leads to improvement of depression (1). Clomipramine is a tricyclic antidepressant like imipramine and is widely used in the therapy for depression (1, 2). It is recognized that clomipramine is also available for treatment of obsessive compulsive disorder (OCD) (2 – 4). In mice, clomipramine reduces immobility in the forced swimming test, which is the behavioral model for antidepressants (5, 6). It also inhibits the OCD animal model, marble burying behavior in mice (7). Antidepressants inhibit reuptake of noradrenaline and/or 5-HT at nerve terminals and elevate these amine levels at the synaptic cleft (1, 8). Clomipramine can inhibit reuptake of both noradrenaline and 5-HT, although clomipramine inhibits 5-HT reuptake more strongly than it inhibits noradrenaline reuptake (1, 8).

It has been reported that 5-HT is involved in glucose regulation. The activation of the central 5-HT1A, 5-HT2A, and 5-HT2B/2C receptor elevates plasma glucose levels of rats (9 – 12). It is well known that stress elevates blood glucose levels. Since depressive patients are considered sensitive to stress, glucose homeostasis may be affected more easily than normal subjects. Therefore, it is important to clarify the effects of antidepressants on blood glucose regulation. We previously reported that antidepressants, selective serotonin reuptake inhibitors (SSRIs) including fluoxetine, fluvoxamine, and zimelidine, cause hyperglycemia in mice, although mechanisms for hyperglycemia elicited by these SSRIs may not be identical (13, 14). Nevertheless, clomipramine is widely used for the therapy of depression. It powerfully inhibits 5-HT reuptake similar to SSRIs, but it is not yet clear whether clomipramine affects plasma glucose levels. In this study, therefore, we investigated the effects of clomipramine on plasma glucose levels of mice.

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Materials and Methods

Animals
Male ddY mice weighing 28 – 32 g were obtained from SLC Japan, Inc. (Hamamatsu). Mice were given 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%. The experimental procedure was approved by the Kobe Pharmaceutical University Animal Care and Use Committee.

Drug treatment
Clomipramine HCl, p-chlorophenylalanine methyl-ester HCl (PCPA), methysergide hydrogen maleate, LY 53857 maleate, SB 206553 HCl, ketanserin tartrate, tropisetron HCl, and (−)-propranolol HCl were obtained from Sigma (St. Louis, MO, USA). Methysergide, LY 53857, ketanserin, and (−)-propranolol were dissolved in saline. Tropisetron was dissolved in a few drops of 0.1 N HCl and diluted with saline. All drugs were injected i.p. at a volume of 0.1 ml/10 g. PCPA was injected i.p. 72, 48, and 24 h before injection of clomipramine. 5-HT-receptor antagonists were given 30 min before the injection of clomipramine.

Determination of plasma glucose levels
Mice were decapitated and blood was collected in tubes containing NaF. Plasma glucose was measured following the method described in our previous study (12).

Adrenalectomy
Adrenalectomy was performed under anesthesia with pentobarbital Na at 50 mg/kg. NaCl (0.5%) was given to adrenalectomized mice to maintain mineral balance. Experiments were carried out one week after the operation. After the experiments, adrenalectomized mice were dissected to confirm that the adrenal gland was completely removed.

Statistics
Dose-related effects on plasma glucose levels were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett’s test. Other results were analyzed by two-way ANOVA followed by Tukey’s test.

Results
Effects of clomipramine on plasma glucose levels of mice
Effects of clomipramine on plasma glucose levels are shown in Fig. 1. Clomipramine above the dosage of 5 mg/kg elicited significant hyperglycemia in mice. The hyperglycemia lasted for at least 60 min at 10 and 20 mg/kg of clomipramine.

Fig. 1. Effects of clomipramine on the plasma glucose of mice. Results are shown as the mean ± S.E.M. (N = 5 – 8). Clomipramine was given i.p. **P<0.01.

Fig. 2. Effects of PCPA on clomipramine-induced hyperglycemia in mice. Results are shown as the mean ± S.E.M. (N = 7 – 9). Clomipramine at 20 mg/kg was given i.p. PCPA at 400 mg/kg was injected i.p. 72, 48, and 24 h before injection of clomipramine. Plasma glucose levels were determined 30 min after the injection of clomipramine. ***P<0.001 vs saline of respective group.
Effects of PCPA on hyperglycemia induced by clomipramine

Figure 2 shows the effects of the 5-HT depleter PCPA on hyperglycemia induced by clomipramine. PCPA did not affect clomipramine-induced hyperglycemia.

Effects of (−)-propranolol, tropisetron methysergide, and LY 53857 on clomipramine-induced hyperglycemia

Figure 3 shows the effects of 5-HT-receptor antagonists on clomipramine (20 mg/kg)-induced hyperglycemia in mice. The 5-HT<sub>1A/1B</sub>-receptor antagonist (−)-propranolol and the 5-HT<sub>3/4</sub>-receptor antagonist tropisetron did not affect hyperglycemia elicited by clomipramine. Hyperglycemia induced by clomipramine was enhanced by pretreatment with the 5-HT<sub>1</sub>/2/5/7-receptor antagonist methysergide and the 5-HT<sub>2A/2B/2C</sub>-receptor antagonist LY 53857.

Fig. 3. Effects of methysergide, tropisetron, (−)−propranolol, and LY 53857 on clomipramine-induced hyperglycemia in mice. Results are shown as the mean ± S.E.M. (N = 5 – 8). Clomipramine was given i.p. at 20 mg/kg. 5-HT-receptor antagonists were injected i.p. 30 min before clomipramine. Plasma glucose levels were determined 30 min after the injection of clomipramine. ***P<0.001 vs respective saline-pretreated group. **P<0.01, ***P<0.001 vs saline + clomipramine-treated group.
Effects of ketanserin and SB 206553 on clomipramine-induced hyperglycemia

The effects of ketanserin and SB 206553 on clomipramine-induced hyperglycemia are shown in Fig. 4. The 5-HT$_{2B/2C}$-receptor antagonist SB 206553 amplified clomipramine-induced hyperglycemia, although the 5-HT$_{2A}$-receptor antagonist ketanserin was without effect.

Effects of clomipramine on blood glucose levels in adrenalectomized mice

Figure 5 demonstrates blood glucose levels in adrenalectomized mice. As shown in the results, clomipramine-induced hyperglycemia was strongly inhibited by prior adrenalectomy.

Discussion

Many antidepressants improve depression by modifying neurotransmission of biogenic amines such as noradrenaline, 5-HT, or dopamine (1, 8). Although 5-HT participates in glucose regulation (9–11), effects of antidepressants on glucose homeostasis are not fully established. The present results indicate that the tricyclic antidepressant clomipramine above the dosage of 5 mg /kg significantly induced hyperglycemia in mice. Previous studies have shown that clomipramine reduces immobility in the forced swimming test or inhibits marble burying behavior (5–7). The doses at which clomipramine inhibited behaviors in these models are higher than those showing hyperglycemia in this study. It was reported that another tricyclic antidepressant imipramine induces hyperglycemia in fasted rabbits and it antagonizes insulin-induced hypoglycemia (15). We previously reported that SSRIs fluoxetine or fluvoxamine also elevated plasma glucose levels in non-fasted...
Our present results with clomipramine further shows that the antidepressants may affect glucose homeostasis. In previous studies, it was recognized that clomipramine strongly blocks reuptake of 5-HT. The 5-HT uptake IC_{50} value of clomipramine was 1.5 nM, which was lower than that of the SSRI, fluvoxamine (3.8 nM) (8). This suggests that clomipramine-induced hyperglycemia may be related to inhibition of 5-HT reuptake. Therefore, we examined the effects of the 5-HT depletor PCPA on hyperglycemia elicited by clomipramine. The dose of PCPA used in this study reduced brain 5-HT levels by 74% and inhibited fluvoxamine-induced hyperglycemia in mice (14). However, PCPA did not affect clomipramine-induced hyperglycemia. Therefore, clomipramine-induced hyperglycemia is not related to its inhibition of 5-HT reuptake. We previously demonstrated that SSRIs, fluoxetine, and fluvoxamine could induce hyperglycemia in mice, although these hyperglycemic effects were not equal (14). The 5-HT depletor PCPA reduces hyperglycemia induced by fluvoxamine, while hyperglycemic effects of fluoxetine are resistant to 5-HT depletion (14). These findings suggest that clomipramine-induced hyperglycemia may be similar to that induced by fluoxetine.

Clomipramine has an affinity for 5-HT_{2} receptors and the direct interaction with this receptor may be related to pharmacological effects of clomipramine (16). Thus, to clarify the involvement of 5-HT-receptor subtypes, we examined effects of several 5-HT-receptor antagonists including antagonists of 5-HT_{2} receptors on clomipramine-induced hyperglycemia. Clomipramine-elicited hyperglycemia was enhanced by the 5-HT_{1A/1B}-receptor antagonist methysergide and the non-selective 5-HT_{2C}-receptor antagonist LY 53857. However, the 5-HT_{1A/1B}-receptor antagonist (−)-propranolol and the 5-HT_{1D/4G}-receptor antagonist tropisetron did not affect hyperglycemia induced by clomipramine. These results suggest that clomipramine-induced hyperglycemia is related to the 5-HT_{2} receptor but not to the 5-HT_{1A/1B} and 5-HT_{3/4} receptors. The present results have shown that blockade of the 5-HT_{2} receptor results in facilitation of hyperglycemia induced by clomipramine. The involvement of 5-HT_{2} receptors in the pharmacological effects of clomipramine has been reported. We previously demonstrated that the 5-HT_{2}-receptor antagonist LY 53857, at the same dose as used in this study, enhanced the anti-immobility effects of clomipramine (6). It indicates that the 5-HT_{2} receptor is involved in the pharmacological effects of clomipramine. Thus, the enhancing effects of LY 53857 on clomipramine-induced hyperglycemia are consistent with results obtained with the forced swimming test. Therefore, hyperglycemia induced by clomipramine is closely associated with direct effects of clomipramine on the 5-HT_{2} receptor.

The 5-HT_{2} receptor is subdivided into the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors (17). As shown in the results, the 5-HT_{2C}-receptor antagonist ketanserin did not affect clomipramine-induced hyperglycemia. The 5-HT_{2B/2C}-receptor antagonist SB 206553 apparently enhanced hyperglycemia induced by clomipramine. These results suggest that the selective blockade of the 5-HT_{2B/2C} receptor increases clomipramine-induced hyperglycemia in mice. It is not known whether clomipramine has affinity with the 5-HT_{2B} receptor. However, a previous report demonstrated that tricyclic antidepressants including imipramine or clomipramine have an apparent affinity for the 5-HT_{3C}-receptor subtype (16). Clomipramine may act as a 5-HT_{3C}-receptor antagonist, since it inhibited 5-HT-stimulated phosphoinositide hydrolysis in the choroid plexus, which is an index of a 5-HT_{3C}-receptor-mediated response (16). Therefore, it is suggested that clomipramine may induce hyperglycemia in mice as a result of blockade of the 5-HT_{3C} receptor.

It is well known that adrenaline released from the adrenal gland has an important role in regulating blood glucose levels and that it elevates plasma glucose levels by inhibition of insulin release, glycolysis, or inhibition of glucose uptake in tissues. Therefore, we studied effects of adrenalectomy on clomipramine-hyperglycemia. As shown in the results, adrenalectomy significantly inhibited clomipramine-induced hyperglycemia. Therefore, adrenal release from the adrenal gland may be involved in hyperglycemic effects of clomipramine. As discussed above, the inhibition of the 5-HT_{3C} receptor may be related to clomipramine-induced hyperglycemia. Recent reports demonstrated that the 5-HT_{3C} receptor tonically inhibits noradrenaline release from the noradrenergic neurons in the brain and that the blockade of the 5-HT_{3C} receptor facilitates noradrenaline release determined by microdialysis (18). These results indicate that the inhibition of the central 5-HT_{3C} receptor by clomipramine may activate the sympato-adrenal system, leading to the facilitation of adrenal release. It is not yet clear whether clomipramine directly stimulates adrenal medulla and adrenomedullary release and further studies are required.

In summary, the present results suggest that clomipramine induced an apparent hyperglycemia in mice. Clomipramine-induced hyperglycemia is not associated with its inhibition of 5-HT reuptake. It is also indicated that clomipramine-induced hyperglycemia is related to its inhibition of the 5-HT_{2B/2C} receptor, probably the 5-HT_{2C} receptor, which is mediated by adrenaline release.
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References