Chlorpromazine (CPZ) is known as a drug which has a wide variety of actions such as dopamine receptor blockade, $\alpha$-adrenergic receptor blockade, and calmodulin antagonistic action (1). We recently reported that CPZ causes hyperglycemia and an increase in plasma cyclic AMP level in mice through stimulation of the sympathetic nervous system (2–4). CPZ-induced hyperglycemia and increases in plasma cyclic AMP level were completely abolished by the treatment of mice with adrenalectomy or hexamethonium bromide, a ganglion blocker (2–4). This indicates that there is no possibility that CPZ can raise the plasma glucose and cyclic AMP level by decreasing the phosphodiesterase activity in the peripheral tissues or blood due to calmodulin antagonistic action.

However, it was recently reported that trifluoperazine, a representative calmodulin antagonist, blocked glucose-stimulated insulin release in experiments in vitro using isolated pancreatic islets (5–7). Thus, we studied the effect of CPZ on insulin release in the experiments both in vivo and in vitro.

In the in vivo experiments, adult male mice of ddY strain, weighing 22 to 28 g, were kept at room temperature (22–23°C). To minimize the influence of hepatic glycogenolysis on plasma glucose levels, the mice were fasted for 18 hr. The mice were treated with CPZ (10 mg/kg, i.p.) 5 min before the administration of glucose (1.0 g/kg, i.p.). After the time indicated, the animals were decapitated and the blood was collected immediately. The plasma insulin level was determined by using a double-antibody radioimmunoassay kit from the Dainabot RI Institute Co., Tokyo, Japan. The plasma glucose was estimated by means of the glucose oxidase procedure using a kit from the Boehringer Mannheim Co., West Germany. In the in vitro experiments, islets were isolated by the method of Lacy and Kostianovsky (8). In brief, the pancreases of 10–12 fed mice were minced with scissors in Hanks’ solution, and then collagenase (6–8 mg per milliliter of remaining tissue) was added. The tube was shaken by hand at 37°C for 4–5 min. After the incubation, the digested tissue was washed well; and the islets were picked up with a pipette under a stereomicroscope (x12). Five islets were preincubated for 50 min at 37°C in 500 $\mu$l of Krebs-Ringer bicarbonate medium supplemented with 0.2% albumin and 3.3 mM glucose under 95% $O_2$ and 5% $CO_2$. The islets were incubated for 60 min in a mixture containing 400 $\mu$l of fresh medium and 100 $\mu$l of test agents dissolved in ascorbic acid solution (0.05%). Incubation was done in darkness. At the end of the incubation phase, 10 $\mu$l of the incubation medium were taken and assayed for insulin with use of a radioimmunoassay kit. Chlorpromazine hydrochloride was obtained from the Shionogi Pharmaceutical Co., Osaka, Japan; L(−)-epinephrine bitartrate and collagenase (Type V) were purchased from the Sigma Chemical
Glucose loading (1.0 g/kg, i.p.) caused a sharp and transient increase in plasma insulin level, with the peak observed 5 min later (Fig. 1). Pretreatment of mice with CPZ (10 mg/kg, i.p.) potentiated the glucose-loading-induced increase in plasma insulin level (Fig. 1). In this time, glucose-loading-induced hyperglycemia was markedly enhanced by CPZ treatment. In addition, we observed similar results by using non-fasted mice without glucose loading (data not shown).

In the in vitro experiment, CPZ (10–100 μM) inhibited the glucose (16.7 mM)-induced release of insulin dose-dependently (Fig. 2-a). Epinephrine (1 μM) inhibited the glucose (16.7 mM)-induced insulin release completely. In this experiment, high doses of CPZ (30–100 μM) partially restored the glucose-induced insulin secretion which was inhibited by epinephrine, although this effect was not statistically significant (Fig. 2-b).

Since the in vitro inhibitory effect of CPZ on glucose (Fig. 2-a) and glibenclamide (data not shown)-induced insulin release was similar to that of trifluoperazine (5–7), a drug which was used to show the implication of calmodulin in insulin release, we think that CPZ inhibited insulin release in the present study (Fig. 2-a) through inhibition of the calmodulin function. It is known that insulin secretion is regulated in both a stimulatory manner and an inhibitory manner by β- and α2-adrenoceptors, respectively (9–13). Thus, α2-selective antagonists overwhelmed epinephrine (1 μM)-caused inhibition of insulin release more effectively than did α1-selective antagonists. CPZ is known to be an α-adrenergic antagonist and is a non-selective α1-antagonist (14). In the present experiments, CPZ (30–100 μM) only partially restored the glucose-induced insulin secretion which was inhibited by epinephrine (Fig. 2-b). Therefore, these results indicate
that the anti-calmodulin effect of CPZ is unable to effectively suppress the regulatory effect of β-adrenoceptors on the insulin release.

As shown in Fig. 1, CPZ did not inhibit but rather enhanced the glucose-loading-induced increase in plasma insulin level. This in vivo result cannot be explained only by the result of the in vitro experiment (Fig. 2-a). In the in vivo experiment, CPZ enhanced the glucose-loading-induced increase in plasma glucose level (Fig. 1). CPZ stimulates the sympathetic nervous system, and it caused the release of catecholamine from the adrenal medulla (2). In the presence of catecholamines, CPZ may tend to stimulate insulin release, perhaps through a weak blockade of α₂-adrenoceptors by CPZ following stimulation of β-adrenoceptors. Released catecholamines may also promote hyperglycemia through an inhibition of peripheral glucose uptake by the stimulation of α₂-adrenoceptors (15). CPZ may enhance the inhibition of peripheral glucose uptake by a blockade of α-adrenoceptors (16). Thus, in the in vivo experiment, the CPZ-induced hyperglycemia might effectively overcome the inhibitory effect of CPZ on the insulin secretion and increase the plasma insulin levels. In addition, we must note that the inhibition of insulin secretion in vitro required a relatively high concentration of CPZ in comparison with the dose used in the in vivo experiment (17). Effects of CPZ on the dynamics of other hormones such as glucagon or somatostatin may be related to the discrepancy of insulin secretion observed in vivo and in vitro.

Ammon et al. (18) have shown that CPZ inhibits the glucose-loading-induced increase in plasma insulin level in rats. They have also shown that CPZ inhibits glucose-induced insulin release from isolated islets in vitro. Some of their results in rats are the opposite of the data we obtained from mice in the present study. We cannot explain the reason for this. Differences in species or experimental conditions may possibly be involved.

REFERENCES


