EFFECT OF DILTIAZEM ON INSULIN SECRETION
II. EXPERIMENTS ON PERFUSED RAT PANCREAS,
ANESTHETIZED DOGS AND CONSCIOUS RATS

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Abstract—Effect of diltiazem on insulin secretion was investigated in the perfused rat pancreas. Experiments were also carried out in anesthetized dogs and conscious rats with and without glucose loading. In the perfused rat pancreas, diltiazem reduced both glucose- and tolbutamide-induced insulin secretion and these effects of diltiazem were reversed with removal of the compound. Inhibition of the glucose-induced insulin secretion caused by diltiazem was counteracted by increasing the concentration of calcium ion. In experiments on intact animals, diltiazem at vasoactive doses produced no significant influence on the basal level of plasma insulin or glucose-induced insulin secretion. These data taken together with findings in previously reported work suggest that diltiazem reduces insulin secretion from pancreatic B-cells in vitro possibly by the calcium-antagonistic property, while the compound exhibits practically no inhibitory action on the insulin secretion in vivo.

In a preceding paper (1), it was shown that in isolated islets of Langerhans from rats, diltiazem produced a concentration-dependent inhibition of the release of insulin following stimulation with glucose. This inhibitory effect of diltiazem was antagonized with increasing concentrations of extracellular calcium ion. We suggested that diltiazem may suppress the glucose-induced insulin secretion by reducing free calcium ion concentration in B-cells. In the present study, the time course for change in insulin secretion from rat pancreas was followed using a perfusion method and the dynamic aspects of the inhibitory effect by diltiazem were studied. In vivo effects of diltiazem on the insulin secretion were also studied in dogs and rats with and without glucose loading.

MATERIALS AND METHODS

1. Perfusion of rat pancreas: The perfused rat pancreas was prepared according to the method described by Sussman et al. (2) with slight modification. Male Sprague-Dawley rats (about 450 g) were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and the abdominal cavity was opened. All abdominal vessels except for the superior mesenteric artery and celiac axis were ligated and cut. A portion of the duodenum tightly adhering to the pancreas was left intact. The thoracic aorta was cannulated to a point just superior of the celiac axis and the perfusate was allowed to flow through the pancreas and a portion of the small intestine. The effluent was continuously collected through a cannulated portal vein at one to two min intervals. At the end of the experiments, the perfusion circuit was confirmed to be complete by perfusion with Evans blue. The perfusate was Krebs-bicarbonate
buffer solution, aerated with 95% O₂ and 5% CO₂, and the flow rate was maintained at 1.6–2.3 ml/min by means of a perfusion pump. Experiments were carried out at 37°C. The composition of Krebs-bicarbonate solution was as follows (in mM): NaCl 119, KCl 4.7, CaCl₂ 1.25, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25.0, and glucose 1.67. Dextran-T70 and bovine serum albumin were added to Krebs-bicarbonate solution, the final concentration being 3% and 1% respectively. The concentration of CaCl₂ or glucose in Krebs-bicarbonate solution was varied according to the experimental conditions.

2. Experiments in dogs: Dogs weighing 12 to 15 kg were anesthetized with sodium pentobarbital (35 mg/kg i.v.) and the level of anesthesia was maintained constant by an intravenous infusion of pentobarbital at a rate of 4 mg/kg/hr. The trachea was cannulated and the animal was ventilated by an artificial respirator with room air. Blood pressure was recorded from a cannulated femoral artery through a pressure transducer. Blood for the analysis of blood sugar and plasma insulin was withdrawn at a given interval from the brachial artery.

Glucose was given into the femoral vein and experiments were carried out under two different conditions. 1). An initial load of 4 g of glucose and a continuous infusion of glucose at a rate of 440 mg/min, in which a single or continuous injection of diltiazem was given into the femoral vein during administration of glucose. Effect of somatostatin was also examined. 2). Loading of glucose (10 g/15 kg body weight) was carried out by means of intravenous infusion of 20% glucose solution at a rate of 20 ml/min. Glucose was administered three times at 140–170 min intervals and the second load served for the experiments with diltiazem. Twenty min before the second administration of glucose, diltiazem was continuously infused into the femoral vein at a rate of 20 μg/kg/min.

3. Experiments in rats: Male rats (Sprague-Dawley strain, about 200 g) were fasted overnight and divided into two groups of 9. In one group, 1.5 g/kg of glucose were given s.c. and these animals served as control. The other group was given 50 mg/kg of diltiazem p.o. plus 1.5 g/kg of glucose s.c.. Blood samples were drawn from the caudal vein after the animals had been maintained at 45°C for 3 min.

4. Analysis: Blood sugar was measured by the modified method of Momose et al. (3) and insulin in plasma or in perfusion effluent was determined using an insulin radioimmunoassay kit (INSI K-3, Midori Juji) (4). In this assay, human insulin was used as a standard and the separation of the free from bound insulin was made by dextran-coated charcoal.

5. Drugs: The compounds used were as follows: Tolbutamide (Ono Pharmaceutical Industry Ltd), somatostatin (Institute for Protein Research, Osaka Univ.) and diltiazem hydrochloride (Tanabe Seiyaku). All compounds were dissolved either in saline or in Krebs-bicarbonate solution.

RESULTS

1. Experiments in perfused rat pancreas

In perfused rat pancreas, elevation of glucose from 1.67 to 16.7 mM elicited a biphasic
pattern of insulin secretion. When diltiazem ($10^{-5}$ M) was perfused before and during stimulation with glucose, the first and second phase of insulin secretion were suppressed (Fig. 1). The inhibitory effect of diltiazem was reversible following perfusion with Krebs-bicarbonate solution without diltiazem. Fig. 2 represents effects of diltiazem during con-

**FIG. 1.** Effect of diltiazem on the glucose-induced insulin secretion in perfused rat pancreas. Diltiazem was added to the perfusate before and during glucose stimulation. Each point is the mean ± S.E. from 4 experiments. IRI: immunoreactive insulin.

**FIG. 2.** Effect of diltiazem on the insulin secretion during glucose stimulation. Each point is the mean ± S.E. from 4 experiments.

**FIG. 3.** Antagonism between diltiazem and CaCl$_2$ on the glucose-induced insulin secretion. Each point is the mean ± S.E. of 4 experiments.
Continuous perfusion of 16.7 mM glucose. Diltiazem at the concentration of $10^{-5}$ M reduced the glucose-induced insulin secretion in the second phase. This decrease was counteracted when the concentration of CaCl$_2$ in the perfusate was increased from 1.25 to 6.25 mM in the presence of diltiazem (Fig. 3).

Tolbutamide (500 μg/ml) stimulated the first phase of insulin secretion. As shown in Fig. 4, the tolbutamide-induced insulin secretion was inhibited by diltiazem ($10^{-5}$ M) and this effect was reversed after removal of the diltiazem.

2. Experiments in anesthetized dogs

It has been reported that intravenous administration of 100 μg/kg of diltiazem caused approx. 100% increase in the coronary sinus outflow in the anesthetized dogs, while the systemic blood pressure and heart rate were only slightly decreased (5). As shown in Fig. 5, however, even at a high dose (300 μg/kg, i.v.), diltiazem produced no significant effect on the level of blood sugar and basal release of insulin, while the systemic blood pressure was reduced. Fig. 6 shows experimental results in which glucose was continuously infused into the femoral vein. Administration of glucose evoked a considerable increase in the insulin secretion (approx. 4 to 5 times of the control) and this increase was maintained during the experiments. As illustrated in Fig. 6, the glucose-induced insulin secretion was not affected by intravenous infusion of diltiazem at a rate of 50 μg/kg/min, whereas the blood pressure fell considerably. A single intravenous dose of 300 μg/kg diltiazem also had no influence on the glucose-induced insulin secretion. On the contrary, somatostatin, a positive control drug, reduced the glucose-induced insulin secretion remarkably.

Fig. 7 represents effect of diltiazem on the insulin secretion following stimulation with intravenous loading of glucose
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FIG. 6. Effects of diltiazem and somatostatin on plasma insulin level during glucose infusion in anesthetized dogs. Diltiazem (50 μg/kg/min) and somatostatin (0.8 μg/kg/min) were continuously infused into femoral vein for 40 min. Glucose infusion: 4 g of glucose was initially loaded, followed by the infusion of 440 mg/kg/min intravenously. Each point is the mean ± S.E. of 4 experiments. B.P.: mean blood pressure, H.R.: heart rate, B.S.: blood sugar, IRI: immunoreactive insulin.

FIG. 7. Effect of diltiazem on glucose-induced insulin secretion in anesthetized dogs. Experiments were divided into two series: control series (○—○) and experimental series (●—●) in which effect of diltiazem was examined on the second response of insulin secretion. Detailed explanation, see text. Each point is the mean ± S.E. from 4 experiments.

(10 g/15 kg body weight). Glucose was administered three times at 140-170 min intervals. In control experiments without diltiazem (Fig. 7, dotted line), application of glucose elicited a prompt increase in blood sugar from 100 to 300 mg %, reaching the peak 2.5 to 5 min after glucose loading. Increase in blood sugar continued for 40 to 60 min. As illustrated in Fig. 7, the same response could be evoked after the second and third application of glucose. In parallel to the increase in blood sugar, plasma insulin level was also elevated. In this case, however, the first insulin response to glucose was more re-
markable than the second and third responses: after the first loading of glucose, the plasma insulin level was increased from 15±2 to 90±7 μU/ml, whereas the second and third loads of glucose stimulated the release of insulin from 8±1.5 to 63±6 μU/ml and from 5±1 to 58±4.2 μU/ml, respectively. There was no statistically significant difference between the second and third insulin secretion.

In the next series of experiments, diltiazem was administered intravenously for 40 min at a rate of 20 μg/kg/min before and during the second load of glucose (Fig. 7, solid line). Fig. 7 illustrates that the pattern of changes in the blood sugar and plasma insulin level following stimulation with glucose in experimental series with diltiazem (solid line) were quite similar to those obtained with the control series of experiments (dotted line). The blood pressure was slightly decreased after infusion of diltiazem. Thus, diltiazem had no influence on the insulin secretion stimulated by glucose.

3. Experiments in conscious rats

The effect of diltiazem on the blood sugar and plasma insulin level was examined in conscious rats in the presence and absence of glucose. When 1.5 g/kg of glucose was given s.c., the blood sugar increased from 83±5 to 153±7 mg % 30 min after the injection (Fig. 8). The increase in blood sugar was followed by a gradual fall to a level which was 121±7 mg % at 120 min after loading glucose. The plasma insulin level also increased and reached a peak value of 24±4.2 μU/ml (basal value, 11±0.9 μU/ml) 30 min after glucose given s.c.. Increase in the glucose-induced insulin secretion was of short duration terminating at 90 min after administration of glucose. Under these conditions, diltiazem (50 mg/kg, p.o.) with glucose (1.5 g/kg, s.c.) had no significant effect on the glucose-induced insulin secretion. On the other hand, in rats without glucose loading, oral administration of 50 mg/kg of diltiazem had no effect on the basal blood sugar and plasma insulin levels.

FIG. 8. Effect of diltiazem on glucose-induced insulin secretion in conscious rats. Glucose was loaded at 1.5 g/kg s.c. and at the same time, 50 mg/kg of diltiazem was given p.o.. Each point is the mean ± S.E. of 10 experiments. B.S.: blood sugar, IRI: immunoreactive insulin.

DISCUSSION

Diltiazem is a 1,5-benzothiazepine derivative with a notable coronary vasodilating activity (5, 6). In isolated myocardium (7, 8) and vascular (9–12) or intestinal (13–16) smooth muscles, it has been reported that this compound antagonizes calcium ion which is essential for muscle contraction, thus producing a decrease in the contractile force. Such a calcium antagonistic property of diltiazem has been shown in isolated pancreatic B-cell (1): the glucose-induced insulin secretion from the isolated islets of Langerhans from rats was suppressed by diltiazem and this effect was counteracted by increasing extracellular concentration of calcium ion.
As demonstrated in the present experiments, glucose produced a biphasic pattern of insulin secretion in the perfused rat pancreas (17, 18) and diltiazem suppressed both the first and second phase. The first phase of tolbutamide-induced insulin secretion was also inhibited by diltiazem. The inhibitory effect of diltiazem on the glucose-induced insulin secretion was reversed by the increase in calcium ion concentration, as examined in the second phase.

In perifused islets of Langerhans from rats, Lacy et al. suggested that the first phase of insulin secretion following stimulation with glucose may be due to the release of beta granules already associated with the microtubular system and the second phase of secretion could be the result of stored or newly synthesized granules becoming associated with the system (19). In any case, activation of the microtubular-microfilament system in the B-cell is assumed to be due to calcium ion which flows into the cell following stimulation with glucose (20). It has also been suggested that the tolbutamide-induced insulin secretion is dependent on the extracellular concentration of calcium ion (21). Therefore, the present perfusion experiments support the assumption (1) that diltiazem interferes with calcium ion in the stimulus-secretion coupling of insulin secretion in the B-cell.

On the other hand, in experiments with anesthetized dogs and conscious rats, diltiazem produced no influence on either the basal plasma insulin level or the glucose-induced insulin secretion. Under the present experimental conditions, however, somatostatin remarkably reduced the glucose-induced insulin secretion in anesthetized dogs. Somatostatin has been shown to inhibit insulin secretion both in vivo and in vitro (22-25). Doses of diltiazem used in the present experiments were sufficient to produce hemodynamic effects. For example, a single injection of diltiazem at the dose of 300 µg/kg i.v. (Fig. 5) or continuous infusion at a rate of 50 µg/kg/min (Fig. 6) decreased the blood pressure by approx. 70 or 50 mmHg respectively. Oral administration of 50 mg/kg to conscious rats produced a 20 to 30 mmHg decrease in blood pressure (Yamaguchi et al. unpublished).

With reference to the present experiments in vivo, it has been reported in chronic experiments (6 months) that oral administration of diltiazem had no influence on the level of blood sugar in dogs (20 mg/kg) or on urinary glucose excretion in rats (125 mg/kg) (26). Thus, it is concluded that although diltiazem antagonizes calcium ion in the insulin secretion in vitro, the compound exerts practically no influence on the basal release of insulin or glucose-induced insulin secretion in vivo.

It has been reported that diltiazem produced potent relaxing effects on the constrictor responses of various isolated vascular strips to spasmogens (9-12). The concentration of diltiazem which inhibited the contraction of these vascular preparations by 50% was less than 10⁻⁶ M. On the other hand, a dose of 10⁻⁵ M of diltiazem was required to induce a similar degree of inhibition in glucose-induced insulin secretion, as shown in the preceding in vitro (1) and present perfusion experiments. These results suggest that the insulin secretory system of the pancreas is less sensitive to diltiazem than is the vascular system. Therefore, the fact that diltiazem produces vascular effects without affecting insulin secretion, as illustrated in the present in vivo experiments, may be ascribed to the organ selectivity of this
compound.

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