INHIBITION OF GLUCAGON AND INSULIN RELEASE FROM THE ISOLATED PERFUSED RAT PANCREAS BY CALMODULIN INHIBITORS

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ABSTRACT

Effects of *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) and trifluoperazine, specific calmodulin inhibitors, on the glucose-induced insulin release and arginine-induced glucagon release from the isolated perfused rat pancreas were studied. These two drugs at 50 μ M significantly suppressed the insulin release in both the first and second phases. The extent of suppression was less with W-7 than with trifluoperazine at equivalent concentrations. Trifluoperazine at 50 μ M reduced the efflux rate to 25% of the initial level, but the available data indicate that the reduction in the insulin release by trifluoperazine is attributable to a direct action of this agent on B cells. Suppression of insulin release by W-7 was initiated within 1 min after introducing the agent into the perfusion medium; withdrawal of W-7 caused an immediate over-secretion of insulin in response to glucose. Arginine-induced glucagon release from the perfused pancreas was also inhibited by trifluoperazine at 25 μ M and 50 μ M; with W-7, however, glucagon release was inhibited only at a higher concentration (100 μ M). These results suggest that calmodulin is involved in the process of insulin release as well as glucagon release from the pancreas.

KEY WORDS calmodulin / calmodulin inhibitor / W-7 / trifluoperazine / insulin release / glucagon release

Ca²⁺ has been implicated as a fundamental regulator in a variety of cellular function including secretion, and the possibility that Ca²⁺ may act through its binding to the specific protein was suggested by Meyer *et al.* (12) in 1964.

Following the discovery of calmodulin by Kakiuchi *et al.* (6) and Cheung (1) as a heat-stable protein activator of brain cyclic nucleotide phosphodiesterase, a wide distribution of this Ca-binding protein has been demonstrated throughout the animal and plant kingdom including rat islets of Langerhans (17, 18).

In isolated islets of rats, calmodulin has been reported to stimulate the activity of phosphodiesterase (17), adenylate cyclase (16, 18) and

Ca²⁺-ATPase (14). Experiments on isolated islets of rat using a specific calmodulin inhibitor such as trifluoperazine (8, 15, 17) suggested a possible important role of calmodulin in Ca²⁺-dependent insulin release. Recently, Hidaka *et al.* (4, 5, 7) described a new calmodulin inhibitor, *N*-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7), which is chemically different from phenothiazine derivatives.

Based on these lines of information we undertook to examine the role of calmodulin in endocrine pancreas, taking advantage of these calmodulin inhibitors. W-7 and trifluoperazine proved to inhibit not only the insulin release but also glucagon release from the isolated perfused pancreas of rats.

MATERIALS AND METHODS

The pancreas with the proximal portion of the duodenum was isolated from the Wistar male rat weighing 250–350 g after an overnight fast, by the method of Grodsky *et al.* (3), with modifications. The basal perfusion medium was Krebs-Ringer bicarbonate buffer (pH 7.4) containing 4.5% dextran T-70, 0.5% bovine serum albumin and 2.5 mM glucose, and continuously gassed with 95% O₂-5% CO₂. The perfusion medium was introduced into the celiac artery at a flow rate of 2.5–3.5 ml/min during the equilibration period of 30 min. Secretagogue used was 10 mM D-glucose for insulin, and 15 mM L-arginine for glucagon. Calmodulin inhibitor was introduced 5 min before the secretagogue. The effluent was separately collected every minute from the portal vein after a single passage through the pancreas. Insulin and glucagon in the perfusate were assayed by radioimmunoassay.

N-(6-Aminoethyl)-5-chloro-1-naphthalenesulfonamide (W-7) was a generous gift from Drs H. Hidaka and N. Aoki. Trifluoperazine dimaleate was obtained from Yoshitomi Pharmaceutical Co.

RESULTS

Effects of W-7 and trifluoperazine on the insulin release induced by 10 mM glucose are shown in Fig. 1, A and B. Both W-7 and trifluoperazine did not affect the basal release of insulin from the pancreas. W-7 at 25 μ M had no apparent effect on glucose-induced insulin release, but trifluoperazine at the same concentration markedly inhibited the release. W-7 at 50 μ M significantly inhibited the glucose-induced insulin release, but the extent of inhibition was less than with 50 μ M trifluoperazine.

When the experiment was done under a constant perfusion pressure, trifluoperazine caused a marked reduction in the efflux rate, and the rate was not restored even when the pressure was raised. This effect of trifluoperazine was dose-dependent.

The inhibitory effects of W-7 and trifluoperazine on the first and second phases of glucose-induced insulin release from the pancreas are summarized in Fig. 2, A and B. In the presence of 25 μ M trifluoperazine, the amount of insulin released in response to glucose was reduced by 75% in the first phase (0–4 min), by 85% in the second phase (5–20 min), and by 80% during the total period of the drug admin-

Fig. 1 Effects of W-7 and trifluoperazine on the insulin release from the isolated perfused rat pancreas. Insulin release was induced by 10 mM D-glucose. A: W-7; and B: trifluoperazine. ●—●, 25 μ M; and □—□, 50 μ M. Values are means $\pm$ SD (vertical bars). Values (means $\pm$ SD) in control experiments are shown by the shaded area.

Fig. 2 Inhibition of glucose-induced insulin release by W-7 (A) and trifluoperazine (B). Total amounts of insulin that were released during the total period (0–20 min), the first phase (0–4 min) and the second phase (5–20 min) are separately shown. Statistical significance of differences between various conditions indicated were analyzed by the Student *t*-test. * $P < 0.05$; and ** $P < 0.01$

Fig. 3 Time course of insulin release affected by W-7. The pancreas was perfused with 10 mM D-glucose for the initial 15 min, with glucose and 50 μ M W-7 for the following 15 min, and again only with glucose thereafter. Values are means $\pm$ SD (vertical bars).

istration (0–20 min). W-7 and trifluoperazine at 50 μ M inhibited the total release of insulin by 65% and 90%, respectively.

Fig. 3 shows the time course of inhibition of insulin release caused by 50 μ M W-7. The pancreas was perfused with 10 mM glucose for 15 min, with glucose plus 50 μ M W-7 for the next 15 min, and again only with glucose for the final 15 min. Insulin release was inhibited by W-7 within 1 min. Within 1 min following the withdrawal of W-7, the amount of insulin

released in the perfusate was abruptly increased to an extraordinarily high level.

The effects of W-7 and trifluoperazine on arginine-induced glucagon release are shown in Fig. 4, A and B. W-7 at 25 μ M and 50 μ M did not inhibit arginine-induced glucagon release, except for a marginal decrease in glucagon release observed 2 min after the introduction of W-7. However, W-7 at a higher concentration (100 μ M) strongly inhibited the glucagon release. Following the withdrawal of

Fig. 4 Effects of W-7 and trifluoperazine on the glucagon release from the isolated perfused rat pancreas. Glucagon release was induced by 15 mM L-arginine. A: W-7; and B: trifluoperazine. ●—●, 25 μ M; □—□, 50 μ M; and ▲—▲, 100 μ M. Values are means $\pm$ SD (vertical bars). Values in control experiments are shown by the shaded area.

arginine, glucagon release from the control pancreas was rapidly reduced to the basal level; in contrast, glucagon release from the pancreas that had been perfused with 100 μ M W-7 remained virtually unchanged during 10 min following the withdrawal of both arginine and W-7.

Trifluoperazine at the concentration of 25 μ M and 50 μ M markedly inhibited the glucagon release induced by arginine with dose-dependency. Trifluoperazine added to the perfusion medium together with 15 mM arginine reduced the flow rate (data not shown), similarly as trifluoperazine administered together with glucose (Fig. 1B).

DISCUSSION

Evidence has accumulated that calmodulin plays an important role in various tissues, and the presence of calmodulin has been demonstrated in rat islets of Langerhans (17, 18). Calmodulin activates many enzymes in normal pancreatic islets (14, 16, 17, 18) and stimulates protein phosphorylation in hamster insulinoma cells (8). Insulin release induced by glucose (8, 15), glyceraldehyde (8), potassium (15) and ouabain (15) in isolated islets is inhibited by trifluoperazine. These observations strongly suggest the possibility that Ca^{2+} -dependent insulin release is

regulated by a specific Ca^{2+} -binding protein, i. e. calmodulin.

We have undertaken to examine this possibility using isolated perfused rat pancreas, which is expected to respond to drugs in a more physiological fashion than the isolated islets. We have compared the effects of trifluoperazine and W-7, a newly synthesized calmodulin inhibitor, on the endocrine pancreas.

Our results clearly demonstrate that W-7 and trifluoperazine inhibit glucose-induced insulin release in both the first and second phases of secretion in isolated perfused rat pancreas, and that W-7 is less potent than trifluoperazine in inhibiting the release of insulin and glucagon. These results lend a strong support to the assumption that Ca^{2+} stimulates the insulin release by its interaction with calmodulin, which somehow regulates the secretory processes in the pancreas.

Phenothiazine derivatives have been reported to act with a high affinity on the cell membrane and cytoskeleton, and cause striking morphological changes in cultured cells (13). In contrast to W-7, trifluoperazine reduced the flow rate in a dose-dependent manner, which may possibly be caused by the action of this agent on smooth muscles of the blood vessel. Trifluoperazine at $50 \mu\text{M}$ reduced the flow rate to 25% of the initial level; but it reduced the insulin release more profoundly, i. e. to less than 10% of the control level (Fig. 1B). This implies that the reduction in the insulin release by trifluoperazine is attributable to a direct action of this agent on B cells, though the influence arising from a lowered flow rate should also be taken into consideration.

The glucose-induced insulin release was suppressed very rapidly by introducing W-7 in the perfusion medium; withdrawal of W-7 caused an immediate over-secretion of insulin (Fig. 3), suggesting that the interaction between W-7 and calmodulin is reversible. In contrast, trifluoperazine has been reported to bind to calmodulin either reversibly or irreversibly (19).

There have been conflicting observations with respect to the relation between calcium ions and glucagon release. In one experiment (10), glucagon release was increased when the calcium concentration was raised; in other experiment, however, the release was potentiated by the removal of calcium ions (9). To our knowledge, there has been no study where the relationship between calmodulin and glucagon release was analyzed. In our results, W-7 at a rather high concentration ($100 \mu\text{M}$) significantly decreased

the arginine-induced glucagon release (Fig. 4A). The glucagon release was suppressed also by trifluoperazine at concentrations effective in inhibiting the glucose-induced insulin release (Fig. 4B; cf. Fig. 1B). These observations support the possibility that calmodulin, together with calcium ions, enhances the glucagon release from the pancreas.

In summary, our present observations clearly indicate the involvement of calmodulin in the secretory process of insulin as well as glucagon in the pancreas. A study on the effect of calmodulin inhibitors on somatostatin release from the pancreas is in progress.

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