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Inhibitory Effect of Zinc on Insulin and Glucagon Release from the Isolated Perfused Rat Pancreas

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Abstract Effect of zinc ion on the glucose-induced insulin and arginine-induced glucagon release from the isolated perfused rat pancreas was studied. Zinc inhibited insulin release dose-dependently at the concentration of 100 and 200 μM. This inhibition by zinc was observed only in the second phase of glucose-induced insulin release, but not in the first phase. The inhibition by zinc was partial and reversible. Zinc, at 200 μM, also inhibited arginine-induced glucagon release reversibly. Zinc inhibited the activity of phosphodiesterase from rat brain activated in the presence of calcium ion and calmodulin in a dose-dependent manner similarly to trifluoperazine and N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7), specific calmodulin inhibitors. These results suggest that the inhibition of insulin or glucagon release by zinc ion is at least due to the interaction of zinc with the phosphodiesterase activating system, calcium-calmodulin in the pancreatic islets of Langerhans.

Key Words: Pancreas; insulin, glucagon, zinc, Zinc; calmodulin, calmodulin inbibitor

Introduction

The presence of a relatively large amount of zinc in the islets of Langerhans[1,2] suggests a possibility that this divalent cation might play a role in the function of endocrine pancreas. In fact, the necessity of zinc had been demonstrated for insulin storage of B-cell[3,4], but not for insulin biosynthesis[4]. Recently, Ghafghazi et al. have reported the inhibitory effect of zinc on insulin secretion using rat isolated islets[5] and isolated perfused rat pancreas[6], but the result against this conclusion has also been reported[7]. The mechanism of this inhibitory action of zinc, however, remains to be clarified. On the other hand, calcium ion has been implicated as a fundamental regulator in many cellular functions including secretion[8], and considered to act through its binding to a specific protein, calmodulin, which has been demonstrated its wide distribution throughout the animal and plant kingdoms including rat islets of Langerhans[9,10]. Calmodulin has been reported to stimulate the activity of phosphodiesterase[9], adenylate cyclase[10,11] and Ca^{+}-ATPase in the isolated islets and zinc has been reported to inhibit erythrocyte Ca^{2+}-ATPase or bovine heart phosphodiesterase[14].

Our previous report on the isolated perfused rat pancreas using a specific calmodulin inhibitor such as trifluoperazine (TFP) or N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) had clearly demonstrated the involvement of calmodulin in the secretory process of insulin as well as glucagon[15]. Based on these observation. we examined the effect of zinc not only on insulin release but also glucagon release from the isolated perfused rat pancreas from the viewpoint of its possible interaction to calcium-calmodulin system.

Materials and Methods

1. Reagents

Bovine albumin (fraction V) was purchased from Reheis Chemical Co. (Phoenix, Arizona, USA) and Dextran T-70 from Pharmacia Fine Chemicals (Uppsala, Sweden). Cyclic AMP was purchased from Boehringer Mannheim (West Germany) and 5′-nucleotidase from Sigma Co, (St. Louis, USA). N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) was a generous gift from Dr. Hidaka (Department of Pharmacology, Mie University) and Dr. Aoki (The Institute of Hematology and Department of Medicine, Jichi Medical School). Calmodulin was prepared and purified from bovine brain according to the method of Yazawa et al[16]. and its purity was ascertained with SDS polyacrylamide gel electrophoresis. Cyclic nucleotide phosphodiesterase was prepared from rat brain with the modified procedure of Cheung[17]. Other reagents were of analytical grade.

2. Perfusion of the rat pancreas

Pancreas with the proximal portion of the duodenum was prepared from the male Wistar rats weighing 300-400 g after an overnight fast by the modification of the method of Grodsky[18]. The basal perfusion medium was Krebs-Ringer bicarbonate buffer (pH 7.4) containing 4.5% dextran T-70, 0.5% bovine albumin and 2.5 mM D-glucose, gassed with 95% O_2 and 5% CO_2 during experimental periods and the flow rate was 2.5-3.5 ml per min. In all experiments, the isolated pancreas was perfused with basal medium for 30 min for the purpose of equilibration followed by stimulation of insulin release by 10 mM D-glucose or of glucagon release by 10 mM L-arginine. To investigate the effect of zinc on insulin release, $ZnCl_2$ was added to the perfusion medium 5 min before, concomitant with, or 15 min after the initiation of glucose perfusion. To examine the effect of zinc on glucagon release, $ZnCl_2$ was introduced 10 min after arginine perfusion. The effluent was collected every minutes through experimental periods and the content of insulin or glucagon in the perfusate were determined by the specific radioimmunoassay respectively.

3. Assay of phosphodiesterase activity

Phosphodiesterase activity was assayed using the procedure of Watterson et al.[19]. The reaction mixture (0.5 ml) contained 40 mM Tris HCl buffer (pH 8.0), 10 mM $MnCl_2$, 0.1 mM $CaCl_2$, 2 mM cyclic AMP, 1 μg calmodulin and appropriate amount of the enzyme which made hydrolysis of cyclic AMP by 15% at maximum. Inorganic phosphate, which was liberated from 5′-AMP produced in the assay mixture by incubation with 5′-nucleotidase at 37°C for 30 min, was determined by the method of Goldenberg and Fernandez[20].

Results

The effect of zinc on the insulin secretion when introduced simultaneously with glucose is shown in Fig. 1. $ZnCl_2$, at 200 μM, suppressed significantly the second phase of insulin release from the pancreas to about a half of that of control, but did not inhibit the first phase of insulin release. Introduction of $ZnCl_2$ 5 min before glucose infusion also did not inhibit the first phase of insulin release produced by 10 mM glucose as shown in Fig. 2, indicating that the lack of zinc inhibition of the first phase was not due to the delayed priming of zinc. No effect of zinc on basal insulin release was observed.

When 100 μM or 200 μM $ZnCl_2$ was introduced during glucose infusion, the inhibition of the second phase of insulin secretion by zinc was observed in a few min. Zinc inhibition of insulin release was immediate and temporary at the concentration of 100 μM, but the inhibition was constantly observed during infusion of 200 μM of $ZnCl_2$, suggesting that zinc inhibition of glu-

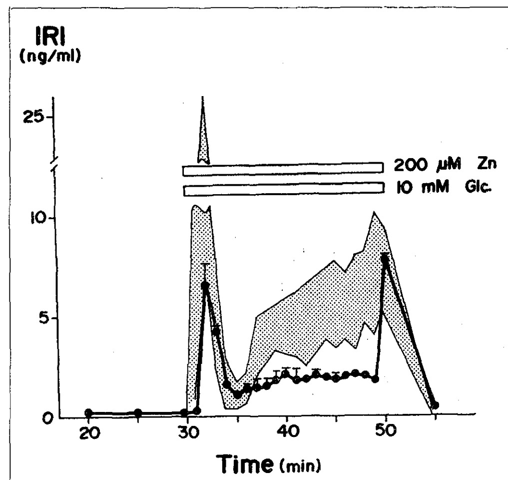

Fig. 1 Effect of 200 μM $ZnCl_2$ on insulin release from the isolated perfused rat pancreas. $ZnCl_2$ was added to the perfusate concomitantly with 10 mM D-glucose. Values express mean ± S.E. (vertical bars) of immunoreactive insulin (IRI) in 4 experiments. Shaded area represents IRI response in 8 control experiments.

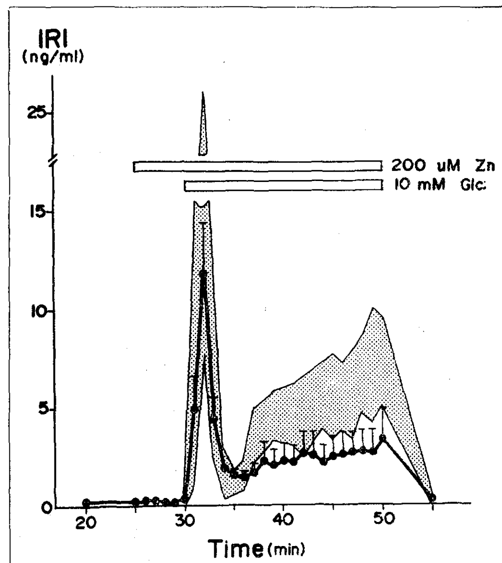

Fig. 2 Effect of 200 μM $ZnCl_2$ on insulin release from the isolated perfused rat pancreas. $ZnCl_2$ was added to the perfusate 5 min before the introduction of 10 mM D-glucose. Values express mean ± S.E. (vertical bars) of immunoreactive insulin in 3 experiments. Shaded area represents IRI response in 8 control experiments.

cose-induced insulin release is dose-dependent. Insulin secretion was restored in a min when $ZnCl_2$ perfusion was discontinued, and the rebound increase of insulin secretion was observed immediately after the termination of zinc infusion (Fig. 3).

The effect of zinc on arginine-induced glucagon secretion is shown in Fig. 4. $ZnCl_2$, at 200 μM, inhibited glucagon secretion when introduced 10 min after the initiation of L-arginine. But this inhibition was transient.

The effects of $ZnCl_2$, TFP and W-7 on phosphodiesterase activity prepared from rat brain are shown in Fig. 5. $ZnCl_2$ inhibited the enzyme activity with 14 μM of a half inhibition concentration in the presence of Ca^{2+} and calmodulin. The half inhibition concentration of TFP or W-7 was 10 μM or 38 μM respectively.

Discussion

There have been conflicting observations with respect to the inhibition of insulin release produced by zinc. Ghafghazi et al. demonstrated first the direct inhibitory effect of zinc on insulin release using the isolated perfused rat pancreas[6] and isolated islets[5]. In their perfusion study, 250 μM of zinc inhibited both the first and second phase of glucose-induced insulin release, though the data were not analyzed statistically[6]. On the other hand, Figlewicz et al. reported that zinc (10–200 μM) did not affect glucose-induced insulin release by isolated islets or perfused pancreas, except for a case of very long incubation (8 h) of isolated iselts[7].

In our results using isolated perfused rat pancreas, zinc suppressed significantly the

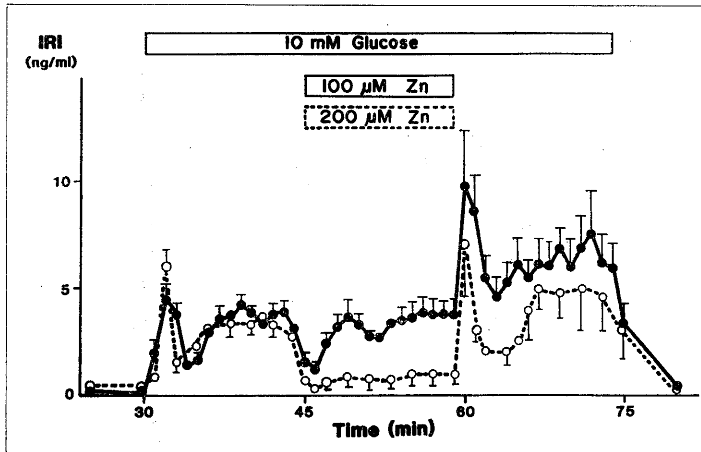

Fig. 3 Effect of 100 or 200 μM $ZnCl_2$ on insulin release from isolated perfused rat pancreas. $ZnCl_2$ was added to the perfusate 15 min after the initiation of 10 mM D-glucose perfusion. ●—● ; 100 μM $ZnCl_2$, and ○----○ ; 200 μM $ZnCl_2$. Values are mean±S.E. (vertical bars) of immunoreactive insulin (IRI) in 4 experiments respectively.

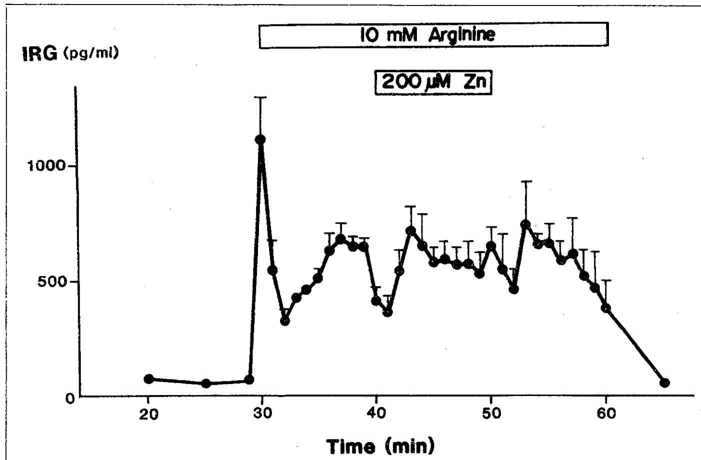

Fig. 4 Effect of 200 μM $ZnCl_2$ on the glucagon release from the isolated perfused rat pancreas. $ZnCl_2$ was added to the perfusate 10 min after the initiation of 10 mM L-arginine perfusion. Values are mean±S.E. (vertical bars) of immunoreactive glucagon in 4 experiments.

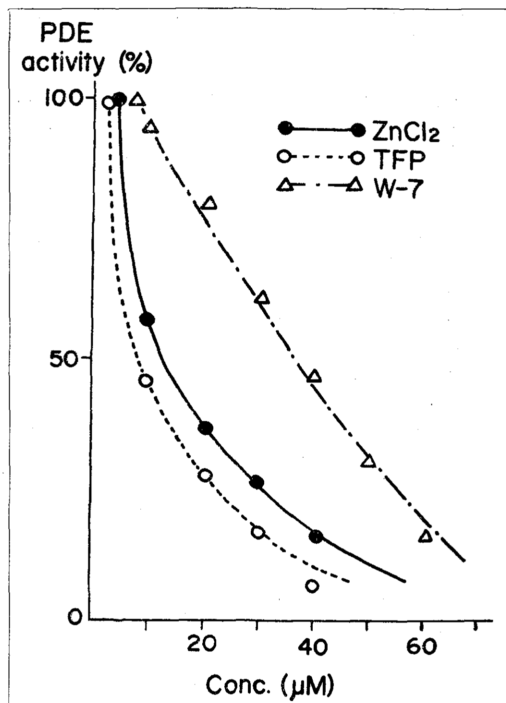

Fig. 5 Inhibition by $ZnCl_2$, TFP or W-7 of phosphodiesterase of rat brain. Measurement of the activity of the enzyme is described in the text. ●—●; $ZnCl_2$, ○---○; TFP, △— — —△; W-7.

second phase of insulin release induced by 10 mM glucose dose-dependently, but it did not affect the first phase of insulin release. No change of basal insulin release and of the first phase of insulin secretion by glucose was observed up to 30 min perfusion of 200 μM of zinc before 10 mM glucose introduction (data not shown). Insulin release was suppressed in a few min when zinc was perfused during the second phase of insulin release induced by glucose. This suppressed insulin release by zinc recovered gradually at the concentration of 100 μM of zinc, and persisted during infusion of zinc at 200 μM. The prompt rebound of insulin secretion was observed immediately after the termination of zinc perfusion in both cases. These phenomena suggest that zinc did not change irreversibly the secretory function of pancreatic B-cells.

Though no report of zinc effect on glucagon release has been published, 200 μM of zinc also inhibited L-arginine induced glucagon release, but this inhibitory effect was rapid and temporary like that seen in the case of zinc at 100 μM inhibition of insulin release.

To our knowledge, there has been no study on the mechanism of action of zinc in the insulin and glucagon release. It has been demonstrated that zinc antagonized calcium mediated functions in various tissues[8], and calcium act through its binding to the specific protein, calmodulin. Zinc inhibited bovine heart phosphodiesterase[14] and erythrocyte Ca^{2+}-ATPase[13], and the activities of these enzymes are regulated by calcium-calmodulin system[21]. Rat pancreatic islets contain calmodulin[9,10], and calmodulin has been reported to stimulate the activities of phosphodiesterase[9], adenylate cyclase[10,11] and Ca^{2+}-ATPase[12] in the islets. The involvement of calmodulin in the process of insulin and glucagon release is supported by the findings that specific calmodulin inhibitors inhibited the release of these hormones as seen in our previous report[15]. Suppression of glucose-induced insulin release by W-7 or TFP, calmodulin inhibitors, was very similar to that of inhibition by zinc.

These observations suggest a possibility that zinc may exert its inhibitory effect through its interaction with calcium-calmodulin function. As shown in Fig. 5, the activation of phosphodiesterase in the presence of calcium and calmodulin was suppressed by adding zinc to the assay mixture with a half inhibition concentration of 14 μM. This inhibitory effect of zinc on phosphodiesterase activity was less than that of TFP and more potent than that of W-7, while TFP inhibition of insulin release was more remarkable than that of W-7[15]. Considering the potentiation of insulin release

by phosphodiesterase inhibitors such as theophylline or IBMX[22], the site of calcium-calmodulin action affected by zinc in the islets may be a process or processes other than phosphodiesterase. It has been demonstrated that calmodulin inhibitor did not inhibit the insulin secretion augumented by agents which cause accumulation of cyclic AMP in cytosol, and calcium-calmodulin stimulated the phosphorylation of the specific protein in the homogenate of hamster insulinoma cells[23]. It is suggested from these observations and my result that zinc inhibition of insulin and glucagon secretion induced by glucose or arginine may be due to the inhibition of the calcium-calmodulin function to some processes other than the first or the last step of the secretory mechanism.

In conclusion, zinc inhibits the second phase of insulin release induced by glucose and also arginine-induced glucagon release, and the results suggest that the inhibitory action of zinc might be mediated by the suppression of calcium-calmodulin function.

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References

1) Figlewicz, D.P., Formby, B., Hodgson, A.T., Schmid, F.G. and Grodsky, G.M.: Kinetics of ^{65}zinc uptake and distribution in fraction from cultured rat islets of Langerhans. *Diabetes*, 29: 767-773, 1980.
2) Havu, N., Lundgren, G. and Falkmer, S.: Zinc and manganese contents of microdissected pancreatic islets of some rodents. *Acta Endocrinol.*, 86 : 570-577, 1977.
3) Maske, H.: Interaction between insulin and zinc in the islets of Langerhans. *Diabetes*, 6 : 335-341, 1957.
4 (Howell, S.L., Tyhurst, M., Duvefelt, H., Anderson, A. and Hellerstrom, C.: Role of zinc and calcium in the formation and storage of insulin in pancreatic β-cell. *Cell Tissue Res.*, 188 : 107-118, 1978.
5) Ghafghazi, T., McDaniel, M.L. and Lacy, P.E.: Zinc-induced inhibition of insulin secretion from isolated rat islets of Langerhans. *Diabetes*, 30 : 341-345, 1981.
6) Ghafghazi, T., Ludvigsen, C.W., McDaniel, M. L. and Lacy, P.E.: The inhibitory effect of zinc on insulin secretion. *IRCS Med. Sci.*, 7 : 122, 1979.
7) Figlewicz, D.P., Heldt, A., Forhan, S.E. and Grodsky, G.M.: Effect of exogenous zinc on insulin secretion in vitro. *Endocrinology*, 108 : 730-732, 1981.
8) Brewer, G.J.: Calmodulin, zinc, and calcium in cellular and membrane regulation; An interpretive review. *Am. J. Hematol.*, 8 : 231-248, 1980.
9) Sugden, M.C., Christie, M.R. and Ashcroft, S. J.H.: Presence and possible role of calcium-dependent regulator (calmodulin) in rat islets of Langerhans. *FEBS Letters*, 105 : 95-100, 1979.
10) Valverde, I., Vandermeers, A., Anjaneyulu, R. and Malaisse, W.J.: Calmodulin activation of adenylate cyclase in pancreatic islets. *Science*, 206 : 225-227, 1979.
11) Sharp, G.W.G., Wiedenkeller, K.E., Kaelin, D., Siegel, E.G. and Wollheim, C.B.: Stimulation of adenylate cyclase by Ca^{2+} and calmodulin in rat islets of Langerhans. *Diabetes*, 29 : 74-77, 1980.
12) Pershadsingh, H.A., McDaniel, M.L., Lacy, P. E. and McDonald, J.M.: Ca^{2+}-activated ATPase and ATP-dependent calmodulin-stimulated Ca^{2+} transport in islets cell plasma membrane. *Nature*, 288 : 492-495, 1980.
13) Brewer, G.J., Aster, J.C., Knutsen, C.A. and Kruckeberg, W.C.: Zinc inhibition of calmodulin; A proposed molecular mechanism of zinc action on cellular functions. *Am. J. Hematol.*, 7 : 53-60, 1979.
14) Donnelly, T.E.: Effect of zinc chloride on the hydrolysis of cyclic GMP and cyclic AMP by activator-dependent cyclic nucleotide phosphodiesterase from bovine heart. *Biochim. Biophys. Acta*, 522 : 151-160, 1978.
15) Yaga, K., Matsutani, A., Inoue, Y., Kobayashi, K. and Kaneko, T.: Inhibition fo glucagon and insulin release from the isolated perfused rat pancreas by calmodulin inhibitors. *Biomed.*

Res., 2 : 418–423, 1981.
16) Yazawa, M., Sakuma, M. and Yagi, K.: calmodulins from muscles of marine invertebrates, scallop and sea anemone. *J. Biochem.*, 87 : 1313–1320, 1980.
17) Cheung, W.Y.: Cyclic 3′, 5′-nucleotide phosphodiseterase; preparation of a partially inactive enzyme and its subsequent stimulation by snake venom. *Biochim. Biophys. Acta*, 191 : 303–315, 1969.
18) Grodsky, G.M., Batts, A.A., Bennet, L.L., Vcella, C., McWilliams, N.B. and Smith, D.F.: Effects of carbohydrates on secretion of insulin from isolated rat pancreas. *Am. J. Physiol.*, 205 : 638–644, 1963.
19) Watterson, D.M., Harrelson, W.J.Jr., Keller, P. M., Sharief, F. and Vanaman, T.C.: Structural similarities between the Ca^{2+}-dependent regulatory proteins of 3′, 5′-cyclic nucleotide phosphodiesterase and actomyosin ATPase. *J. Biol. Chem.*, 251 : 4501–4513, 1976.
20) Goldenberg, H. and Fernandez, A.: Simplified method for the estimation of inorganic phosphorus in body fluid. *Clin. Chem.*, 12 : 871–882, 1966.
21) Klee, C.B., Crouch, T.H. and Richman, P.G.: Calmodulin. *Ann. Rev. Biochem.*, 49 : 489–515, 1980.
22) Hedeskov, C.J.: Mechanism of glucose-induced insulin secretion. *Physiol. Rev.*, 60 : 442–509, 1980.
23) Schubart, U.K., Erlichman, J. and Fleischer, N.: The role of calmodulin in the regulation of protein phosphorylation and insulin release in hamster insulinoma cells. *J. Biol. Chem.*, 255 : 4120–4124, 1980.