Zinc-Induced Inhibition of Insulin Secretion from Isolated Rat Islets of Langerhans

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SUMMARY

The present experiments indicate that $ZnCl_2$ (0.015–0.50 mM) inhibits in a dose-dependent manner insulin secretion from isolated rat islets stimulated by D-glucose, L-leucine, and potassium. This inhibitory effect is partially reversed by washing and antagonized by high calcium concentrations in the medium. Zinc levels that inhibit insulin release do not affect ^{45}calcium uptake, and zinc will not replace calcium in triggering insulin release. The conversion of ^{14}C-D-glucose to $^{14}CO_2$ by islets is not modified by zinc (0.12 mM or 0.50 mM) following either 2- or 0.5-h incubation periods, respectively. It is concluded that the inhibitory effect of zinc on insulin secretion may, in part, be mediated through interference with an intracellular function of calcium by the β-cell. DIABETES *30*:341–345, April 1981.

In 1934, Scott[1] determined that crystalline insulin prepared by various procedures contained zinc and that amorphous insulin would not crystallize without the presence of this element. Later, it was found that pancreatic islets of most vertebrae, including man, contained zinc, as determined by histochemical procedures.[2–4] The presence of zinc in pancreatic islets has focused attention on possible roles that this trace metal may play in the biosynthesis, storage, and release of insulin. It has been shown that zinc is involved in the storage of insulin,[4–6] whereas insulin biosynthesis is apparently unaffected by a deficiency in this metal.[6]

The effect of zinc on carbohydrate homeostasis has been studied employing both in vivo and in vitro conditions. Some investigators have observed reduced glucose clearances in zinc-deficient rats given either i.v. or i.p. glucose administration.[7–10] A reduced concentration of plasma insulin has also been reported in zinc-deficient animals.[10] However, others[11,12] have found no differences in tolerance following i.p. glucose administration between zinc-deficient and control rats fed ad libitum. In vitro studies by Huber and Gershoff[8] indicated that pancreatic slices from zinc-deficient rats release less insulin following glucose stimulation than slices obtained from pair-fed controls. In this study, there was no significant difference reported in the insulin concentration between normal and zinc-deficient pancreatic tissue. We have previously reported that zinc inhibits glucose-induced insulin release from both the isolated, perfused rat pancreas and collagenase-isolated rat islets.[13] The objective of the present study was to examine in more detail the possible inhibitory effects of zinc on insulin secretion from isolated pancreatic islets.

MATERIALS AND METHODS

Medium and chemicals. All incubations were accomplished with a modified Krebs-Ringer bicarbonate medium (KRB) containing 115 mM NaCl, 24 mM $NaHCO_3$, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, and albumin, 0.5% (w/v) bovine plasma albumin (Armour Pharmaceutical Co., Chicago, Illinois). The medium was equilibrated to pH 7.4 with a mixture of O_2 (95%) and CO_2 (5%) and maintained at a temperature of 37°C. Chemical and radioactive isotopes were obtained from the following sources: D-glucose (dextrose, National Bureau of Standards, Washington, D.C.), L-leucine (Sigma Chemical Co., St. Louis), sucrose, KCl, $ZnCl_2$, and toluene (Fisher Scientific Co.), theophylline (K and K Laboratories, Plainview, New Jersey), and ^{3}H-sucrose, D-(U-^{14}C)-glucose, and $^{45}CaCl_2$ (New England Nuclear Corp., Boston, Massachusetts).

In vitro determination of insulin release. Pancreatic islets were isolated by the collagenase technique from adult male Wistar rats (200–300 g) that were allowed food and water ad libitum.[14] Isolated islets were transferred with the aid of a Pasteur pipette and dissection microscope to round-bottom vials (11 mm diameter × 20 mM height) which contained 200 μl of KRB medium. Each vial contained 20–25 islets

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and 12–15 vials were used per experiment. The glass incubation vials were inserted into scintillation vials equipped with rubber stoppers, gassed with O_2/CO_2 (95%/5%), and shaken in a Dubnoff metabolic shaker (70–100 cycles/min). The islets were then preincubated for 30 min in 200 μl of glucose-free medium. The preincubation medium was removed and replaced with 200 μl KRB incubation medium containing glucose (27.5 mM) for 30 min in the presence of different concentrations of $ZnCl_2$ (0.015, 0.030, 0.062, 0.125, 0.25, and 0.50 mM). Control groups were treated identically except in the absence of the test agent. At the end of the stimulation period, the medium was removed and frozen for subsequent insulin assays by the method of Wright et al.[15] using crystalline porcine insulin as standard and ^{125}I-labeled porcine insulin as tracer. Neither zinc nor other experimental test agents studied interfered with the measurement of insulin by the radioimmunoassay.

^{45}Ca uptake. ^{45}Ca uptake by isolated islets was determined with a double isotope procedure described previously.[16] Twenty-five islets were preincubated for 30 min at 37°C in 200 μl of glucose-free medium followed by incubation for 30 min in 200-μl medium containing zinc (0.50 mM), $^{45}CaCl_2$ (2.5 mM, 6 mCi/mmol) and [^3H]-sucrose (5 mM, 8 mCi/mmol) as the extracellular marker, and glucose (27.5 mM). ^{45}Ca uptake by islets in the control group was determined in the absence of zinc.

Glucose oxidation. Batches of 20–25 islets were preincubated in glass incubation vials in glucose-free medium. At the end of the preincubation period, the medium was removed and 200 μl of incubation medium containing zinc (0.125 or 0.50 mM) and uniformly labeled ^{14}C-D-glucose (16.5 mM, 0.6 mCi/mmol) was added. The measurement of $^{14}CO_2$ production was performed over 0.5- and 2-h incubation periods as described previously.[17]

Statistical analysis. The results of insulin release are expressed as μU insulin/islet/min and statistical analysis was determined by the unpaired Student's *t* test unless otherwise stated.

RESULTS

Table 1 summarizes the effect of zinc on glucose-induced insulin release by isolated rat islets. The average rate of insulin secretion observed in the presence of 5.5 mM glucose (i.e., a nonstimulatory level) with 2.5 mM calcium was 0.61 ± 0.03 μU/islet/min. Zinc (0.062 mM) did not alter basal insulin secretion, whereas at higher concentrations (0.125–0.50 mM), significant inhibition was observed. The average rate of insulin secretion following stimulation with glucose (27.5 mM) in the presence of 2.5 mM calcium was 2.7 ± 0.05 μU/islet/min. Zinc at concentrations of 0.015–0.50 mM inhibited insulin release in a dose-dependent manner with 92% inhibition at the highest concentration of zinc.

Insulin release induced by potassium and L-leucine was also significantly inhibited by zinc (0.50 mM). The percent inhibition of insulin secretion was 90 and 86 for potassium (50 mM) and L-leucine (15 mM) induced insulin release, respectively (Table 2).

The reversibility of the inhibition produced by zinc on glucose-induced (27.5 mM) insulin release was evaluated under conditions described in Table 3. The inhibitory effect produced by 0.12 and 0.50 mM zinc during the first stimulation period of glucose-induced insulin release was 48% and 95%, respectively, whereas the inhibition of insulin secretion during the second stimulation period performed in the absence of zinc decreased to 34% and 61%, respectively. Thus, the inhibitory effect by zinc on insulin secretion

TABLE 1
Effect of zinc on glucose-induced insulin release

	Ionic composition of medium (mM)		Insulin release (μU/islet/min)		% Inhibition
	Zinc	Calcium	Control	Experimental	
			Glucose (5.5 mM)		
1	0.062	2.5	0.68 ± 0.05 (8)	0.63 ± 0.08 (8)	8
2	0.125	2.5	0.64 ± 0.04 (18)	0.51 ± 0.04 (12)	20
3	0.250	2.5	0.60 ± 0.05 (13)	0.37 ± 0.05 (13)	38*
4	0.500	2.5	0.53 ± 0.06 (14)	0.11 ± 0.03 (14)	80*
			Glucose (27.5 mM)		
5	0.015	2.5	2.84 ± 0.05 (10)	2.58 ± 0.08 (10)	10
6	0.030	2.5	2.84 ± 0.05 (10)	2.26 ± 0.09 (10)	20*
7	0.062	2.5	2.56 ± 0.14 (9)	1.46 ± 0.10 (10)	33*
8	0.125	2.5	2.75 ± 0.08 (42)	1.41 ± 0.06 (39)	49*
9	0.250	2.5	2.64 ± 0.12 (19)	0.72 ± 0.04 (19)	73*
10	0.500	2.5	2.58 ± 0.06 (42)	0.20 ± 0.02 (42)	92*
11	0.125	5.0	2.75 ± 0.14 (16)	1.98 ± 0.09 (17)	28* 11 versus 8, $P < 0.05$
12	0.125	7.5	3.20 ± 0.29 (16)	2.08 ± 0.12 (14)	35* 12 versus 8, $P < 0.05$
13	0.125	12.5	2.68 ± 0.11 (17)	1.93 ± 0.14 (18)	28* 13 versus 8, $P < 0.05$

After preincubation for 30 min in a glucose-free KRB medium (200 μl), the medium was removed from the islets (20 per incubation vial) and replaced with incubation medium containing glucose (27.5 mM) and the indicated zinc concentration for a stimulation period of 30 min. Values represent the mean ± SE with the number of observations in parentheses.

* Statistical comparison with the corresponding control group showed significant differences ($P < 0.005$). The significance of differences among the groups with increasing calcium concentrations was also compared by factorial analysis of variance ($F = 28.27$) and the Newman-Keuls test for multiple comparisons.

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TABLE 2
Effect of zinc (0.50 mM) on insulin secretion stimulated with potassium or L-leucine

Experimental condition	Insulin (μU/islet/min)		% Inhibition
	Control	Experimental	
Potassium (50 mM)	2.40 ± 0.06 (12)	0.23 ± 0.02	90
L-leucine (15 mM)	2.63 ± 0.10 (12)	0.36 ± 0.04	86

After preincubation for 30 min in KRB medium containing glucose (5.5 mM), the medium was removed from the islets (20 per incubation vial) and replaced with medium containing glucose (5.5 mM) and potassium or L-leucine in the absence (control) and presence of zinc (experimental) for a stimulation period of 30 min.

following these washing procedures was partially reversible.

To test the possibility of an antagonistic action between zinc and calcium, the effect of increasing medium calcium concentrations on zinc inhibition of glucose-induced insulin release was determined (Table 1). In this study, the medium calcium concentration was raised from 2.5 to 5.0, 7.5, and 12.5 mM in the absence and presence of zinc (0.12 mM). Although insulin release in the absence of zinc was not statistically altered by these increasing calcium concentrations, the inhibition produced by zinc (0.12 mM) in the presence of calcium (2.5 mM) was diminished from 49% to 28% in the presence of calcium (5.0 mM). An increase in calcium levels to 7.5 and 12.5 mM at this zinc concentration, however, did not further diminish inhibition of insulin release. These results suggested that the inhibitory effect of zinc may be mediated through an interaction with calcium.

To clarify the interaction between calcium and zinc, the effect of zinc on ^{45}Ca uptake by islets was studied. Islets were preincubated for 30 min in KRB medium, and ^{45}Ca uptake (2.5 mM) was determined after incubation for 30 min with glucose (27.5 mM) in the presence and absence of zinc (0.5 mM). ^{45}Ca uptake in the absence and presence of zinc was 10.6 ± 1.1 versus 11.2 ± 1.0 pmol/islet, respectively, with 15 observations performed at each condition. These results indicate that zinc has no inhibitory effects on total uptake of ^{45}Ca induced by glucose in islets.

To determine whether zinc would substitute for calcium in provoking an insulin secretory response, glucose-induced (27.5 mM) insulin release was measured in the presence of 2.5 mM calcium and compared with that obtained with either 0.5 mM zinc in a calcium-free medium, or 0.5 mM calcium. A reduction in calcium concentration from 2.5 to 0.5 mM resulted in a 56% decrease in insulin secretion. The presence of zinc (0.5 mM) in the medium, however, failed to replace calcium and resulted in a 90% decrease in insulin release by this same comparison (10 observations performed at each condition).

Effect of zinc on ^{14}C-D-glucose metabolism. The effect of different zinc concentrations on islet glucose metabolism was determined by measuring the conversion of uniformly ^{14}C-labeled D-glucose to $^{14}CO_2$. These measurements were made at intervals of either 0.5 or 2 h. The 0.5-h incubation was used to compare more directly any changes in glucose metabolism with both insulin release and ^{45}Ca measurements, as these were performed at this same time interval. Zinc (0.12 mM) did not modify the rate of ^{14}C-D-glucose (16.5 mM) oxidation by isolated islets at 2 h (Table 4). Zinc (0.5 mM) inhibited $^{14}CO_2$ production by 52% when measured at 2 h, but this zinc concentration failed to affect $^{14}CO_2$ production following a 0.5-h incubation.

DISCUSSION

The present experiments were designed to evaluate the effect of zinc on insulin secretion stimulated by different agents, and to study further the mechanism of zinc action on insulin secretion. Zinc inhibited insulin secretion from islets stimulated with glucose, L-leucine, and potassium. Basal insulin secretion was also inhibited by zinc. The inhibitory effect of zinc on glucose-induced insulin release was partially reversed by removing zinc from the incubation medium. This partial reversibility of zinc inhibition of insulin release is important, as it indicates that the insulin secretory mechanism has not been irreversibly altered. In these in vitro studies, zinc inhibited insulin release most significantly at concentrations that are in excess to that present in rat and human serum, i.e., 0.01–0.03 mM.[18] The degree to which these in vitro concentrations of zinc compare with physiologic conditions is difficult to assess, since the endogenous zinc level per islet is estimated to be 11.1 pmol (mean intracellular volume = 3.8 nl/islet) and binding of exogenous zinc to both albumin and insulin present in the incubation media are significant factors in affecting free versus bound forms of this cation.[19,20]

The antagonism of calcium and zinc was evaluated in the present study by increasing the medium calcium concentration from 2.5 to 5.0, 7.5, and 12.5 mM in the presence of zinc (0.12 mM). The higher calcium concentrations partially but significantly antagonized the inhibitory effect of zinc on insulin secretion. This antagonistic action of calcium and zinc has also been reported in in vivo studies. The simultaneous

TABLE 3
Reversal of zinc inhibition of glucose-induced insulin release

Experimental condition	Insulin (μU/islet/min)			
	First stimulation period	% Inhibition	Second stimulation period	% Inhibition
Control	2.56 ± 0.13 (14)		2.96 ± 0.15 (14)	
Zn (0.125 mM)	1.34 ± 0.09 (15)	48	2.26 ± 0.23 (14)	34
Zn (0.500 mM)	0.14 ± 0.03 (15)	95	1.16 ± 0.10 (15)	61

Experimental procedures for the first stimulation period were identical to those in Table 1. For the second stimulation period, the islets were rinsed once with KRB medium (250 μl), and then incubated in 200 μl of KRB medium containing glucose (5.5 mM) for 30 min. This medium was then removed and replaced with incubation medium with 27.5 mM glucose for a second stimulation period of 30 min. Statistical comparison with the corresponding control group showed significant differences (P < 0.025–0.001) in all cases.

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TABLE 4
Effect of zinc on ^{14}C-D-glucose oxidation to $^{14}CO_2$

Treatment, zinc (mM)	pmol D-glucose oxidized per islet			
	30 min		120 min	
	Control	Experimental	Control	Experimental
0.125	—	—	102.64 ± 3.8 (14)	102.26 ± 4.67 (15)
0.500	15.16 ± 1.5 (10)	13.19 ± 0.69 (10)	92.25 ± 6.8 (12)	44.60 ± 2.71 (15)

Isolated islets (20 per vial) were preincubated for 30 min in the KRB medium. The preincubation medium was removed and replaced with medium containing 16.5 mM ^{14}C-D-glucose (0.6 mCi/mmol) in the absence and presence of zinc for a 30- and/or 120-min incubation period. Values represent the mean ± SEM with the number of observations indicated in parentheses.

administration of $CaCl_2$ subcutaneously and zinc sulfate orally in the rat prevented the hyperglycemic action of the latter.[21]

The results observed in the present study with increased calcium concentration are compatible with the hypothesis that zinc may either inhibit calcium entry into the β-cell or alter the intracellular translocation of calcium. The direct determination of the effect of zinc (0.5 mM) on glucose-induced ^{45}Ca uptake indicated that zinc does not directly affect ^{45}Ca uptake under conditions in which insulin secretion is almost totally inhibited.

Metabolic studies indicated that inhibition of insulin release by zinc could be dissociated from any marked inhibitory effects on glucose metabolism. Zinc concentrations of 0.12 and 0.50 mM at 2- and 0.5-h incubation intervals, respectively, significantly inhibited glucose-induced insulin release without altering ^{14}C-D-glucose oxidation to $^{14}CO_2$. Since zinc and calcium are both divalent cations, it was also of interest to test whether zinc could replace calcium and trigger insulin release. When the calcium concentration in the medium was reduced from 2.5 to 0.5 mM, glucose-induced insulin release was diminished. However, zinc (0.5 mM) was unable to substitute for calcium in the secretory mechanism under the conditions of these experiments.

The divalent cations nickel, cobalt, manganese, and magnesium have been reported to exert an inhibitory effect on insulin release induced by glucose and other secretagogues from either islets or the perfused pancreas.[22-25] The inhibition of insulin release by cobalt, manganese, and magnesium has also been reported to decrease with increasing concentrations of calcium in the media.[23,24] In the case of cobalt and nickel, the inhibitory effects on insulin release by this cation showed no parallel alteration on the oxidation of ^{14}C-D-glucose to $^{14}CO_2$.[22,23] These observations are similar to the results obtained with zinc in the present study. The cations cobalt, manganese, and magnesium have been reported to compete with calcium for entry into the β-cell, presumably by blocking the slow calcium channel.[23-25] Manganese apparently also permeates the slow calcium channel and subsequently displaces calcium from intracellular sites.[24] The alterations of calcium influx or entry by cobalt, manganese, and magnesium are in contrast with the present results, as zinc exerted no detectable effect on calcium net uptake in the intact islet. These results may indicate different mechanisms for the membrane transport of zinc and calcium. The net uptake of these cations by islets has been determined and the rates at which cellular equilibration is obtained differ significantly.[16,19,26]

The partial antagonism of zinc-induced inhibition of insulin release by increased calcium concentration and the lack of an inhibitory effect of zinc on glucose metabolism are consistent with the hypothesis that zinc may inhibit insulin release by an intracellular interaction with calcium. Interestingly, zinc inhibition of calmodulin has been proposed as a molecular mechanism to explain both the diverse cellular inhibitory effects of zinc and the antagonism between zinc and calcium, as observed in a variety of cell types.[27] Other alternate or probable mechanisms that cannot be presently excluded for the inhibition of insulin release by zinc from the β-cell include membrane stabilization by interactions of zinc with sulfhydryl-containing membrane proteins[28] and/or disruption of microtubules by zinc with the formation of sheets of protofilaments, as reported with neurotubules by Larsson et al.[29] As to whether inhibition of insulin release by zinc reported in this study reflects a regulatory component for controlling insulin release in vivo requires further evaluation.

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