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Calcium Modulation of Insulin Secretion in Perfused Pancreata of Obese Zucker Rats

Hyun-Ju Choi

Dept. of Human Nutrition and Food Science, Chungnam Sanup University, Hongsung 350-800, Korea

Abstract

Insulin secretory response to various calcium concentrations was investigated in 10- to 12-week old male lean and obese Zucker rats using an *in vitro* pancreatic perfusion procedure. There was no significant difference in insulin secretion response to low, medium, and high calcium concentrations in the lean rat. However, the obese rat shows a characteristics of hypersecretion of insulin. The obese rat pancreas perfused with the low calcium concentration released as low insulin as the lean rat. When perfused with the medium calcium concentration, the obese rat pancreas released twice as much insulin as the lean rat. The hypersecretory phenomenon was also seen in the obese rat pancreas perfused with the high calcium concentration during the first phase of perfusion period, but this phenomenon was gradually diminished during the second phase of perfusion period. These results indicate that there may be a selective insulin secretory response to the extracellular calcium in the obese Zucker rat pancreas.

Key words: calcium, insulin, pancreas perfusion, obese rat

INTRODUCTION

It has been reported that insulin secretion requires external calcium concentration(1-5). There has been several thoughts as to how the calcium influx is regulated. The primary means of calcium entry appears to be *via* voltage-gate calcium channels(6). A calcium channel has been found on the normal mouse pancreatic β-cells(7), and its inactivation is almost purely Ca^{2-} dependent(8). Glucose stimulation of the β-cells leads to an increase of free cytosolic calcium, resulting in an occurrence of the insulin secretion(9). There is an evidence that intracellular organelles may also play a role in controlling cytosolic calcium concentration. The major organelles are the mitochondria(10) and endoplasmic reticulum(1,11). Influx into the endoplasmic reticulum has been shown to be controlled by a Ca^{2+}-ATPase(1), while efflux appears to be controlled by the second messenger inositol 1,4,5-triphosphate(IP_3) system(12,13). It is hypothesized that receptor-generated IP_3 stimulates an efflux of calcium from the endoplasmic reticulum, which in turn leads to an increase in cytosolic calcium triggering the insulin release.

Genetically obese Zucker rats exhibit typically hyperinsulinemia, and one of its causes is a direct pancreatic β-cell hypersecretion. The hypersecretion of insulin in obese Zucker rats was proved in our previous studies of *in vivo* and *vitro* perfused pancreas(14,15). This hypersecretion may be caused by following several factors; hypertrophy(16) and hyperplasia(17) of the pancreas tissue, a decreased hepatic insulin clearance(18), the sensitive response of β-cells to the neurosignal for the insulin secretion(19), and the abnormally balanced autonomic nervous system(14,15,20). It is hypothesized that there may be hyper-responsiveness of the obese rat pancreas to physiological stimuli. The purpose of this study is to examine any discrepancies of the pancreas secretory response to the low, medium, and high calcium levels in the perfusate between the genetically lean and obese Zucker rats.

MATERIALS AND METHODS

In vitro pancreatic perfusion procedure

Genetically lean and obese male Zucker rats(10~12 week old) were used in the present study. They were anesthetized with intraperitoneal injection of sodium pentobarbital of 65mg/kg body weight. The *in vitro* pancreatic perfusion system was performed. In brief, the pancreas and adjacent portion of the duodenum, the spleen and the stomach were excised. And the pancreas was placed on a warmed perfusion platform. The celiac artery and portal vein were cannulated to serve as arterial input and venous output, respectively. Via-

bility of the pancreas tissue was ensured by measuring the anoxic time which ranged from six to nine minutes. Temperature of the pancreas was also maintained at 37°C through a thermostatically controlled heating source.

Perfusate preparation

As for a perfusate preparation, the modified Krebs-Ringer bicarbonate solution with 4% dextran was used. The $CaCl_2$ concentration in the perfusate was varied to produce three different solutions as follows: low calcium solution of 1.625meq/L, medium calcium of 3.25 meq/L, and high calcium solution of 8.125meq/L. The resulting medium contained the following in meq/L: sodium 143, potassium 5.9, phosphate 2.4, sulfate 2.4. chloride 126, bicarbonate 25, and magnesium 1.3. In each perfusion, a constant glucose stimulus of 300mg/dl was maintained for 70min of the entire period. The perfusate was continually oxygenated with 95% O_2 and 5% CO_2, and warmed using a circulating water bath. The perfusate flow rate was maintained at a 5ml/min using a constant flow pump(Masterflex, Cole Parmer Instrument Co., Chicago, U.S.A.). After a ten-minute equilibration period, during which the temperature of the preparation was stabilized at 37°C, the entire venous effluent from the portal vein cannula was collected in a series of graduated cylinder at 1min interval for the first 10min, and 5~10min interval for the next 60min. The effluent samples were frozen at -20°C for later insulin analysis.

Insulin secretory rate

Insulin in venous effluent samples was determined by the radioimmuno-assay. Purified rat insulin was used as the reference standard(21.3U/mg, Novo, Copenhagen, Denmark), and human ^{125}I insulin(Amersham Co., Arlington Heights IL, U.S.A.) was used as a tracer. Insulin secretory rates(ng/min) were calculated by multiplying the insulin concentration(ng/ml) by the perfusate flow rate(ml/min).

All data are expressed as the means±SEM. The ANOVA and Scheffe F-test were used to determine statistical significance between means of groups at $p<0.05$.

RESULTS AND DISCUSSION

Table 1 shows that the body weight of obese rats was greater than that of lean rats. However, in the same phenotype, there was no difference in body weight among the three different calcium groups. There was also no significant difference in the pancreas weight among all groups, regardless of lean and obese rats.

Table 1. Body weight and pancreas weight of the male lean and obese Zucker rats in three groups perfused with differnt calcium(Ca) concentrations

Group(n)	Body weight(g)	Pancreas weight(g)
Lean rats		
Perfused with low Ca(7)	216± 6[a]	0.96±0.07
Perfused with medium Ca(7)	223± 8[a]	1.01±0.03
Perfused with high Ca(8)	216±12[a]	0.83±0.13
Obese rats		
Perfused with low Ca(6)	329±12[b]	0.91±0.05
Perfused with medium Ca(6)	324±12[b]	0.84±0.06
Perfused with high Ca(7)	340±11[b]	0.84±0.07

Values represent mean±SEM

Values with the different superscripts within a column are significantly different($p<0.05$)

Table 2 shows an insulin secretion by pancreas during 0~10min period which is the first phase, and during 11~70min period in the second phase of secretion. When the pancreas was perfused with the solution of low calcium concentration, the amount of insulin released from the obese rat pancreas was comparable to that from the lean rat pancreas during both the first and second phases. The obese/lean hypersecretory factors during the first and second phases were 1.3 and 1.6,

Table 2. Intergration of insulin secretion over time in 10- to 12-week-old male lean and obese Zucker rat pancreata perfused with three different calcium(Ca) concentrations

Group(n)	Insulin secretion(μg/time period)	
	0 to 10min	11 to 70min
Perfused with low Ca		
Lean(7)	2.06±0.50[a]	10.75±1.96[a]
Obese(6)	3.23±0.47[a,d]	14.12±2.56[a]
Obese/Lean[1)]	1.6	1.3
Perfused with medium Ca		
Lean(7)	2.60±0.35[a]	12.76±1.91[a]
Obese(6)	6.50±0.66[b,d]	31.44±4.01[b]
Obese/Lean	2.5	2.5
Perfuse with high Ca		
Lean(8)	2.00±0.23[a]	10.80±1.15[a]
Obese(7)	5.82±1.35[c,d]	22.00±4.51[a,b]
Obese/Lean	2.9	2.0

[1)]Hyperecretory factor: Obese insulin secretion amount divided by lean insulin secretion amount

Values represent mean±SEM. Values with the different superscripts within a column are significantly different($p<0.05$)

respectively. However, when the pancreas was perfused with the solution of medium calcium concentration, obese rats released twice as much insulin as lean rats during both phases of secretion($p<0.05$). This hypersecretory phenomenon was also seen in the obese rat pancreas perfused with the solution of high calcium concentration during the first phase of secretion. And, the obese/lean hypersecretory factor was 2.9. However, the hypersecretory phenomenon with a high calcium solution in the obese was not significant during the second phase of secretion, even though the obese/lean hypersecretory factor was 2.0. In obese rats, the glucose stimulus with less than 300mg/dl concentration causes hypersecretion of insulin(21). However, in the present study, a pancreatic hypersecretory response of the obese rat to glucose of 300mg/dl was abolished with a low calcium perfusion. Although the glucose may be the primary stimulus for the insulin secretion, an appropriate amount of calcium seems to be necessary in the insulin hypersecretion in obese rats. A cell culture study using genetically obese (ob/ob) mice showed that their islets were less dependent on glucose, suggesting a different degree of glucose contribution to insulin secretion(22). It has been reported *that a decreased cytoplasmic free calcium ion* caused a reduction in insulin secretion and this phenomenon occurred through an activation of alpha$_2$-adrenoreceptor of β-cells of obese hyperglycemic mice(23).

Fig. 1 shows a total insulin amount secreted for entire 70min of the perfusion period. In lean rats, there was no difference in total insulin amount among three groups of low, medium, and high calcium concentrations. However, in obese rats, the pancreata perfused with medium calcium concentration released significantly more insulin than those with low or high calcium concentration. Insulin hypersecretion from the obese rat pancreas perfused with a medium concentration of calcium solution in the present study is in agreement with our previous results in which the same calcium concentration was used(14,15,19).

Figs. 2 and 3 show dynamics of the insulin secretion with respect to perfusion time. A typical biphasic insulin secretory pattern of the first and second peaks of insulin secretion can be seen in both figures. The dynamic of

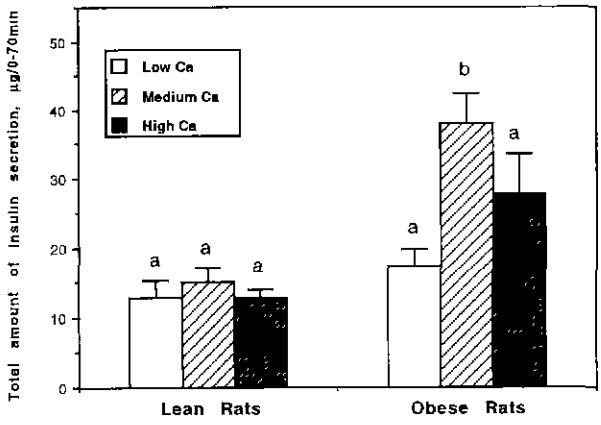

Fig. 1. Total amount of insulin secretion from pancreata perfused with three different calcium(Ca) concentrations in 10- to 12- week-old lean and obese Zucker rats.
Values represent mean±SEM. Values with the different superscripts are significantly different($p<0.05$).

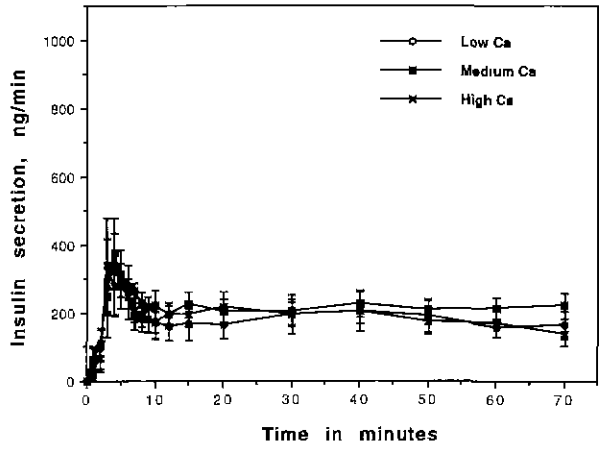

Fig. 2. Insulin secretory profiles of pancreata perfused with three different calcium(Ca) concentrations in 10- to 12- week-old lean Zucker rats.
Values represent mean±SEM.

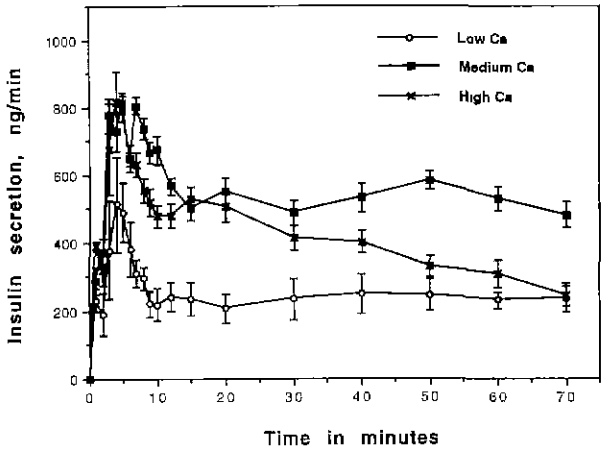

Fig. 3. Insulin secretory profiles of pancreata perfused with three different calcium(Ca) concentrations in 10- to 12- week-old obese Zucker rats.
Values represent mean±SEM.

the lean rat pancreas appears similar among three groups with different calcium concentrations(Fig. 2). In the obese rat pancreas with a low calcium concentration, the insulin secretion is low during 0~10min period, and remained at a low level thereafter(Fig. 3). Interestingly, the obese rat pancreas with a high calcium concentration solution shows a different secretory dynamics from that with either low or medium calcium concentration, in which the insulin secretion was gradually diminished as the perfusion time elapsed.

A general secretory response of the endocrine pancreas to the glucose has two phases: the sharp peak and decline in the first 10min(the first phase), followed by a more steady state or even increasing rate of release for the next 60min(the second phase). In the previous studies, the insulin secretion rate remained nearly constant in lean and obese Zucker rats(14,15,19). Therefore, the difference in secretory dynamics between the lean and obese rats shown in Fig. 3 appears to be striking in the high concentration of calcium perfused condition. In other words, insulin secretion in obese rat pancreas was continuously declined when obese rats were exposed to the high calcium concentration, while it remained at a plateau when exposed to the low calcium concentration. These results indicate that the secretory process in the obese rat pancreas may be influenced by a subtle change in the calcium concentration.

Glucose-induced secretion is dependent on the presence of extracellular calcium ions, which enter into the β-cell mainly through voltage-dependent calcium channel. It has been reported that an impaired calcium metabolism, which is a decreased activity of the Ca-ATPase in obese Zucker rats(24). A calcium entry into β-cells through voltage-dependent calcium ion channel(25) is regulated by a cyclic AMP level. It has been reported that an operation of the voltage-dependent calcium ion channel (26) and a regulation of cAMP accumulation(27) are impaired in the hyperinsulinemic obese(ob/ob) mice. The defective ion channel was also founded in the familial persistent hyperinsulinemic hypoglycemia patient(28). The physiological mechanism of the hypersecretion in the obese rat pancreas perfused with medium and high calcium concentrations could not be elucidated in the present study. A possible explanation is a potential selectivity of calcium channel in the islets of obese pancreas, although further studies are needed in the light of the intracellular events. It has been reported that the pancreatic islet acid amyloglucosidase and acid alpha-glucosidase, which are related to the insulin secretion, were highly dependent upon calcium ion(29). It was also found that the glucose metabolism is enhanced in islets of the hyperinsulinemic obese mice(30). Therefore, other possible explanations are that in islets of the obese rat, a coupling reaction of the glucose and calcium ion may be different in the complex process of glucose-induced insulin release, and the calcium dependency on intracellular enzymes related to secretion may be different from islets of the lean rat.

In summary, this study demonstrates a profound inhibitory effect of either low or high calcium on the insulin secretion in the genetically obese Zucker rats. This inhibitory effect of calcium appears to be different in secretion dynamics between the low and high calcium concentration. A tonic inhibition occurs throughout the entire perfusion period in the obese rat pancreas with the low calcium concentration, while a gradual inhibition occurs as the perfusion time elapsed in the obese rat pancreas with the high calcium concentration.

REFERENCES

1. Prentki, M. and Matschinsky, F. M. : Ca^{+2}, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. *Physiol. Rev.*, **67**, 1185(1987)
2. Wollheim, C. B., Ullrich, S., Meda. P. and Vallar, L. : Regulation of exocytosis in electrically permeabilized insulin-secreting cells. Evidence for Ca^{2+} dependent and independent secretion. *Bioscience Reports*, **7**, 443(1987)
3. Nilsson, T., Arkhammar, P. and Berggren, P. : Dual effect of glucose on cytoplasmic free Ca^{2+} concentration and insulin release reflects the β-cell being deprived of fuel. *Biochemical and Biophysical Research Communications*, **153**, 984(1988)
4. Pralong, W. F., Bartley, C. and Wollheim, C. B. : Single islet β-cell stimulation by nutrients: relationship between pyridine nucleotides, cytosolic Ca^{2+} and secretion. *EMBO J.*, **9**, 53(1990)
5. Yada, T., Kakei, M. and Tanaka, H. . Single pancreatic β-cells from normal rats exhibit an initial decrease and subsequent increase in cytosolic free Ca^{2+} in response to glucose. *Cell Calcium*, **13**, 69(1992)
6. Tabcharani, J. A. and Misler, S. : Ca^{2+}-activated K^+ channel in rat pancreatic islet B cells : permeation, gating and blocked by cations. *Biochimica et Biophysica Acta*, **982**. 62(1989)
7. Roraman, P., Ashcroft, F M. and Trube, G. : Single Ca channel currents in mouse pancreatic β-cells. *Pflugers Arch.*, **412**, 597(1988)
8. Plant, T. D. : Properties and calcium-dependent inactivation of calcium currents in cultured mouse pancreatic β-cells. *J. Physiol.*, **404**, 731(1988)

9. Smith. P. A., Rorsman, P. and Ashcroft, F. M. : Modulation of dihydropyridine-sensitivie Ca^{2+} channels by glucose metabolism in mouse pancreatic β-cells. *Nature*, **342**, 550 (1989)
10. Babcock, D. F., Herrington, J., Goodwin, P. C., Park, Y. B. and Hille, B. : Mitochondrial participation in the intracellular Ca^{2+} network. *J. Cell Biology*, **136**, 833(1997)
11. Sabramanian, K. and Meyer, T. : Calcium-induced restructuring of nuclear envelope and endoplasic reticulum calcium stores. *Cell*, **89**, 964(1997)
12. Prentki. M., Biden, T. J., Janjic, D., Irvine, R. F., Berridge, M. J. and Wolljeim, C. B. : Rapid mobilization of Ca^{2+} from rat insulinoma microsomes by inositol-1,4,5-triphosphate. *Nature*, **309**, 562(1984)
13. Nilsson. T., Arkhammar, P., Hallberg, A., Hellman, B. and Berggren. P. : Characterization of the inositol 1,4,5-triphosphate-induced Ca^{2+} release in pancreatic β-cells. *Biochem. J.*, **248**, 329(1987)
14. Choi-Lee, H. J., Curry, D. L. and Stern, J. S. : Direct effect of CNS on insulin hypersecretion in obese Zucker rats : involvement of vagus nerve. *Am. J. Physiol.*, **256** (Endocrinol Metab. 19), E439(1989)
15. Choi-Lee, H J., Curry, D. L. and Stern, J. S. : Tonic sympathetic nervous system inhibition of insuin is diminished in obese Zucker rats. *Obesity Research*, **1**, 371(1993)
16. Shino, A., Matsuo, T., Iwatsuka, H and Suzuoki, Z. : Structural changes of pancreatic islets in genetically obese rats. *Diabetologia*. **9**, 413(1973)
17. Larsson, L. I., Boder, G. B. and Shaw, W. N. : Changes in the islets of Langerhans in the obese Zucker rat. *Laboratory Investigation*, **36**. 593(1977)
18. Rossel, R., Gomis, R., Casamitjana, R., Segura, R., Vilardell, E. and Rivera, F. : Reduced hepatic insulin extraction in obesity : relationship with plasma insulin levels. *J. Clin. Endocrinol. Metab.*, **56**, 608(1983)
19. Choi-Lee. H. J., Curry, D. L. and Stern, J. S. : Selective muscarinic sensitivity in perfused pancreata of obese Zucker rats. *Intl. J. Obesity*, **17**, 569(1993)
20. Rohner-Jeanrenaud, F., Hochstrasser, A. C. and Jeanrenaud. B. : Hyperinsulinemia of preobese and obese(fa/fa) rats is partly vagus nerve mediated. *Am. J. Physiol.*, **244**(Endocrinol. Metab. 7). E317(1983)
21. Curry. D. L. and Stern, J. S. : Dynamics of insulin hypersecreton by obese Zucker rats. *Metabolism*, **34**, 791(1985)
22. Black, M. A., Heick, H. M. C. and Begin-Heick, N. : Abnormal regulation of insulin secretion in the genetically obese(ob/ob) mouse. *Biochem. J.*, **238**, 863(1986)
23. Nilsson, T , Arkhammar, P., Rorsman, P. and Berggren, P. : Inhibition of glucose-stimulated insulin release by alpha$_2$-adrenoreceptor activation is parallelled by both a repolarization and a reduction in cytoplasmic free Ca^{2+} concentration. *J. Biological Chem.*, **263**, 1855(1988)
24. Zemel, M. B., Sowers, J. R., Shehin, S., Walsh, M. F. and Levy, J. : Impaired calcium metabolism associated with hypertension in Zucker obese rat. *Metabolism*, **39**, 710(1990)
25. Wang, J. L., Corbett, J. A., Marshall, C. A. and McDaniel, M. L. : Glucose-induced insulin secretion from purified β-cells: a role for modulation of Ca^{2+} influx by cAMP and protein kinases C-dependent signal transduction pathways. *J. Biological Chem.*, **268**, 7785(1993)
26. Black. M. A., Fournier, L. A., Heick. H. M. and Begin-Heick, N. : Different insulin-secretory response to calcium-channel blockers in islets of lean and obese(ob/ob) mice. *Bioichem. J.*, **249**, 401(1988)
27. Black, M. A., Fournier, L. A., Heick, H. M. and Begin-Heick, N. : Abnormal regulation of cAMP accumulation in pancreatic islets of obese mice. *Am. J. Physiol.*, **255** (Endocrinol. Metab. 18), E8333(1988)
28. Thomas, P. M., Cote, G. I. and Wohllk, N. : Mutations in the sufonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science*. **268**, 426(1995)
29. Salehi, A. and Lundquist, I. : Ca^{2+} deficiency, selective alpha-glucosidehydrolase inhibition, and insulin secretion. *Am. J. Physiol.*, **265**(Endocrinol. Metab. 28), E1(1993)
30. Khan, A., Chandramouli, V., Otenson, C. G., Berggren. P. O., Low, H., Landau, B. R. and Efendic, S. : Glucose cycling is markedly enhanced in pancreatic islets of obese hyperglycemic mice. *Endocrinology*, **126**, 2413(1990)

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