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GLUCOSE STIMULATES INSULIN RELEASE WITHOUT ALTERING CYCLIC AMP PRODUCTION OR INOSITOLPHOSPHOLIPID TURNOVER IN FRESHLY OBTAINED HUMAN INSULINOMA CELLS

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SUMMARY: Glucose, forskolin, IBMX and carbachol all stimulated insulin release from freshly obtained human insulinoma cells. In these same cells, cellular cyclic AMP levels were raised by forskolin and IBMX but not by glucose and carbachol. On the other hand, of all the insulin secretagogues examined, only carbachol stimulated the formation of ^{3}H-inositol trisphosphate in these cells. Thus, in these insulinoma cells, glucose apparently induces insulin secretion without altering cyclic AMP production or inositolphospholipid turnover.

Glucose is a potent physiological stimulator of insulin secretion from the β cells of the pancreatic islets. Several mechanisms for this glucose-induced insulin release have been postulated. Some investigators have shown an increase in cyclic AMP production on insulin release by glucose(1-3). Other recent reports have demonstrated that glucose stimulates inositolphospholipid turnover in pancreatic islets, suggesting important roles for inositol trisphosphate and protein kinase C in the regulation of insulin secretion by glucose(4-7). However, it is still unclear whether the increase in cyclic AMP production and/or the stimulation of inositolphospholipid turnover represent

Abbreviations: IBMX, isobutylmethyl xanthine; BSA, bovine serum albumin; FCS, fetal calf serum; IP, inositol monophosphate, IP_2, inositol bisphosphate; IP_3, inositol trisphosphate; IP_4, inositol tetraphosphate.

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indispensable mechanisms for glucose-induced insulin secretion from β cells in pancreatic islets.

In this study, by using freshly obtained human insulinoma cells, we report that glucose induced insulin release without affecting cyclic AMP production or inositolphospholipid turnover, suggesting the presence of an alternative mechanism for insulin secretion by glucose in these cells, independent of these changes.

MATERIALS AND METHODS

Isolation and incubation of human insulinoma cells: Tumor tissue (920 mg) obtained from the pancreas of a insulinoma patient was minced immediately and washed twice in ice-cold RPMI-1640 supplemented with 10% FCS(RPMI). The minced tumor was then placed into RPMI(10 ml) containing dispase(800 PU/ml) and was mechanichally shaken for 20 min at 37°C in a water bath. After the digestion, the supernatant, containing mostly single cells, was taken out, and the remaining tumor pieces were redigested for 10 min. The combined supernatant digestants were centrifuged at 1000 rpm for 7 min. The cell pellet was then washed twice with RPMI, and after resuspension in RPMI(20 ml), the dispersed cells were incubated in a flask for 2 hours at 37°C in an atmosphere of 95% O_2-5% CO_2. Following incubation, the cells were washed twice with HEPES-buffered Krebs-Ringer bicarbonate buffer with 1 mg/ml BSA and 2.5 mM glucose pH 7.4(HKRBG). The cells were then resuspended in HKRBG for use in the following experiments. By this procedure, the tumor yielded 55 × 10^6 cells with a viability of more than 90% by trypan blue exclusion.

Release of insulin: An aliquot(1 ml) of cell suspension(0.2 × 10^6 cells/ml) was incubated with various test agents under atmosphere of 95% O_2-5% CO_2 at 37°C in a shaking bath. After 30 min the cells were separated by centrifugation and the medium was stored at -20°C for assay of insulin. The measurement of insulin was performed by polyethyleneglycol radioimmunoassay(RIA) as described in a previous report(8). These insulinoma cells were found to contain 8.42 ng/mg wet weight of insulin. In contrast, neither immunoreactive glucagon nor immunoreactive somatostatin was detected in these cells by their specific RIAs (9).

Production of cyclic AMP: The cells(10^6) were incubated in 0.9 ml of HKRBG for 10 min at 37°C under 95% O_2-5% CO_2 and then various test materials(100 µl) were added. After 15 additional min, the incubations were terminated by adding 1 ml of ice-cold 12% trichloroacetic acid(TCA), centrifuging the mixture at 3000 rpm, and washing the supernatant three times with diethylether. Concentrations of cyclic AMP were determined by RIA using the antiserum provided by K. Martin(10).

Release of ^{3}H-inositolphosphates: Cells(10^6) were prelabeled for 2h at 37°C in 0.4 ml HKRBG containing 25 µCi ^{3}H-inositol. At

110 min of incubation, lithium chloride was added to the mixture to achieve a final concentration of 10 mM. The incubation was then initiated by adding various test substances(100 µl), and after 1 min the incubations were terminated by adding 1.5 ml of chloroform:methanol(1:2). Aqueous products were separated on Dowex-1 resin columns(formate form) into IP, IP_2, IP_3 and IP_4 (11-13). The fraction denoted as containing IP_3 may consist of a mixture of the 1,4,5 and 1,3,4 isomers and the fraction labeled IP_4 may also contain higher phosphorylated forms of inositol.

Chemicals: Forskolin, IBMX, carbachol, BSA and TCA were purchased from Sigma Chemicals. RPMI-1640 was a product of Flow Laboratories and FCS was from Grand Island Biological CO.. $Na^{125}I$ and (2-^{3}H)myo-inositol(15.8 Ci/mmol) were products of Amersham. Dowex-1(100-200 mesh; x 8 in the formate form) was obtained from Bio Rad.

RESULTS

Addition of forskolin(10^{-4} M) or IBMX(10^{-4} M) to the medium containing 2.5 mM glucose induced significant increase in both insulin release from($P < 0.01$ and $P < 0.01$ vs control, respectivly) and cyclic AMP content($P < 0.01$ and $P < 0.01$, respectively) in these insulinoma cells as shown in Table 1. Insulin release was also enhanced by adding carbachol(10^{-4} M)($P < 0.01$) to the medium or by elevating the glucose concentration from 2.5 mM to 16.5 mM($P < 0.01$). None of these secretagogues for insulin, however, stimulated any increase in cyclic AMP levels in the cells. Furthermore, 16.5 mM glucose did not have any additional effect on the IBMX-induced cyclic AMP production, though it markedly potentiated IBMX-induced insulin release(Table 1).

On the other hand, significant increases in the formation of ^{3}H-IP_2 and ^{3}H-IP_3 were observed by 1 min of incubation with carbachol(10^{-4} M)($P < 0.01$ and $P < 0.01$ vs control, respectively) (Table 2). In contrast, forskolin, IBMX or high glucose(16.5 mM) had no effect on the formation of ^{3}H-inositolphosphates.

DISCUSSION

The response of the freshly obtained human insulinoma cells to glucose and other insulin secretagogues made it possible to

Table 1. Effects of various secretagogues on insulin release and cyclic AMP production in human insulinoma cells

Glucose (mM)	Test Agents	Insulin (μU/tube/30 min)	cyclic AMP (pmol/tube/10 min)
2.5	(control)	2.43 ± 0.18	2.58 ± 0.17
2.5	+ carbachol(10^{-4} M)	5.96 ± 0.26*	2.41 ± 0.19
2.5	+ forskolin(10^{-4} M)	6.35 ± 0.66*	10.93 ± 1.23*
2.5	+ IBMX(10^{-4} M)	4.24 ± 0.33*	6.88 ± 0.51*
16.5		4.73 ± 0.21*	2.55 ± 0.17
16.5	+ IBMX(10^{-4} M)	7.23 ± 0.89*	7.00 ± 0.61*

Results are listed as means ± SEM(n=4). Student's t test was used for statistical analysis. *P < 0.01 vs control(2.5 mM glucose alone).

investigate the mechanism of action of glucose on insulin release in these insulinoma cells. The most striking finding from the present experiment was the absence of any increase in cyclic AMP production or formation of ^{3}H-inositolphosphate on significant glucose stimulation of insulin release. Several investigators have suggested an involvement of cyclic AMP in glucose-induced insulin release, based on the fact that in intact islets, there is a modest increase in cyclic AMP production especially in the

Table 2. Effects of various secretagogues on the formation of ^{3}H-inositolphosphates in human insulinoma cells

Glucose (mM)	Test Agents	^{3}H-IP	^{3}H-IP$_2$	^{3}H-IP$_3$	^{3}H-IP$_4$
2.5	(control)	574 ± 23	238 ± 22	140 ± 16	57 ± 11
2.5	+ carbachol(10^{-4} M)	632 ± 36	421 ± 26*	274 ± 14*	68 ± 8
2.5	+ forskolin(10^{-4} M)	528 ± 45	235 ± 22	138 ± 13	60 ± 5
2.5	+ IBMX(10^{-4} M)	565 ± 46	248 ± 31	144 ± 10	52 ± 10
16.5		544 ± 41	240 ± 21	136 ± 8	58 ± 6

Values represent cpm/tube. The means ± SEM(n=4) are shown. Student's t test was used for statistical analysis. *P < 0.01 vs control.

presence of phosphodiesterase inhibitors(1-3). Recent reports, on the other hand, have identified a role for glucose in promoting the breakdown of phosphatidylinositol bisphosphate(14) and in the formation of inositolphosphates(6). This suggests a role for inositolphospholipid turnover in the insulinotropic action of glucose(4-7,14). In our human insulinoma cells, these two cellular signal transduction mechanisms: adenylate cyclase-cyclic AMP-dependent pathway and membrane inositolphospholipid turnover-dependent pathway appeared to be working, because both forskolin and IBMX increased cellular cyclic AMP content, and carbachol stimulated the formation of ^{3}H-IP_3. The absence of an increase in cyclic AMP production or formation of inositolphosphates by glucose in our insulinoma cells does not argue against the important role for cyclic AMP and inositolphospholipid turnover in the normal process of glucose-induced insulin secretion from intact islets. Nevertheless, the present study strongly suggests the presence of an alternative signal transduction mechanism unrelated to cyclic AMP production or to inositolphospholipid turnover mediating the insulinotropic action of glucose in these human insulinoma cells.

The precise mechanism by which glucose induced insulin release from these insulinoma cells is not clear. The major role of the rise in cytosolic free calcium concentration in glucose-induced insulin release has been well established(15,16). Although recent studies suggest that mobilization of intracellular calcium probably due to the activation of inositolphospholipid turnover occurs as an early event(4-7,15,16), it is also evident that calcium influx from the extracellular space through calcium channels plays an important role in insulin release by glucose (15,16). Therefore, the stimulation of calcium influx into the cell through calcium channels might have been mainly responsible

for glucose-induced insulin secretion from our human insulinoma cells though we have not measured the intracellular calcium levels in the present study.

REFERENCES

1. Charles, M.A., Lawecki, J., Pictet, R., and Grodsky, G.M. (1975) J. Biol. Chem. 250, 6134.
2. Valverde, I., Vandermeers, A., Anjaneyulu, R., and Malaisse, W.J. (1979) Science 206, 225.
3. Rabinovitch, A., Grill, V., Renold, A.E., and Cerasi, E. (1976) J. Clin. Invest. 58, 1209.
4. Tanigawa, K., Kuzuya, H., Okamoto, M., and Imura, H. (1983) Biochem. Biophys. Res. Commun. 112, 419.
5. Zawalich, W., Brown, C., and Rasmussen, H. (1983) Biochem. Biophys. Res. Commun. 117, 448.
6. Best, L., and Malaisse, W.J. (1983) Biochem. Biophys. Res. Commun. 116, 9.
7. Biden, T.J., Prentki, M., Irvine, R.F., Berridge, M.J., and Wollheim, C.B. (1984) Biochem. J. 223, 467.
8. Chiba, T., Chihara, K., Minamitani, N., Goto, B., Kadowaki, S., Taminato, T., Matsukura, S., and Fujita, T. (1982) Horm. Metab. Res. 14, 57.
9. Kadowaki, S., Taminato, T., Chiba, T., Mori, K., Abe, H., Goto, Y., Seino, Y., Matsukura, S., Nozawa, M., and Fujita, T. (1979) Diabetes 28. 600.
10. Martin, K.J., Freitag, J.J., Conrades, M.B., Hruska, K.A., Klahr, S., and Slatopolsky, E. (1978) J. Clin. Invest. 68, 256.
11. Berridge, M.J., Dawson, R.M.C., Downes, C.P., Heslop, J.P., and Irvine, R.F. (1983) Biochem. J. 212, 473.
12. Batty, I.R., Nahorski, S.R., and Irvine, R.F. (1985) Biochem. J. 232, 211.
13. Chiba, T., Park, J., Sugano, K., and Yamada, T. (1987) AM. J. Physiol. (in press).
14. Laychock, S.G. (1983) Biochem. J. 216, 101.
15. Prentki, M., and Wollheim, C.B. (1984) Experientia(Basel) 40, 1052.
16. McDaniel, M.L., Colca, J.R., Kotagal, M., and Lacy, P.E. (1985) in The Diabetic Pancreas(Volk, B.W. and Arquilla, E.R., eds) pp213, Plenum Press, New York.