Cell Swelling-Induced Signaling for Insulin Secretion Bypasses Steps Involving G Proteins and PLA₂ and is N-ethylmaleimide Insensitive

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Key Words

Insulin secretion • Glucose • Cell swelling • G proteins
• PLA₂ • SNARE • Intracellular signaling

Abstract

Background: This study was undertaken to examine putative mechanisms of calcium independent signal transduction pathway of cell swelling-induced insulin secretion. **Methods:** The role of phospholipase A₂, G proteins, and soluble N-ethylmaleimide-sensitive-factor attachment protein receptor (SNARE) in insulin secretion induced by 30% hypotonic medium was studied using isolated rat pancreatic islets. **Results:** In contrast to glucose stimulation, osmotically induced insulin secretion from pancreatic islets was not inhibited by 10 µmol/l bromoenol lactone, an iPLA₂ (Ca²⁺ independent phospholipase) inhibitor. Similarly, preincubation of islets for 20 hours with 25 µg/ml mycophenolic acid to inhibit GTP synthesis fully abolished glucose-induced insulin secretion but was without effect on hypotonicity stimulated insulin release. Glucose-induced insulin secretion was prevented by preincubation with 20 nmol/l tetanus toxin (TeTx), a metalloprotease inactivating soluble SNARE. Cell swelling-induced insulin secretion was

inhibited by TeTx in the presence of calcium ions but not in calcium depleted medium. The presence of N-ethylmaleimide (NEM, 5 mmol/l, another inhibitor of SNARE proteins) in the medium resulted in high basal insulin secretion and lacking response to glucose stimulation. In contrast, high basal insulin secretion from NEM treated islets further increased after hypotonic stimulation. **Conclusion:** G proteins and iPLA₂ – putative mediators of Ca²⁺ independent signaling pathway participate in glucose but not in hypotonicity-induced insulin secretion. Hypotonicity-induced insulin secretion is sensitive to clostridial neurotoxin TeTx but is resistant to NEM.

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Introduction

Although first report of hyposmolarity-induced insulin secretion was published more than 30 years ago [1], molecular mechanisms of this phenomenon remain largely unknown [2]. Comparison with glucose-induced insulin secretion revealed the involvement of a separate signal transduction pathway [2, 3] with specific features of osmotically stimulated peptide hormone release [2, 4].

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On the other hand, the mechanism of glucose-induced insulin secretion is much better understood. In the fast first phase, the enhanced glucose metabolism increases cellular ATP/ADP ratio, closes the ATP-sensitive K⁺ channels and depolarizes the cell membrane. Activation of calcium voltage-gated channels increases Ca²⁺ entry promoting insulin release (for review see [3, 5]). In contrast, cell swelling-induced insulin secretion is independent from extra- and intra-cellular Ca²⁺ and resistant to noradrenalin inhibition, thus indicating a novel transduction pathway [2].

To disclose a mechanism of swelling-induced signal transduction, the involvement of Ca²⁺ independent steps should be considered. Insulin secretion is known to be modulated by nutrients, incretins, and growth factors. Some of their modulatory effects are mediated via membrane receptors coupled primarily to either G proteins or tyrosine kinases, which subsequently activate respective second messenger cascades [6, 7]. Glucose induces parallel changes in guanine and adenine nucleotides in a concentration dependent manner correlating with insulin secretion from pancreatic islets [8]. The exocytosis can be induced by intracellular addition of GTP or its poorly hydrolysable analog GTPγS in a calcium independent mechanism in various cells types, including primary β-cell [9-12]. Cho et al. [13] showed participation of GTP in the mechanism of secretory vesicle swelling, which precedes exocytosis, by rapid gating of water by aquaporin 1. G proteins have also been shown to participate in cell volume regulation. They increase an intracellular Ca²⁺ concentration, mitogen-activated protein kinase (MAPK) activity, vesicular acidification, and swelling-induced taurocholate secretion suggesting that G proteins may mediate some effects associated with cell swelling (for review see [14]). Pertussis toxin or cholera toxin known to affect G proteins blunt the regulatory volume decrease (RVD) as well as swelling-induced osmolyte efflux. Altogether, these findings make GTP and G proteins likely candidate as mediators of cell swelling-induced Ca²⁺ independent insulin secretion from pancreatic β-cells.

Another Ca²⁺ independent mechanism leading to exocytosis involves phospholipase A₂ (PLA₂, [15]). Glucose induces hydrolysis of membrane phospholipids in β-cells that is accompanied by accumulation of phospholipids derived mediators [15]. Pancreatic islets contain at least five different types of PLA₂. Type IB is localized on secretory granules of β-cells and has been shown to be co-secreted with insulin after stimulation with specific secretagogues [16].

In glucose-stimulated islets, hydrolysis of arachidonic acid (AA) from membrane phospholipids appears to be mediated at least partially by a PLA₂β that is catalytically active in the absence of Ca²⁺ [17]. Pancreatic islets and insulinoma cell lines express iPLA₂β mRNA, and its enzymatic activity can be inhibited by bromoenol lactone (BEL) [18, 19]. iPLA₂ in rat and human β-cells is implicated as a putative glucose sensor coupling alterations in glycolytic metabolism to the generation of biologically active eicosanoids and thus facilitating glucose-induced insulin secretion [19, 20]. Metabolites of polyunsaturated fatty arachidonic acid can play role in the cell volume regulation [21-23]. The activation of iPLA₂ is essential for regulation of cell volume changes in G proteins mediated process [21].

To analyze the mechanism of cell swelling-induced insulin secretion, we have examined the role of G proteins and iPLA₂ in Ca²⁺ independent signaling pathways involved in exocytosis. In addition, we tested the participation of SNARE proteins in cell swelling-induced exocytosis of insulin using SNARE inhibitors tetanus toxin and N-ethylmaleimide (NEM).

Material and Methods

Male Wistar rats (Charles River, Sulzfeld, Germany) weighing 300-350g were kept under controlled temperature (22-24°C) and constant 12h light/dark cycle, fed with Purina Chow and tap water *ad libitum*. The animal procedures were performed in accordance with national law and were approved by ethical committee.

Chemicals

Mycophenolic acid, adenine, guanine, bromoenol lactone, tetanus toxin, N-ethylmaleimide, collagenase, trypsin inhibitor type I-S were purchased from Sigma, Germany.

Isolation of islets of Langerhans

Islets were isolated from adult male Wistar rats according to the method by Lacy and Kostianovsky [24]. Briefly, under pentobarbital anesthesia (3.7 mg/100g body mass) the common bile duct was cannulated proximally near the hilus of the liver, a tie was placed adjacent to the duodenum, and pancreas was distended by injection of approximately 10 ml of Hank's Balanced Salt Solution (HBSS) with 1% BSA. The pancreas was then dissected out, minced, and digested with 1 mg/ml collagenase with 0.008 g soybean trypsin inhibitor type I-S in 100 ml HBSS for 10 min at 37°C. The tissue was then separated from the remaining acinar tissue under a dissecting microscope and used immediately for the experiment. Islets were cultured overnight in RPMI 1640 medium (containing 10% fetal calf serum, 11.1 mmol/l glucose, 100 units/ml penicillin, and 100 pg/

Fig. 1. Effect of G proteins inhibition on insulin secretion from isolated islets. Fresh isolated islets (5 per tube) were divided into five groups according the composition of incubation medium (20 hours). Control: RPMI medium, Et-OH + A: RPMI + 0.05% ethanol (MPA solvent) + 150 μ mol/l adenine, MPA + A: RPMI + 25 μ g/ml MPA + 150 μ mol/l adenine, Et-OH + G: RPMI + 0.05% ethanol + 100 μ mol/l guanine, MPA + G: RPMI + 25 μ g/ml MPA + 100 μ mol/l guanine. Next day islets were preincubated for 60 min in Krebs-Ringer HEPES buffer (basal) followed by the two subsequent 30 min incubation periods: A: basal - high glucose medium, B: basal - hypotonic medium. Results are shown as ng insulin released by one islet during 30 min incubation, n = 7. * P<0.05, ** P<0.005 compared to previous basal incubation (paired t-test).

ml of streptomycin), except in studies involving freshly isolated islets.

Incubation media

Krebs-Ringer HEPES buffer (KRH - basal) of following composition: 118 mmol/l NaCl, 4 mmol/l KCl, 1.2 mmol/l MgSO_4 , 1.2 mmol/l KH_2PO_4 , 5 mmol/l NaHCO_3 , 25 mmol/l HEPES, 3 mmol/l D-glucose, 2.5 mmol/l CaCl_2 , 0.1% BSA, pH 7.4.

Glucose medium

This medium was prepared by increasing D-glucose concentration to 20 mmol/l. To maintain physiological osmolarity, the NaCl concentration was reduced to 109.5 mmol/l. Other components were the same as in the basal medium.

Hyposmotic medium

(202 mOsm/kg H_2O): 30% hyposmotic medium was prepared by appropriate dilution of basal medium with deionized water.

Depolarization medium

This medium was prepared by increasing KCl concentration to 50 mmol/l. To maintain physiological osmolarity, the NaCl concentration was reduced to 72 mmol/l. Other components were the same as in the basal medium.

GTP proteins

Mycophenolic acid (MPA) prevents GTP synthesis de novo [25]. Isolated pancreatic islets (5 per tube) were incubated in medium with 25 μ g/ml MPA or 0.05% ethanol (MPA solvent),

with 150 μ mol/l guanine or 100 μ mol/l adenine, thermostated at 37°C and 5% CO_2 /95% air for 20 hours according Komatsu et al. [26]. Next day islets were preincubated for 60 min in basal medium and then incubated for two subsequent 30 min periods alternatively in basal medium and stimulating medium (30% hyposmolar medium or 20 mmol/l glucose medium) in a final volume of 200 μ l. At the end of each incubation period medium was saved for insulin radioimmunoassay.

Bromo-enol lactone

We used 10 μ mol/l bromoenol lactone (BEL) to inhibit calcium independent cytosolic PLA_2 [27]. DMSO concentration was 0.1%. The same DMSO concentration was used also in the second control group. During 60 min preincubation BEL was present in basal medium. During two subsequent 30 min periods, basal medium and stimulating medium (30% hyposmolar medium or 20 mmol/l glucose medium) were without inhibitor. Final volume was 200 μ l and insulin secretion was measured by RIA.

Tetanus toxin

Tetanus toxin (20 nmol/l) was present during islets preincubation in depolarization medium to induce depolarization and exocytosis followed by endocytosis thus ensure toxin transport into the cells. Control islets were preincubated in depolarization medium without toxin. Preincubation was followed by next 60 min incubation in 200 μ l basal medium. Two subsequent 30 min incubation periods (in basal and stimulating medium respectively) were performed in calcium containing or calcium depleted medium.

Fig. 2. Effect of PLA₂ inhibition on insulin secretion from isolated islets. Fresh isolated islets were divided into three groups with different composition of preincubation medium. Control: basal medium in total volume 200 μ l. DMSO: medium contained 0.1% DMSO (BEL solvent). BEL: medium contained 10 μ mol/l bromoenol lactone. 60 min preincubation was followed by two 30 min incubations: A: basal - high glucose or B: basal - hypotonic medium without calcium with 1 mM EGTA. Results are shown as ng insulin released by one islets during 30 min incubation, n = 10. * P<0.05, *** P<0.001 compared to previous basal incubation (paired t-test).

N-ethylmaleimide (NEM)

Isolated Langerhans islets were 90 min preincubated in 200 μ l basal medium containing 5 μ mol/l NEM followed by two 30 min incubation periods (basal and stimulating medium respectively) with the same NEM concentration. Control islets were incubated in media without NEM.

Radioimmunoassay for insulin

Insulin released into medium was determined directly by specific radioimmunoassay. All samples from each experiment were analyzed in the same assay to avoid interassay variation. Insulin standards were prepared in each type of medium we utilized and thus the correction for potential effect of different media on the antibody binding was included. Radioactive ¹²⁵I-insulin was prepared in our Institute as previously described [28]. Rabbit anti-insulin antibody was provided by Dr. Štolba (Institute of Endocrinology, Prague). Synthetic insulin was obtained from Novo Nordisk (USA). All assays were performed in total volume of 400 μ l 0.01 mol/l PBS (pH 7.4). After overnight incubation of samples with the antibody at 4°C, bound and free peptides were separated by cold 200 μ l dextran coated charcoal (500 mg Norit + 50 mg dextran in 100 ml H₂O). After 15 min centrifugation at 3000xg, the radioactivity of pellet was measured.

Data analysis

Results are shown as means $\pm$ SE. Data were compared by paired (for comparison with data from previous incubation) or unpaired Student's t-test where applicable and were considered significantly different at level P<0.05.

Results

The pretreatment of β -cells with MPA, an inhibitor of GTP synthesis, in the adenine supplemented medium, which was added to compensate possible secondary effect of MPA on adenine synthesis, prevented glucose-induced insulin secretion (Fig. 1A). When adenine was replaced by guanine, the inhibiting effect of MPA pretreatment was reversed. Hypotonicity-induced insulin secretion (Fig. 1B) was not inhibited by MPA either in presence of guanine or adenine. Neither treatment affected insulin content in the islets as measured at the end of experiment (data not shown).

BEL, an inhibitor of calcium independent cytosolic PLA₂, fully abolished glucose-induced insulin secretion (Fig. 2A), but not that induced by cell swelling (Fig. 2B).

Glucose-induced insulin release was prevented by preincubation with tetanus toxin, a metalloprotease which cleaves SNARE proteins (Fig. 3A). Cell swelling-induced insulin secretion was inhibited by preincubation with toxin only if calcium was present in the medium. Tetanus toxin was not effective in the calcium-depleted medium (Fig. 3B).

The presence of another inhibitor of SNARE proteins, NEM, in the medium resulted in high basal insulin secretion (Fig. 4). Glucose stimulation (Fig. 4A) did not

Fig. 3. Effect of SNARE proteins inhibition on insulin secretion from isolated islets. Isolated islets (5 per tube) were divided into two groups; control was incubated for 30 min in 100 μ l depolarization medium and TeTx group was incubated in the same medium with 20 nmol/l tetanus toxin. Islets were then preincubated for 60 min in 200 μ l Krebs-Ringer HEPES buffer (basal) followed by two 30 min incubations: A: basal – high glucose, B: basal – hypotonic medium with (+Ca²⁺) or without (-Ca²⁺) calcium. Calcium depleted medium contained 1 mM EGTA. Results are shown as ng insulin released by one islet during 30 min incubation, n = 7. * P<0.05, ** P<0.005 compared to previous basal incubation (paired t-test).

significantly increase insulin secretion in the presence of NEM. In contrast, high insulin secretion from NEM treated islets was further increased after hypotonic stimulation (Fig. 4B).

Discussion

Swelling-induced peptide secretion represents important cellular reaction when material stored in intracellular secretory vesicles is expelled as a secretory burst of peptides and proteins (including hormones and enzymes) from various types of cell [4, 29]. It is a broad phenomenon possessing only limited selectivity [29]. The most intriguing feature of swelling-induced protein and peptide secretion is Ca²⁺ independence. Insulin secretion from β -cells is usually accompanied by ATP release. Mechanical stresses which include cell swelling were shown to result in the release of ATP from a variety of cells. However, Boudreault and Grygoczyk [30] showed that, in contrast to peptide secretion, calcium dependent exocytosis plays a major role in hypotonicity-induced ATP release indicating that different mechanisms are involved in ATP and peptide released by cell swelling.

To examine mechanisms of signal transduction involved in the swelling-induced peptide secretion we compared the effects of various inhibitors on glucose- and hypotonicity-induced insulin release. Both glucose and cell swelling induce immediate release of insulin from pancreatic β -cells. It is well established that glucose and other metabolizable nutrients depolarize the β -cell membrane by closing ATP-sensitive K⁺ channels resulting in Ca²⁺ influx through voltage-dependent channels, which constitutes a main stimulus for insulin exocytosis [5]. However, this pathway is not involved in cell swelling-induced insulin secretion since it is independent from extra- and intra-cellular Ca²⁺ [2]. Calcium independent exocytosis can also be induced by intracellular addition of GTP or its poorly hydrolysable analog GTP γ S in various cells types, including primary β -cell [9, 10], suggesting that G proteins may regulate final steps of secretion [31]. It is supposed that GTP and its analog react with different GTP-ases including heterotrimeric G proteins and monomeric proteins like Ras and dynamin. It is interesting that secretion induced by GTP γ S is tetanus toxin [32] and NEM insensitive [33].

MPA is a selective inhibitor of inosine monophosphate dehydrogenase, which is a rate-limiting enzyme of *de novo* GMP synthesis. MPA inhibits also GMP synthetase

Fig. 4. Effect of N-ethylmaleimid on insulin secretion from isolated islets. Fresh isolated islets (5 per tube) were divided into two groups; without (left part) or with 5 mmol/l N-ethylmaleimid (NEM) in preincubation and incubation media. 90 min preincubation in 200 μ l basal medium was followed by two 30 min incubations: A: basal – high glucose, B: basal – hypotonic medium without calcium with 1 mM EGTA. Results are shown as ng insulin released by one islet during 30 min incubation, $n = 10$. * $P < 0.05$, *** $P < 0.001$ compared to previous (basal) incubation (paired t-test), ### $P < 0.001$ compared to control basal incubation without NEM (unpaired t-test).

thus potentiating depressing effect on the level guanosine nucleotides [25]. MPA reduces GTP and indirectly and only moderately it reduces also ATP levels [25] and increases UTP concentration. MPA does not affect glucose metabolism [26]. We have used MPA to test participation of GTP and G proteins in cell swelling-induced insulin secretion. To compensate its possible effect on ATP, adenine supplemented medium was used in our experiments. Presence of MPA in medium during 20 hours preincubation period (MPA was not present in media during our experiments since its effect is irreversible) completely inhibited glucose induced insulin secretion in the presence of adenine. Komatsu et al. [26] showed, that 25 mg/ml of MPA in 20 h incubation reduced GTP content by 30–40% and completely abolished glucose induced insulin release. We expect that by using the same experimental conditions the MPA induced a similar reduction of GTP content in β -cells. This is supported by data showing a reversion of MPA effects on insulin release by the addition of guanine to the incubation medium. Thus, essential role of GTP in glucose signaling was confirmed in present experiments. Similarly as Metz et al. [25] we did not find change of the insulin content in the islets suggesting that exocytosis but not insulin

synthesis was affected by MPA (insulin releasable pool represents only negligible part of its content). In contrast to glucose-induced insulin release, hypotonicity-induced insulin secretion was not inhibited by the reduction of GTP in β -cells after MPA pretreatment. We therefore conclude that GTP and G proteins are not involved in signalling of swelling-induced insulin exocytosis.

Phospholipase A_2 activity is increased in mammalian cells in response to osmotic challenge (for review see [34]). The increased PLA_2 activity is seen as an increased release of free polyunsaturated fatty acids, e.g. arachidonic acid (AA) and membrane bound phospholipids. AA may also play a role as second messenger in glucose stimulated insulin secretion [35–37]. AA could be released from membrane phospholipids by Ca^{2+} -dependent PLA_2 [38, 39] or ATP-stimulated Ca^{2+} -independent PLA_2 [19]. The most important isoenzyme of A_2 in islets of Langerhans is $iPLA_2$ which is expressed in β -cells [17, 19]. BEL as inhibitor of this isoform [17, 40–42] inhibits glucose-induced insulin secretion [17, 43]. However, the effect of BEL could also be mediated by the decrease of ATP/ADP ratio [44]. Since $iPLA_2$ is independent from intracellular Ca^{2+} , and cell swelling-induced insulin release is also Ca^{2+} independent, we

examined a possibility of $iPLA_2$ involvement in the mechanism of cell swelling induced insulin secretion. Moreover, PLA_2 as mechanosensor could be directly activated by lateral crowding of membrane lipids after its stretching due to osmotic effect [45]. Bromoenol lactone (BEL) in a 10 $\mu\text{mol/l}$ concentration fully inhibited glucose- but not swelling-induced insulin secretion. While effect on glucose-induced insulin secretion could be result also of the change of ATP/ADP ratio, our data showed that PLA_2 is not involved in signal transduction in hypotonicity-induced insulin release. This is in harmony with our previous findings showing that AA metabolites such as prostaglandins and leukotrienes are not important components of the mechanism by which cell swelling induces prolactin secretion in pituitary cells [46]. Another enzyme, phospholipase D (PLD) plays an essential role in the cell swelling-induced vesicle cycling and in the activation of volume-sensitive anion channels [47]. PLD may be stimulated via PLA_2 [48], however participation of this step of signal transduction in swelling-induced insulin release was excluded by its resistance to BEL. Recently BEL has been found to inhibit another key enzyme in cellular phospholipid metabolism, the Mg^{2+} -dependent phosphatidic acid phosphohydrolase (PAP-1) [49]. The latter enzyme catalyzes the dephosphorylation of phosphatidic acid to yield 1,2-diacylglycerol (DAG), a central intermediate in glycerolipid synthesis as well as an important intracellular messenger molecule. We can therefore exclude also this enzyme as signal transmission step in hypotonicity-induced insulin secretion.

PLA_2 and its stimulation of AA production is known to be engaged in the RVD after cell swelling [50, 51]. Our results confirm that the swelling-induced effect on cell volume regulation could be dissociated from that on swelling-induced secretion (for review see [29]) and both mechanisms could react independently; an inhibition of RVD may not affect swelling-induced secretion.

Regulated exocytosis of secretory granules involves exocytosis of a premade pool of stable granules (for review see [52]). In many cell types large reserve pool exists and only a small portion of granules are initially rapidly releasable. The first-phase insulin secretion is attributable to the release of readily releasable pool (RRP) of granules representing less than 50 granules out of more than 10,000 granules [53]. A remarkable feature of the β -cell is that nearly all RRP granules are situated in a close proximity to the few Ca^{2+} channels; they are rapidly exposed to exocytotic levels of Ca^{2+} and undergo exocytosis within 100-200 ms [53]. Ca^{2+} independent swelling-induced peptide release is also very rapid [4], indicating different

signaling [2] and possibly exocytotic pathway.

Exocytotic secretion depends on the functioning of a mechanism involving membrane SNARE proteins. β -cells contain several types of SNARE proteins including SNAP-25, syntaxin 1-3, VAMP-2 and synaptotagmin [54]. SNARE proteins are proteolytic substrates for the clostridial neurotoxins [54], particularly their light chain subunit. Preincubation of β -cells with neurotoxins TeTx and botulotoxin B was shown to block Ca^{2+} -induced insulin secretion but not that induced by GTP γ S [32]. These data suggested parallel existence of Ca^{2+} -activated pathway involving VAMP-2 and cellubrevin as well as existence of G proteins-induced pathway bypassing SNARE proteins.

Our data confirmed that TeTx inhibits glucose induced insulin secretion [55]. Swelling-induced insulin secretion was blocked only in presence of Ca^{2+} in the incubation medium. In Ca^{2+} free medium TeTx did not affect hypotonicity-induced secretion. It is likely that TeTx as a Zn^{2+} dependent metalloproteinase [56] was not active in the presence of chelator EGTA in the Ca^{2+} depleted medium. Hausinger et al. [57] observed similar results when EGTA and EDTA blocked proteolysis of synaptotagmin by endogenous protease. We therefore believe that tetanus toxin sensitive SNARE proteins are involved in the cell swelling-induced insulin secretion.

N-ethylmaleimide-sensitive-factor (NSF) and soluble NSF-attachment factor (α SNAP) are soluble proteins that act to disassemble SNARE complexes. They are sensitive to NEM [33], which blocks disassembly of complexes and their further participation in exocytosis [58]. Although at the beginning the number of vesicles available for exocytosis increases [59], subsequent steps are blocked by NEM [60]. This effect was apparently responsible for initial increase of basal secretion and subsequent absence of the response to glucose stimulation in presence of NEM in our experiments. It is of much interest that despite high basal insulin secretion, islets were still able to release more insulin in response to hypotonicity. This may suggest the presence of a separate pool of secretory granules available for swelling-induced but not glucose-induced exocytosis after treatment. NEM in low concentration stimulates [61] and in concentration over 0.5 mM inhibits K/Cl co-transport [62] which is important for the RVD to occur. Therefore, it is likely that 5 mM NEM used in our experiments resulted in the inhibition of the RVD in swollen cells and increased the number of swollen secretory vesicles in the proximity of plasma membrane. NEM inhibits also V-type H^+ ATPase [63] which keeps pH in secretory vesicles of β -cells close to

or below 6 [64, 65]. Increase of pH resulting from enzyme inhibition is connected by changes of solubility of stored insulin and acceleration of insulin release [63]. We can speculate that these mechanisms are more likely to promote insulin secretion in hypotonicity- than in glucose-stimulated islets. Alternatively, a novel NEM insensitive pathway of exocytosis can be involved after cell swelling.

It is of interest that rising of glucose concentration in the medium also increases rat pancreatic β [66] or insulinoma cell [67] volume. In contrast to hypotonic cell swelling, no significant RVD is evident during glucose-induced cell swelling [66, 67]. The fact that glucose-induced insulin secretion is completely blocked at conditions when swelling-induced secretion is active (such as Ca^{2+} depletion, MPA and BEL treatment) shows that swelling does not play important role in glucose-stimulated insulin release.

Conclusion

We found that G proteins and iPLA_2 participate in glucose but not hypotonicity induced insulin secretion. Osmotically induced insulin secretion is sensitive to clostridial neurotoxin TeTx but is resistant to NEM suggesting specific exocytotic pathway.

Acknowledgements

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