Original article

Intracellular changes of Na\(^+\), K\(^+\), Ca\(^{++}\) & Mg\(^{++}\) in rat pancreatic islets in different glucose concentration and their relation with insulin secretion

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Abstract:
Impairment of insulin secretion from pancreatic β–cell constitutes an important pathophysiological factor in the development of diabetes mellitus. The changes of intracellular concentration of Na\(^+\), K\(^+\), Ca\(^{++}\) and Mg\(^{++}\) were observed in substimulatory and stimulatory different glucose concentrations. Pancreatic islets from Long-Evans rats were isolated by collagenase digestion. The concentrations of ions expressed in terms of islet protein in the homogenized islets were measured by using an ion-sensitive electrode based autoanalyzer. In the physiological medium, the islet content of all the four ions increased significantly in response to glucose with maximum level at 11 mM and no further increase at 20 mM. Initial depolarizing effect of glucose is due to reduction of K\(^+\) permeability. The reduction of K\(^+\) permeability by glucose in β–cell is a major step in stimulus-secretion coupling for insulin release.

Keyword: Pancreatic Islets

Introduction
Diabetes mellitus is a heterogeneous metabolic disease, characterized by persistent hyperglycemia. The syndrome may develop due to inadequate insulin secretion (insulin deficiency) or defective insulin action (insulin resistance) or both\(^1\). However, it is now well established that an impairment of the insulin secretion from the pancreatic β–cells constitutes important pathophysiological factors in the development of diabetes mellitus\(^3\). The β–cells of islets of Langerhans contribute to glucose homeostasis by sensing changes in the plasma glucose concentration and accordingly adjusting the rate of insulin release\(^3\). Previous study suggests that to be recognized as stimulus for insulin release, glucose must be metabolized first\(^2\). Glucose is the physiologically most important secretagogue. In line with the early observation that increased ATP-ADP ratio by glucose metabolism (glycolysis) causes membrane depolarization. Glucose induced depolarization and insulin secretion is initiated by a reduction of the K\(^+\) permeability of the β–cells mediated by closure of ATP-sensitive K\(^+\) channels. When the resulting depolarization reaches a threshold potential, the voltage dependent Ca\(^{++}\) channel opens with a subsequent influx of the ion\(^5\).

The increases of intracellular Ca\(^{++}\) concentration initiate contractile process and move insulin granules to plasma membrane for exocytosis into the circulation\(^6\). The changes of membrane potential play an important role in the regulatory process of insulin secretion\(^7\)-\(^12\). The process of glucose induced insulin release is associated with an intracellular accumulation of calcium within the pancreatic β–cell\(^13\).

Materials and Methods

Materials:
Reagents of analytical grade, deionized water, collagenase, Hepes buffered medium, bovine serum albumin, glucose, sucrose were used. The study was conducted in Research Division, BIRDEM, Dhaka, during the period of 2001 to 2002.

Media:
All the experiments were performed with a Hepes buffered medium as the physiological preincubation medium. Hepes buffered medium was also used as incubation media containing the different concentration of Na\(^+\) & K\(^+\) in different glucose concentration. All media were supplemented by bovine serum albumin 1 mg/ml.
Isolation & preparation of pancreatic islets:
Pancreatic islets were isolated with collagenase digestion from normal Long-Evans rat weighing 180 – 250 gm after ether anesthesia & cervical dislocation. The abdomen was opened and pancreas was taken out. The pancreas was taken in 1 ml Hepes buffer and 1 ml collagenase solution & chopped out. Then it is transferred into two shaking vials and shaken. The intact islets were allowed to settle down. Islets were then transferred to a petry dish containing Hepes buffer and supplemented with bovine serum albumin. From the petry dish islets were picked up again into a medium containing 3 mM glucose. After preincubation in 3 mM glucose, batches of islets were transferred to petry dishes each containing 1 ml of 0 mM, 3 mM, 11 mM and 20 mM glucose solution and incubated for 1 hour. After appropriate incubation, the islets were rinsed for 2 minutes in ice-cold sucrose solution of 300 mM for removing the extracellular electrolytes. The concentration of intracellular Ca++, K+, Na+ and Mg++ present in this mixture measured by Ion sensitive Electrode based autoanalyzer (NOVA-8, USA).

Statistical Analysis:
All analysis was done using the statistical package for Social Science (SPSS) software for windows. All variables were expressed as medium ISD. To compare the differences between median, non-parametric Mann-Whitney test were performed.

Results
Glucose induced Na+, K+, Ca++ and Mg++ changes in rat pancreatic islets with different concentrations in physiological medium (Table – I).

Concentration of Na+ increased gradually with the increasing concentration of glucose up to 11 mM and it became almost steady at 20 mM. Na+ concentration was found to be increased significantly when the islets were incubated in 11 mM glucose as well as in 20 mM glucose as compared to both 0 mM (P=0.001, P=0.001 respectively) and 3 mM glucose (P=0.001, P=0.002 respectively). No significant difference was observed when the islets were exposed to 3 mM and 20 mM glucose as compared to 0 mM and 11 mM of glucose respectively.

Table – I: Electrolytes content of rat pancreatic islets when incubated in different glucose concentrations.

<table>
<thead>
<tr>
<th>Glucose Concentration</th>
<th>Na+ (mmol/g pr)</th>
<th>K+ (mmol/g pr)</th>
<th>Ca2+ (µmol/g pr)</th>
<th>Mg2+ (µmol/g pr)</th>
</tr>
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<tbody>
<tr>
<td>0 mM (n=8)</td>
<td>0.986</td>
<td>0.098</td>
<td>7.835</td>
<td>7.367</td>
</tr>
<tr>
<td>3 mM (n=8)</td>
<td>(0.63-1.59)</td>
<td>(0.06-0.15)</td>
<td>(5.89-15.77)</td>
<td>(5.89-9.74)</td>
</tr>
<tr>
<td>11 mM (n=8)</td>
<td>1.354</td>
<td>0.139</td>
<td>13.445</td>
<td>12.871</td>
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<td>(0.64-2.45)</td>
<td>(0.07-0.20)</td>
<td>(7.20-20.41)</td>
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<tr>
<td>20 mM (n=8)</td>
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<td>0 mM vs 3 mM</td>
<td>18/0.141</td>
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<td>9/0.016</td>
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<td>0 mM vs 20 mM</td>
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<td>3 mM vs 11 mM</td>
<td>10/0.021</td>
<td>5/0.005</td>
<td>32/1.00</td>
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<tr>
<td>3 mM vs 20 mM</td>
<td>6/0.006</td>
<td>5/0.005</td>
<td>29.50/0.793</td>
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<td>11 mM vs 20 mM</td>
<td>28/0.674</td>
<td>18/0.141</td>
<td>23/0.345</td>
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U/p value

Like K+, concentration of Ca++ increased gradually with the increasing concentration of glucose. Ca++ concentration was found to be increased significantly when the islets were incubated in 11 mM glucose as well as in 20 mM glucose as compared to both 0 mM (P=0.001, P=0.001 respectively) and 3 mM glucose (P=0.001, P=0.002 respectively). No significant difference was observed when the islets were exposed to 3 mM and 20 mM glucose as compared to 0 mM and 11 mM of glucose respectively.

Concentration of Mg++ was already increased at 3 mM glucose and it became steady at 11 mM and 20 mM glucose. Mg++ concentration was found to be increased significantly when incubated in 3 mM, 11 mM as well as in 20 mM glucose as compared to 0 mM (P=0.016, P=0.021, P=0.046 respectively). No significant difference was observed when the islets were exposed to 20 mM glucose as compared to 3 mM and 11 mM glucose respectively.
Discussion:
When β–cells are exposed to glucose a complex series of cellular events occur culminating in a rise in cytosolic free Ca++ concentration that has been correlated with the insulin release. Calcium flux studies shows that as glucose increases there is a progressive increase in Ca++ spiking activity and this is due to increase in Ca++ uptake15,18.

In this study, Ca++ increased to almost significant level (P=0.059, Table – I) already at a glucose concentration as low as 3 mM. It is known that the voltage gated Ca++ channels in β–cell open at around 5 mM of glucose. A sharp rise of Ca++ is seen in the present study at 11 mM of glucose, which then becomes steady up to 20 mM glucose. Thus, it seems that intracellular Ca++ rises even before the opening of the voltage – gated Ca++ channels, and probably explained by the inhibition of outward transport of Ca++ from the cell by glucose18.

One of the major initial events in glucose mediated insulin release is the rise of intracellular K+ due to closure of ATP sensitive K+ channels15. This fact is obvious, again, by the almost significant rise of K+ already at 3 mM of glucose (P=0.074, Table – I), which becomes highly significant at 11 mM of glucose and then becomes steady at 20 mM glucose. The changes of Na+ in β–cells in response to glucose is always a complex and less understood issue. In the present data, the increased Na+ concentration may contribute to the K+ mediated depolarization of the β–cells.

The issue of Mg++ in pancreatic β–cell is the least understood one and studies are rare regarding its changes in β–cell. It is interesting to note that the increased Mg++ level becomes steady after 3 mM glucose, a fact indicating that unlike Ca++, Mg++ is unaffected by the opening of the voltage-gated Ca++ channels. An alternate possibility is that a voltage-gated Mg++ channels operates at a lower level of depolarization. Some authors claim that Mg++-ADP rather than ATP is the regulating molecule of K+-ATP channels19,20. There is a direct relation among calcium, electrical activity & insulin secretion. Knowledge, so far gained from different studies, can explain the role of Ca+ & K+ in insulin release. But the role of other ions are only partially known & remains to be elucidated.

Conclusion:
The feasibility of simultaneous measurement of Na+, K+, Ca++ and Mg++ in rat pancreatic islets is confirmed by the present study. Data shows maximum effect of intracellular K+ and Ca++ obtained by the sugar at 11 mM glucose. Rise of Na+ is probably due to mechanism other than depolarization. Intracellular Mg++ also rises but the effect is maximum at 3 mM glucose. In the present study, the intracellular responses of the ions to different concentration of glucose in a physiological medium were found which is due to underlying ionic events for insulin release.

Reference:


