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Original Article

Glucose induced intracellular ionic changes in pancreatic islets of rat on inhibition of Na^+/K^+ pump by ouabain and its relation with insulin secretion

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Abstract:

Glucose is the primary physiological stimulator of insulin release. Na^+/K^+ pump has a major role in modulating the glucose action. Rat pancreatic islets were isolated by collagenase digestion. The concentration of ions in the homogenized islets were measured by Ion-sensitive Electrode based autoanalyzer. Inhibition of Na^+/K^+ pump by ouabain resulted in gradual increase of intracellular Na^+, decrease of K^+, increase of Ca^{++} and decrease of Mg^{++} compared to the physiological medium. The changes of K^+ paralleled by a rise of Na^+ in the media indicate that a pump-related effect seems to be more plausible for the difference in ionic changes.

Keyword: Na^+/K^+ pump, Ouabain, Pancreatic islets.

Introduction:

Diabetes mellitus may be either due to defective insulin secretion (insulin deficiency) or in improper insulin action (insulin resistance) or both. Glucose is the primary physiology stimulator of insulin release[1] and has a permissive and regulatory role in insulin secretion[2]. The Na^+/K^+ pump operates as an antiport activity, pumping $3Na^+$ out of the cell against its steep electrochemical gradient and pumping $2K^+$ in[3]. The Na^+/K^+ pump is also involved in the generation of resting membrane potential. Insulin release is increased by ouabain (Na^+/K^+ pump inhibitor) due to continuous spike activity that results from gradual decrease of repolarization potential[4]. Changes in glucose induced secretion of β–cell is observed in absence of K^+, which is due to intracellular modification of Na^+ and K^+ concentration due to decreased activity of Na^+/K^+ pump and possibly by altering glucose oxidation[5].

Inhibition of Na^+/K^+ pump by ouabain resulted in gradual increase of intracellular Na^+ in β–cell, when exposed to 3mM glucose. In individual β–cell this action of ouabain was paralleled by closure of ATP regulated K^+ channels and a slow elevation of the cytoplasmic Ca^{++} concentration. Ouabain had dual action on this glucose induced oscillation in promoting their appearance and at higher concentration transforming them into a sustained increase in Ca^{++}. Although ouabain seems to have initial effects on the β-cell membrane potential not only by inhibiting the electrogenic component of the Na^+/K^+ pump but also by preventing its ATP consumption and 0.1mM ouabain was found to cause a slight and gradual stimulation of insulin secretion and it was observed, a slow increase Ca^{++}, which was paralleled by a rise of intracellular Na^+. It seems likely from this observation that deterioration of the Na^+ gradient results in the increase of Ca^{++} by suppression of Na^+/Ca^{++} exchange[6].

Materials and Method:

The pancreatic islets from Long-Evans rat were isolated by collagenase digestion. Islets were then transformed to a petry dish containing Hepes buffer medium (25mM Hepes solution, pH-7.4, 125mM Na^+, 5.9 mM K^+, 1.28 mM Ca^{++}, 1.2 mM Mg^{++}) & supplemented with bovine serum albumin & 3mM glucose as physiological preincubation medium at 37^0C & 28 RPM for 45 minute. After preincubation, batches of islets (20 islets in each batch) were transferred to Hepes buffered medium with ouabain (1mM) containing 1 ml of 0mM, 3mM, 11mM, 20mM glucose for incubation. This incubation medium also was supplemented with bovine serum albumin (1mg/ml) and the islets were incubated for one hour at 37^0C & 45 RPM.

After incubation extracellular ions were washed out with 300mM ice-cold sucrose solution. Each batch of islets was transferred to aliquot containing 85 µl deionized

water & 80 µl additional buffer (175 mM Na^+, 12 mM K^+, 1.28 mM Ca^{++}, 1.2 mM Mg^{++}). 5 µl at 100% Titron-X was added for lysis of intact islets. Then the aliquot was vortexed for homogenization of islets and the concentration of ions present in this mixture were measured using an ion-sensitive electrode based autoanalyzer. This study was conducted in Research Division, BIRDEM, Dhaka, during the period of 2007 – 2008.

Statistical Analysis :

Analysis was done using the SPSS software for Windows. All variables were expressed as median±SD. To compare the differences between median, non-parametric Mann-Whitney Test were performed. $P<0.005$ was considered as the level of significance in all cases.

Result :

Effect of Glucose On Intracellular Ionic Concentration On Inhibition of Na^+/K^+ pump by Ouabain:

The concentration of Na^+ increased compared to the physiological medium. The rising pattern was maximum at 11mM and had a falling tendency at 20mM. Na^+ concentration was found to be increased significantly when the islets were incubated in 3mM, 11mM & 20 mM glucose as compared to both 0mM (p=0.036, p=0.001, p=0.001 respectively) and 3mM (p=0.002, p=0.006 respectively). No significant difference was found in other concentration of glucose.

Table-I: Ionic content of rat pancreatic islets when incubated in different glucose concentration as physiological medium:

Glucose Concentration	Na^+ (mmol/g pr)	K^+ (mmol/g pr)	Ca^{++} (µmol/g pr)	Mg^{++} (µmol/g pr)
0 mM (n=8)	0.986 (0.63 – 1.59)	0.098 (0.06 – 0.15)	7.835 (5.89 – 15.77)	7.367 (5.89 – 7.94)
3 mM (n=8)	1.354 (0.64 – 2.45)	0.139 (0.07 – 0.20)	13.445 (7.20 – 20.41)	12.871 (6.41 – 14.96)
11 mM (n=8)	2.245 (1.22 – 3.24)	0.276 (0.24 – 0.32)	19.736 (17.89 – 28.37)	12.512 (6.11 – 16.22)
20 mM (n=8)	2.150 (1.90 – 3.50)	0.258 (0.15 – 0.29)	21.953 (13.49 – 28.63)	11.117 (6.80 – 19.01)
U/p value				
0mM vs 3mM	19/0.172	15/0.074	14/0.059	9/0.016
0mM vs 11mM	2/0.002	0.000/0.001	0.00/0.001	10/0.021
0mM vs 20mM	0.000/0.001	1/0.001	2/0.002	13/0.046
3mM vs 11mM	10/0.021	0.000/0.001	5/0.005	32/1.00
3mM vs 20mM	6/0.006	2/0.002	5/0.005	29.5/0.793
11mM vs 20mM	28/0.674	18/0.141	23/0.345	30/0.834

Table-II: Ionic content of rat pancreatic islets when incubated in different glucose concentration with ouabain:

Glucose Concentration	Na^+ (mmol/g pr)	K^+ (mmol/g pr)	Ca^{++} (µmol/g pr)	Mg^{++} (µmol/g pr)
0 mM (n=8)	1.676 (1.38 – 2.23)	0.051 (0.05 – 0.10)	10.387 (9.17 – 15.12)	5.193 (4.59 – 12.21)
3 mM (n=8)	2.169 (1.62 – 2.83)	0.081 (0.05 – 0.12)	16.377 (10.59 – 23.01)	10.270 (5.75 – 11.40)
11 mM (n=8)	3.247 (2.59 – 4.01)	0.111 (0.10 – 0.16)	27.066 (20.76 – 34.36)	10.826 (5.13 – 15.57)
20 mM (n=8)	2.911 (2.49 – 4.69)	0.111 (0.06 – 0.18)	23.292 (16.63 – 30.77)	5.798 (5.13 – 11.33)
U/p value				
0mM vs 3mM	12/0.036	11/0.027	4/0.003	9/0.016
0mM vs 11mM	0.00/0.001	0.00/0.001	0.00/0.001	11/0.027
0mM vs 20mM	0.00/0.001	4/0.003	0.00/0.001	17/0.115
3mM vs 11mM	2/0.002	11/0.027	2/0.002	31/0.916
3mM vs 20mM	6/0.006	16/0.093	10/0.021	10/0.021
11mM vs 20mM	21/0.248	26.5/0.563	18/0.141	16/0.092

K^+ concentration dropped in all aspects than that of the physiological medium. K^+ increased gradually upto 11mM with no further tendency to rise at 20 mM. K^+ concentration was found to be increased significantly when the islets were incubated in 3mM, 11mM & 20 mM glucose as compared to 0mM (p=0.027, p=0.001, p=0.003 respectively) and it also significantly increased in 11mM glucose as compared to 3mM glucose (p=0.027). No significant difference was found in other different concentration of glucose.

Ca^{++} concentration was higher than that of physiological medium. It gradually increased upto 11mM and showed a falling tendency at 20mM glucose. Ca^{++} concentration was found to be increased significantly when the islets were incubated in 3mM, 11mM & 20 mM glucose as compared to both 0mM (p=0.003, p=0.027, p=0.001 respectively) and 3mM (p=0.002, p=0.021 respectively). No significant difference was found in 20mM of glucose as compared to 11mM of glucose.

Mg^{++} concentration decreased as compared to the physiological medium even in the absence of glucose. Mg^{++} concentration was found to be increased significantly when the islets were incubated in 3mM and 11mM glucose as compared to 0mM (p=0.016, p=0.027 respectively) and it also significantly increased in 20 mM glucose as compared to 3 mM glucose (p=0.021). No significant difference was observed in other concentration of glucose.

Discussion :

The Na^+/K^+ pump is one of the most important process that may affect Na^+ content of the β–cells and consequently, transport of other ions. It's importance was illustrated by the significant increase of intracellular Na^+ obtained by blocking the pump by the addition of ouabain (Table - I & II). This effect was evident already at 0mM glucose indicating the independent role of the pump. The K^+ content fall when the Na^+/K^+ pump was inhibited by addition of ouabain (Table - I & II). The changes in K^+ paralleled by a rise of Na^+ indicating that a pump related effect (where Na^+ and K^+ changes almost parallely) seems to be more plausible for the difference in ionic changes in media.

With the inhibition of the pump by addition of the ouabain there was a substantial rise of intracellular Ca^{++}. This inhibition of Na^+/K^+ pump was pronounced effect on ionized Ca^{++} which is paralleled by increased duration of the time spent at the depolarized plateau phase with superimposed action potential[7] and marked stimulation of insulin release[8].

Insulin release is increased by ouabain[4]. After removal of ouabain burst pattern reappears & repolarization potential gradually increases again. The release of insulin evoked by glucose and other secretogogues is critically dependent on the presence of calcium in the extra-cellular fluid[9-10]. The burst activity ceased after omission of calcium and is followed by an abolition of insulin release[10-12]. The process of glucose induced insulin release is associated with an intracellular accumulation of calcium within the pancreatic β–cell[13]. The omission of Ca^{++} or Na^+ deficiency as well as ouabain partially inhibited glucose oxidation and the glycolytic pathway[14]. The association of Mg^{++}, in contrast with the Na^+/K^+ pump is exemplified by the fact that the intracellular content shows as significant rise in the absence of glucose.

Conclusion :

Na^+/K^+ pump plays an important role in maintaining the ionic balance in pancreatic β–cell. It is now known that Ca^{++} plays the central role in modulating insulin secretion and the concentration of the ion is modulated by other ions and intracellular messengers. There is, however, major deficiencies in understanding the details of these ionic events in the cellular and molecular levels. The present study explores the simultaneous changes at Na^+, K^+, Ca^{++} and Mg^{++} in rat pancreatic islets in response to glucose and their modulation by Na^+/K^+ pump. On inhibition of Na^+/K^+ pump by ouabain, the intracellular Na^+ increased significantly and fall of K^+ content was more pronounced compared to the physiological medium. Inhibition of the pump resulted in a substantial rise of Ca^{++} and fall of Mg^{++} compared to physiological medium. However, the contribution of the Na^+/K^+ pump varies in case of different ions with a substantial pump independent component for Ca^{++} transport & insulin release.

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