ABNORMAL REGULATION OF NA,K-ATPASE IN GLUCOSE INTOLERANT RATS

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Introduction: Glucose is the most important physiological insulin secretagogue. However, the mechanisms underlying glucose-induced insulin release are not fully understood. The role of electrogenic systems such as ionic pumps, to these events remains essentially uninvestigated. Na,K-ATPase, responsible for maintaining Na^+ and K^+ gradients across the plasma membrane and generates a net outward current, thus changes in its activity may contribute to the early ionic events regulating insulin secretion (Therien and Blostein, 2000).

Objective: The aim of this work was to evaluate the regulation of Na,K-ATPase activity by glucose in intact β-cells of normal and glucose intolerant (GI) rats and its putative contribution to the regulation of insulin secretion.

Material and Methods: Pancreatic β-cells, from normal or control or GI rats, were isolated and cultured (48h). Cell batches were pre-incubated (30min) with 2mM glucose to reach basal. Afterwards cells were challenged with glucose in the interval 0-11mM for 60min, for dose-dependence evaluation, or with 8mM glucose for 5-120min, for time-dependence evaluation. ATPase activity was assessed in intact cells by colorimetric quantification of Pi formed in 30min. Na,K-ATPase activity was calculated by the difference between the activities obtained in the absence and in presence the of 1mM ouabain (Costa et al., 2009).

Results: In β-cells from normal rats, glucose induced a bimodal regulation of Na,K-ATPase. In the absence of glucose, Na,K-ATPase activity was 0.056±0.015 U/mg. Stimulation with 2mM glucose induced an increase of Na,K-ATPase activity of ~4 fold whereas for [glucose] above 2mM it was observed a significant inhibition of Na,K-ATPase activity (0.061±0.013, 0.080±0.009 and 0.064±0.005 U/mg for 5.6, 8.4 and 11mM glucose, respectively, compared to 0.188±0.035 U/mg observed in 2mM G; n=3-8). β-cells from GI rats does not present this profile; in the absence of glucose, Na,K-ATPase activity was 0.202±0.036 U/mg and no significant differences from this value were observed with the other glucose concentration tested.

Addicionally, in β-cells from normal rats, glucose (8mM) induced a time-dependent inhibition, with a biphasic profile, of Na,K-ATPase - it was observed a decrease in the pump activity between 0 and 20min stimulation where it reached a minimum value (77%). For incubation periods over 20min, the pump activity slowly and partially recovered (54%, 55% and 52%, for 30, 60 and 120min, respectively; n=7). In β-cells from GI animals, an less accentuated decrease of Na,K-ATPase activity between 0 ans 20min was also observed (34%), and is not observed further recover in activity.

Conclusions: This work demonstrates there Na,K-ATPase is strictly regulated by glucose in pancreatic β-cell. This regulation is unpaired in GI animals. Na,K-ATPase contribution to glucose-induced ionic events and insulin secretion might be relevant and must be explored as a possible therapeutic target in TD2 .

1. Therien AG, Blostein R (2000) Mechanisms of sodium pump regulation. Am J Physiol Cell Physiol 279:C541-C566

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