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Poster Abstract Submission

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Abstract

Title: Implication of AMPK in glucose-evoked modulation of Na,K-ATPase

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Background and aims: Na,K-ATPase is an integral membrane protein that maintains the gradients of Na^+ and K^+, using the energy of ATP hydrolysis, maintaining the ionic gradients that allow electrical activity to occur. It has been demonstrated that, in pancreatic β-cells, Na,K-ATPase is regulated by glucose and that this phenomenon is impaired in glucose intolerant subjects. However, the mechanism underlying glucose-induced modulation of Na,K-ATPase is still unclear.
The AMP-activated protein kinase (AMPK) is a molecular key player in energy homeostasis, providing exquisite sensitivity to small changes in intracellular AMP levels and thus to intracellular [ATP]/[ADP] ratio, that is known to activate protein regulatory pathways. Since in pancreatic β-cell, glucose has marked effects on oxidative metabolism and total intracellular ATP and AMP levels, the involvement of AMPK in the cascade of events regulating Na,K-ATPase regulation in pancreatic β-cells was postulated. The aim of this work was to evaluate the putative role of AMPK in the glucose-evoked regulation of Na,K-ATPase activity in the pancreatic β-cell.
Materials and methods: Pancreatic β-cells from normal (control) or glucose-intolerant Wistar rats (GIR) were isolated and cultured (48h). Cell batches were pre-incubated (30min) with 2.1mM glucose to reach basal activity. Afterwards cells were challenged to 8.4mM glucose for 20min, in the presence or absence of AMPK agonists (AICAR) and antagonists (compound C; CC). 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.
Results: In basal conditions the activity of Na,K-ATPase from normal and GIR pancreatic β-cell was similar (0.184±0.030 and 0.186±0.020 □molPi/min/mgProt, respectively). Challenging the control β-cells with glucose 8.4mM evoked a 62% reduction of Na,K-ATPase activity whereas in GIR β-cells a significantly lower inhibition (40%) was observed. The addition of AICAR 1mM abolished glucose-induced Na,K-ATPase inhibition (0,166±0.011 □molPi/min/mg). In control β-cell, the addition of CC 10 μM had no effect on glucose-induced inhibition of Na,K-ATPase. In the contrary, in GIR β-cells it significantly potentiated glucose-evoked inhibition of Na,K-ATPase reaching values similar to that

observed in the controls (66%).
Conclusions: The AMPK agonist AICAR counteracts the inhibitory action of glucose on Na,K-ATPase of control β-cells whereas CC amplified the glucose-induced inhibition of Na,K-ATPase in GIR β-cells. These results suggest that AMPK plays a central role in the cascade of events underlying glucose-induced modulation of Na,K-ATPase and that the defect must be upstream of AMPK. Finally, abnormal glucose-induced regulation of Na,K-ATPase occurs prior to overt type 2 diabetes and might be a feature in the disease development.