

Glucose-evoked Na^+, K^+ -ATPase modulation in pancreatic β -cells from normal and impaired glucose tolerance: role of AMPK

C. Roque¹, A. R. Costa¹, C. M. Antunes^{1,2}

¹ICAAM & Chemistry Department, ECTUE; ²Centro de Neurociências e Biologia Celular, Universidade de Coimbra

m10237@alunos.uevora.pt

Na^+, K^+ -ATPase is regulated by glucose in pancreatic β -cells, a process that is altered in glucose impaired tolerance. Although AMP dependent protein kinase (AMPK), a metabolic sensor, is believed to be central in the signal transduction cascade underlying the Na^+, K^+ -ATPase regulation in pancreatic β -cells, its role remains unknown. The aim of this work was to clarify the role of AMPK in glucose-evoked inhibition of Na^+, K^+ -ATPase and to evaluate whether AMPK is differently regulated in pancreatic β -cells from subjects with normal and impaired glucose tolerance. Pancreatic β -cells or islets from normal (control) or glucose-intolerant Wistar rats (GIR) were isolated and cultured. After a pre-incubation (30min) with 2.1mM glucose (G2), batches were challenged for 20min with 2.1 or 8.4mM glucose (G8) in the presence or absence of AMPK agonist (AICAR, 1mM) and antagonist (Compound C (CC), 10 μ M). Na^+, K^+ -ATPase activity was assessed by quantification of Pi, in the absence and in presence of 1mM ouabain. Phosphorylation levels of α_1 subunit of Na^+, K^+ -ATPase-(Ser-23) and α AMPK-(Thr-172) was evaluated by *Western blot* (WB).

In G2 Na^+, K^+ -ATPase activity from normal and GIR β -cell was similar (0.184 \pm 0.030 and 0.186 \pm 0.020 $\mu\text{molPi}/\text{min}/\text{mgProt}$, respectively). Challenging the β -cells with G8 evoked a lower inhibition of Na^+, K^+ -ATPase activity in GIR (40%) compared to controls (62%). In control β -cell, AICAR abolished glucose-induced Na^+, K^+ -ATPase inhibition (0,166 \pm 0.011 $\mu\text{molPi}/\text{min}/\text{mg}$) whereas CC had no effect. In the contrast, CC significantly potentiated glucose-evoked inhibition of Na^+, K^+ -ATPase in GIR β -cells, reaching values similar to the controls (66%), For both GIR and control islets, G8 induced a 50% decrease of AMPK phosphorylation level compared to G2. CC mimicked the effect of G8, but was less efficient in GIR. Concomitantly, α_1 - Na^+, K^+ -ATPase-(Ser-23) phosphorylation level was increased upon G8 or CC stimulation, compared to G2 or AICAR.

These results suggest that AMPK plays a key role in the signaling mechanism underlying glucose-induced modulation of the pump, a process dependent on phosphorylation cascades, and that the defect in GIR must be upstream of AMPK. Glucose-induced inhibition of Na^+, K^+ -ATPase may result from AMPK inhibition by the fuel metabolism and subsequent activation of PKC, known to phosphorylate α_1 - Na^+, K^+ -ATPase-(Ser-23). This mechanism is impaired in GIR, thus potentially contributing to the impaired glucose-induced insulin secretion in IGT. Occurring prior to overt type 2 diabetes, this might be a feature in the disease development.

Acknowledgments: This work is funded by ICCAM, by CNC and by Chemistry Department of ECTUE.