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

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Na^+,K^+-ATPase is regulated by glucose in pancreatic ß-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), 10µM). 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 αAMPK-(Thr-172) was evaluated by *Western blot* (WB).

In G2 Na^+,K^+-ATPase activity from normal and GIR β-cell was similar (0.184±0.030 and 0.186±0.020 µmolPi/min/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±0.011 µmolPi/min/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, α1-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 α1-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.

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