"Nicotinamide nucleotide transhydrogenase and the glucose regulation of mitochondrial glutathione oxidation, NADPH concentration and insulin secretion in mouse islets"

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Abstract

Background and aims: Changes in glutathione redox state (EGSH) were recently measured with the redox-sensitive GFP2 probe fused to glutaredoxin 1 (GRX1-roGFP2) and its mitochondrial form (mt-GRX1-roGFP2) in rat islet cell clusters and human islets. We found an inverse correlation between 1) the acute glucose-mediated reduction in EGSH and 2) the acute rise in NAD(P)H autofluorescence and glucose stimulation of insulin secretion (GSIS). Nicotinamide nucleotide transhydrogenase (NNT) is one of several NADPH-producing enzymes in mitochondria that could contribute to these acute glucose effects. Its spontaneous inactivating mutation in C57BL6/J mice was shown to reduce the GSIS due to reductions in ATP production, β-cell depolarization and Ca2+ influx. Here, we tested the impact of a lack of NNT on the acute glucose regulation of mitochondrial EGSH and its role in GSIS and key stimulus-secretion coupling events by comparing islets from C57BL6/J mice with islets from closely-related C57B...

Document type: Communication à un colloque (Conference Paper)

Référence bibliographique

Nicotinamide nucleotide transhydrogenase and the glucose regulation of mitochondrial glutathione oxidation, NADPH concentration and insulin secretion in mouse islets

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Background and aims: Changes in glutathione redox state (GSH) were recently measured with the redox-sensitive GFP2 probe fused to glutaredoxin 1 (GRX1-roGFP2) and its mitochondrial form (mt-GRX1-roGFP2) in rat islet cell clusters and human islets. We found an inverse correlation between 1) the acute glucose-mediated reduction in GSH and 2) the acute rise in NAD(P)H autofluorescence and glucose stimulation of insulin secretion (GIS). Nicotinamide nucleotide transhydrogenase (NNT) is one of several NADPH-producing enzymes in mitochondria that could contribute to these acute glucose effects. Its spontaneous inactivating mutation in C57BL6/J mice was shown to reduce the GSIS due to reductions in ATP production, β-cell depolarization and Ca²⁺ influx. Here, we tested the impact of a lack of NNT on the acute glucose regulation of mitochondrial GSH and its role in GSIS and key stimulus-secretion coupling events by comparing islets from C57BL6/J mice with islets from closely-related C57BL6/N mice that express wild-type NNT (j vs. N islets).

Materials and methods: Islets were isolated from 8-16 week-old female C57BL6/J and N mice. After isolation, the islets were (co)infected with adenoviruses coding (mt-)GRX1-roGFP2, NNT+mcRerry (to express wild-type NNT in J islets) or mcRerry alone as control, and cultured for 2-4 days. The mitochondrial and cytosolic GSH, the reduced and oxidized forms of NADP and NADT, and the sum ATP+ADP, the intracellular Ca²⁺ concentration, and insulin secretion were measured after 30-60 min incubation or during perfusion at increasing glucose concentrations from 0.5 (G0.5) to 30 mmol/L (G30) in the presence of 4.8 or 30 mmol/L extracellular K⁺ (K4.8 or K30).

Results: In N islets, glucose acutely and significantly reduced mitochondrial GSH as in rats and humans while increasing NADH/(NADH+NAD⁺) (G30 0.22±0.03 vs. G0.5 0.05±0.01, p<0.01), NADPH/(NADPH+NADP⁺) (G30 0.72±0.08 vs. G0.5 0.48±0.06, p<0.05) and ATP/(ATP+ADP) ratios (G30 0.83±0.03 vs. G0.5 0.69±0.003, p<0.001), Ca²⁺ and insulin secretion in K4.8 or K30 (p<0.05). In J islets, the glucose-induced rises in NADH/(NADH+NAD⁺) (0.21±0.04 vs. 0.04±0.01, p<0.01), ATP/(ATP+ADP) (0.85±0.01 vs. 0.71±0.02, p<0.001) and [Ca²⁺]i were similar to those in N-islets. There were also no significant differences between mice for their lack of cytosolic GSH response to glucose. In contrast to N islets, mitochondrial GSH was low at all glucose concentrations and NADPH/(NADPH+NADP⁺) was not increased in G30 compared to G0.5 (0.83±0.03 vs. 0.78±0.03) and not decreased by 10 µM of mitochondrial uncoupler FCCP in G30 (0.83±0.02). The GSIS in K4.8 and K30 was 66% lower (p<0.05) in J vs. N islets, but the relative amplifying action of glucose in K30 was preserved (fold-increase vs. G0.5). Importantly, adenovirus-mediated expression of NNT in J islets restored the glucose regulation of mitochondrial GSH and the glucose (G30 0.84±0.01 vs. G0.5 0.52±0.04, p<0.001) and FCCP (0.64±0.05, p<0.05 vs. G30) regulation of the NADPH/(NADPH+NADP⁺) ratio as observed in N islets, and significantly increased GSIS in both K4.8 and K30 conditions.

Conclusion: NNT is responsible for the glucose-induced rise in NADPH/(NADPH+NADP⁺) ratio and reduction in mitochondrial GSH in pancreatic islets, and increases the GSIS by acting at a site distal to Ca²⁺ influx. The results also suggest that NNT works in the reverse mode of operation in islets exposed to a low glucose concentration or to FCCP.

Supported by: FRS/FNRS/ARC/CFB