Universität Basel

Research Project

Impact of the NADPH Pool in the Endoplasmic Reticulum on Metabolic and Hormonal Regulation

Third-party funded project

Project title Impact of the NADPH Pool in the Endoplasmic Reticulum on Metabolic and Hormonal Regulation
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An impaired redox control in the endoplasmic reticulum (ER) with unfolded-protein response (UPR) and ER-stress has been associated with major cardio-metabolic diseases, chronic inflammatory disorders and cancer. Thus, it is crucial to elucidate the mechanisms underlying ER-redox control and identify the affected biological reactions. The $NAD(P)^+$/NAD(P)H redox couple plays an essential role in many biological functions. In contrast to the cytoplasm, the role of the $NAD(P)^+$/NAD(P)H redox couple in the ER and the relevance of luminal NADPH for essential biological functions is insufficiently studied.

The discovery of hexose-6-phosphate dehydrogenase (H6PDH) revealed a mechanism for NADPH generation in the ER and provided a link between energy status and glucocorticoid signaling. The glucocorticoid activating 11b-hydroxysteroid dehydrogenase 1 (11b-HSD1) is, so far, the only well characterized NADPH-dependent luminal enzyme. However, the impact of H6PDH on macrophage function and inflammatory mediators and its effect on corticosteroid signaling remain unknown. Also, there must be other NADPH-dependent enzymes because 11b-HSD1 cannot account for the myopathy and the increased susceptibility of hepatocytes observed in situations of H6PDH-deficiency.

Based on previous results, we hypothesize that **1)** NADPH-dependent 11b-HSD1 function (glucocorticoid-dependent and -independent) essentially modulates macrophage polarization and activity, and inhibition of 11b-HSD1 exerts anti-inflammatory and anti-infective effects, **2)** inhibition of 11b-HSD1 has glucocorticoid-independent metabolic effects by modulating oxysterol and bile acid homeostasis, and **3)** ER-luminal short-chain dehydrogenase/reductase (SDR) enzymes other than 11b-HSD1 are responsible for effects in macrophage, adipocytes, adrenal cells, hepatocytes and myocytes in situations of H6PDH-deficiency.

Therefore, we propose to investigate the consequences of NADPH depletion in the ER on hormonal and metabolic functions and to characterize enzymatic reactions that are dependent on ER luminal NADPH. Specifically, we propose to:

- investigate the role of luminal NADPH supply on macrophage polarization and function. We will distinguish between 11b-HSD1-dependent and -independent effects.
- assess a potential role of 11b-HSD1 in the metabolism of EBI-2 ligands
- elucidate the impact of luminal NADPH supply and 11b-HSD1 on the metabolism of 7-ketocholesterol (7KC) and on bile acid homeostasis
- establish a method using redox-sensitive green-fluorescent proteins (roGFP) to determine the topology of ER membrane proteins in living cells and attempt to identify ER luminal enzymes other than 11b-HSD1

– characterize the NADPH-dependence of identified luminal enzymes
– develop a strategy to identify novel substrates of SDR enzymes

The role of ER luminal NADPH and the consequences of its depletion on metabolic and hormonal responses will be studied in transfected cells using recombinant enzymes, in cell lines with endogenous expression of the relevant enzymes and treated with siRNA, in primary cells from wild-type and H6PDH knockout mice, and in vivo in wild-type and transgenic mice. Structural modeling will be applied in a search for novel SDR substrates and to further study experimentally verified target-ligand interactions.

The proposed research should significantly enhance our current knowledge on the role of NADPH in the ER. The expected findings are relevant regarding the understanding of the coupling between cellular energy state, hormonal regulation, ER redox regulation, and oxidative stress-induced damage. Disturbed functions of the enzymes investigated are associated with impaired inflammatory responses and with cardio-metabolic disorders, and the results of the proposed project should support the future development of therapeutic interventions.

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