with 0.5mM of NAD+ precursors nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN). FK866 (100nM, 48h) was effective at depleting NAD+ by approximately 90% in C2C12 myotubes and 50% in HepG2 liver cells. In each cell line, 11β-HSD1 reductase activity was decreased by FK866 compared to untreated controls (80% in C2C12 P<0. 0001, 55% in HepG2, P=0. 03 and 65% in HDFn P=0. 002). Restoring NAD+ level with NR (0.5mM, 6h) in C2C12 and (0.5mM, 24h) in HepG2 and HDFn rescued 11β-HSD1 enzyme activity compared with no precursor treatment (60% in C2C12 P=0. 0007, 52% in HepG2 P=0. 03 and 68% in HDFn P=0. 0006). Human dermal fibroblasts treated with TNFa (10nM, 24h) boosted 11β-HSD1 expression 4-fold, however this was not able to overcome the FK866 induced drop-in reductase activity. These data show that depletion of NAD+ and impaired 11β-HSD1 reductase activity in the ER is a generalised phenomenon across cell types. This suggests a crosstalk between cytosolic and ER NAD(P)(H) pools that can respond rapidly to changes in NAD+ precursor availability. This could provide new insight into the regulation of cellular glucocorticoid metabolism and ER NAD(P)(H) homeostasis.

Presentation: No date and time listed

Abstract citation ID: bvac150.1485

Steroid Hormones and Receptors

ODP443

Targeting NAD+ availability modulates 11β-Hydroxysteroid dehydrogenase 1 activity in mouse and human cell lines and primary cells

Ali Kabli, Yasir Elhassan, Silke Heising, Samuel Heaselgrave, Rowan Hardy, David Hodson, Stuart Morgan, and Gareth Lavery

Four nicotinamide adenine dinucleotide coenzymes – NAD +, NADH, NADP+, and NADPH – are the central catalysts of metabolism. The enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is NADPH-dependent reductase that catalyses the reduction of the inactive glucocorticoid cortisone) to active cortisol (11-dehydrocorticosterone to corticosterone in rodents). 11β-HSD1 is in the endoplasmic reticulum (ER) lumen, and the level of NADPH in the ER regulates enzyme activity. Little is known about the biochemical mechanisms controlling cytosolic/ER NAD(P)(H) crosstalk. Here we examined how perturbed cellular NAD + availability modulates 11β-HSD1 enzyme activity in the ER across a range of mouse and human cell types. 11β-HSD1 activity was measured in a series of transformed and primary mouse and human liver (HepG2), muscle, and Human Dermal Fibroblast cells (HDFn) depleted for NAD+ using FK866 to inhibit nicotinamide phosphoribosyl transferase (NAMPT), the rate-limiting enzyme in NAD+ biosynthesis. To replete NAD+ we supplemented cells