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Cooperativity between 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase is based on a common pyridine nucleotide pool in the lumen of the endoplasmic reticulum

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Abstract

11β-Hydroxysteroid dehydrogenase type 1 (11βHSD1) is a NADP(H)-dependent oxidoreductase of the ER lumen, which may have an important role in the pathogenesis of metabolic syndrome. Here, the functional coupling of 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase (H6PDH) was investigated in rat liver microsomal vesicles. The results demonstrate the existence of a separate intraluminal pyridine nucleotide pool in the hepatic endoplasmic reticulum and a close cooperation between 11βHSD1 and H6PDH based on their co-localization and the mutual generation of cofactors for each other.

Keywords: 11β-Hydroxysteroid dehydrogenase type 1; Hexose-6-phosphate dehydrogenase; Pyridine nucleotides; Rat liver microsomes

1. Introduction

The endoplasmic reticulum is a separate subcellular compartment with properties different from those of the cytosol (see Benedetti et al., 2005). The characteristic redox potential of its lumen is maintained by the relative impermeability of the endoplasmic reticulum membrane toward numerous redox-active compounds, including pyridine nucleotides. Because the oxidoreductases of the lumen have to use a separate NAD(P)/NAD(P)H pool, a functional cooperation between intraluminal oxidases and reductases has been hypothesized. Hexose-6-phosphate dehydrogenase (H6PDH) is supposed to have a key role in the generation of reducing equivalents, which can be used by several reductases present in the lumen that include, among others, 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1; Atanasov et al., 2004; Bánhegyi et al., 2004; Hewitt et al., 2005).

2. Methods

We have investigated the functional coupling of 11βHSD1 and H6PDH in rat liver microsomal vesicles. Microsomes were prepared from livers of overnight fasted male Wistar rats, using fractional centrifugation (Bánhegyi et al., 2004). In native microsomal vesicles, cortisone reductase activity of 11βHSD1 was measured on the basis of cortisol formation (detected by an enzyme-linked fluorescent assay; see Bánhegyi et al., 2004), and H6PDH was evaluated by rapid-filtration measurement of the radioactivity entrapped by vesicles (as C^{14}-6-phosphogluconate) upon addition of C^{14}-glucose-6-phosphate (Gerin and Van Schaftingen, 2002). Latency of 11βHSD1 and H6PDH was measured by fluorimetric detection of NADPH formation in microsomal vesicles, which have been permeabilized with alamethicin to allow the free access of the cofactor to the intraluminal enzymes. In the case of 11βHSD1 and H6PDH, microsomes were treated before the permeabilization with 10 μM cortisol plus 1 mM $NADP^+$ or 10 μM glucose-6-phosphate plus 100 μM $NADP^+$, respectively.

3. Results and discussion

Ninety percent of the activity of both 11βHSD1 and H6PDH in liver microsomes was latent, which indicates the intraluminal localization of the active sites. However, the addition of 10 μM cortisone to intact liver microsomes resulted in a measurable cortisol production (75 ± 20 pmol/mg protein/10 min, mean ± S.D., $n = 4$), which strongly suggests the presence of an endogenous intraluminal NADPH pool. The addition of glucose-6-phosphate (10 μM), a substrate for H6PDH, stimulated the cortisone reductase activity of 11βHSD1, measured as cortisol production (290 ± 75 pmol/mg protein/10 min,

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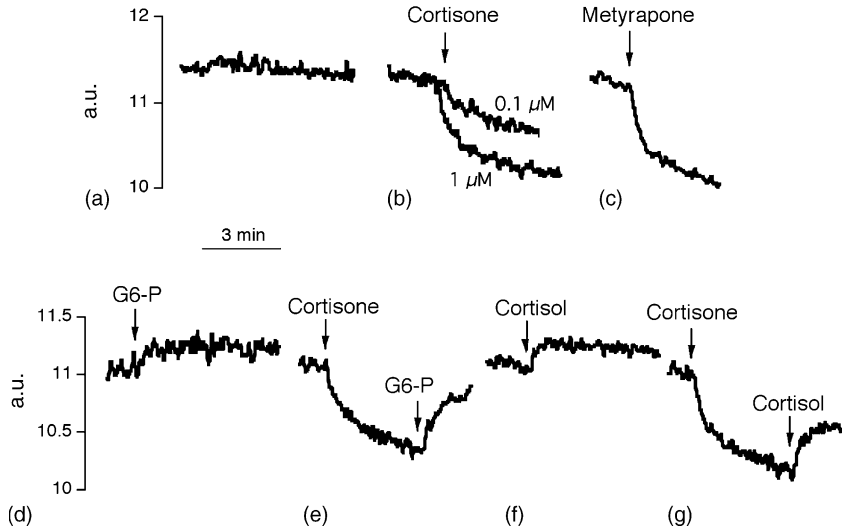

Fig. 1. (a–g) Effect of cortisone, metyrapone, cortisol and glucose-6-phosphate (G6-P) addition on the redox state of intraluminal pyridine nucleotides in rat liver microsomal vesicles. Representative traces out of four to six similar experiments are shown. A.u., arbitrary units.

mean ± S.D., $n = 4$). Similarly, cortisone increased the intravesicular accumulation of radioactivity upon the addition of radiolabelled glucose-6-phosphate (data not shown), indicating the stimulation of H6PDH activity. Inhibition of glucose-6-phosphate uptake by S3483 – a specific inhibitor of the microsomal glucose-6-phosphate transporter – abolished this effect; the cortisol production in the presence of 50 μM S3483 was 80 ± 32 pmol/mg protein/10 min (mean ± S.D., $n = 3$). A correlation was previously shown between glucose-6-phosphate-dependent cortisone reduction and cortisone-dependent glucose-6-phosphate oxidation (Bánhegyi et al., 2004).

Direct fluorimetric analysis of native microsomal vesicle suspensions (2 mg protein/ml, 350 nm excitation and 460 nm emission wavelength) showed a sustained signal (Fig. 1a), which was reduced by the addition of cortisone in a concentration-dependent manner (Fig. 1b). Metyrapone (5 μM), another substrate of 11βHSD1, produced a similar effect (Fig. 1c). On the other hand, reducing agents caused a moderate increase in the signal. Glucose-6-phosphate caused a slight increase in the signal (Fig. 1d), but could revert the effect of cortisone (Fig. 1e). Addition of cortisol also resulted in a minor increase of the fluorescence signal (Fig. 1f). However, the addition of a high concentration of cortisol (10 μM) could also revert the effect of 1 μM cortisone (Fig. 1g).

Collectively, these results demonstrate a close cooperation of 11βHSD1 and H6PDH based on their co-localization and the mutual generation of cofactors for each other.

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