The redox systems of the endoplasmic reticulum and the metabolic syndrome

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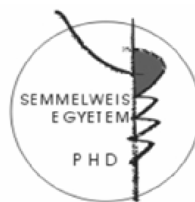

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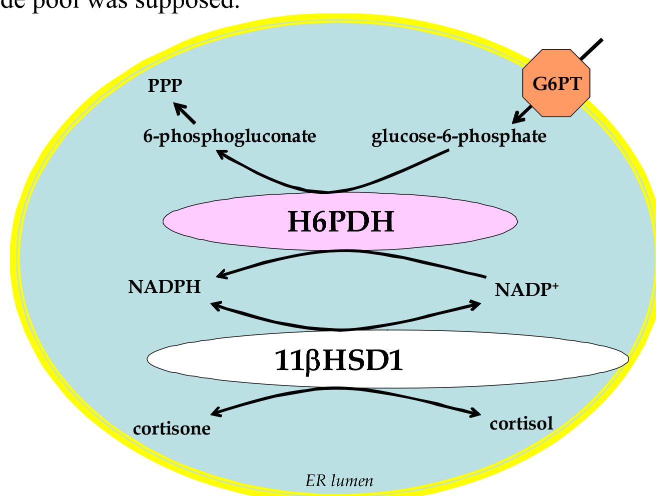
Introduction

The metabolic syndrome is one of the most common clinical features with prevalence about 20-25%. Its symptoms are insulin resistance, obesity, hypertension and dyslipidaemia. The most important complication of metabolic syndrome is the increased risk for the development of ischemic heart disease. In the focus of the pathogenesis of the disease appeared the 11β-hydroxysteroid dehydrogenase type 1.

The 11β-hydroxysteroid dehydrogenase type 1 is expressed in many organs and tissues. The enzyme that expressed in the liver and adipose tissue plays an important role in the pathogenesis of the metabolic syndrome. Its main importance is the regulation of glucocorticoid effect in tissue level. It uses NADPH as a cofactor. The active site of 11β-hydroxysteroid dehydrogenase type 1 is located in the lumen of the endoplasmic reticulum. The regulation of its enzyme activity is possible via the modification of its cofactor supply. The cooperativity between 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase is proved on a biochemical, as well as in a genetical way. The basis of their cooperation is the mutual cofactor generation for each other. Many other reactions are known in the lumen of the endoplasmic reticulum, which requires NADPH as a cofactor. On the basis of these data, despite the more oxidized redox potential of the endoplasmic reticulum compared to the cytosol, the existence of a reduced intraluminal pyridine nucleotide pool was supposed.



The exact role of 11β-hydroxysteroid dehydrogenase type 1 in the development of metabolic syndrome was investigated in transgenic animals as well. Many results prove the role of the hepatic and adipose tissue enzyme in the pathogenesis of the metabolic syndrome. 11β-hydroxysteroid dehydrogenase type 1 overexpressing and knockout transgenic animals exist, and the function of the enzyme was also investigated in type 2 diabetes model animals. The transgenic mice that overexpress 11β-hydroxysteroid dehydrogenase type 1 in adipose tissue show every symptoms of the metabolic syndrome: obesity, dyslipidaemia, insulin resistance, glucose intolerance, and hypertension. Despite these data the mice that overexpress 11β-hydroxysteroid dehydrogenase only in liver shows the symptoms and metabolic abnormalities of the disease except for obesity. On the type 2 diabetic model obese Zucker rat the activity of 11β-hydroxysteroid dehydrogenase is decreased in liver but increased in adipose tissue.

The results of studies on obese patients showed the same features. The enzyme activity measurements that based on urine cortisol metabolite investigation showed decreased hepatic 11β-hydroxsteroid dehydrogenase type 1 activity. The enzyme activity in their subcutaneous adipose tissue was increased, compared to normal body weight controls. The activity of 11β-hydroxysteroid dehydrogenase type 1 in the subcutaneous adipose tissue of obese women showed positive correlation to BMI.

Goals

The proof of the cooperation besides the known biochemical and genetical data could be the existence of an intraluminal pyridine nucleotide pool. Until now the composition and the redox state of the intraluminal pyridine nucleotides was unknown. The redox state of the lumen of the endoplasmic reticulum was thought to be oxidative according to the luminal components that were known until now. But some intraluminal reactions were also known that requires reducing equivalents.

We have supposed that the cooperation between 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase is based on an intraluminal pyridine nucleotide pool. Our goal was the determination of the pyridine nucleotide pool in microsomal vesicles derived from the endoplasmic reticulum to clear the redox state of the lumen. We wanted to calculate intraluminal pyridine nucleotide concentration, then the redox potential of the lumen according to the $NAD(P)^+$/NAD(P)H ratio. Our aim was to examine the influence of 11β-

hydroxysteroid dehydrogenase and hexose-6-phosphate dehydrogenase on the intraluminal pyridine nucleotide pool.

We have supposed that the activity of 11β-hydroxysteroid dehydrogenase is determined by the redox state of the lumen via its cofactor supply. The redox state of the endoplasmic reticulum may have an effect on the development of the metabolic syndrome, and the intraluminal pyridine nucleotides may give an opportunity to the pharmacological treatment of the disease.

We have planned to examine the role of different tissue (liver, adipose tissue) type 1 11β-hydroxysteroid dehydrogenases in the development of different phenotypes of the metabolic syndrome. We have examined the expression and enzyme activity of 11β-hydroxysteroid dehydrogenase type 1 in two type 2 diabetes model rat strains.

Methods

Preparation of microsomal vesicles

Endoplasmic reticulum derived microsomal vesicles were produced from the liver and epididymal adipose tissue of Sprague-Dawley, Wistar, Zucker and Goto-Kakizaki rats. The intactness of the vesicles was examined with the determination of the latency of two intraluminal enzymes, the mannose-6-phosphatase and the UDP-glucuronyltransferase. The microsomes were frozen and stored in liquid nitrogen until they were used.

Fluorimetric NADPH measurements

We have investigated the reduced pyridine nucleotide content of the microsomal vesicles in their characteristic excitation (350 nm) and excitation (460 nm) wavelength. Our experiments were performed in a Cary Eclipse (Varian) fluorescent spectrophotometer.

Transport measurements with light scattering technique

The light scattering technique is based on the light dispersion capacity of systems consist of small vesicles. The light scattering capacity of the system is measured in arbitrary units. The treatment of the vesicles with an osmotically active compound causes water loss and shrinkage of the vesicles which increases the light scattering signal. If the membrane of the vesicle is impermeable to the compound, the increased light scattering signal

exists permanently. If it is permeable, the osmotic gradient disappeared, the vesicles dilate and the signal falls.

Transport measurements by rapid sedimentation technique

Rapid sedimentation technique is a useful method for the detection of microsomal transport mechanism and intravesicular pools. In our experiments we have used this method to the measurement of microsomal pyridine nucleotide transport. The $NADP^+$ concentration was measured via an enzymatic way with the 6-phosphogluconate-dehydrogenase enzyme and its substrate, the 6-phosphogluconate. The NADPH production was detected by fluorimeter.

Enzyme activity measurements

The cortisone reductase activity of 11β-hydroxysteroid dehydrogenase type 1 was detected via cortisol production. The cortisol production was detected by ELFA method (enzyme-linked fluorescent assay). The measurement of the activity of 11β-hydroxysteroid dehydrogenase is possible on the basis of the NADPH produced by the enzyme. The direction of the enzyme activity is determined by its cofactor supply: it can act as a reductase, as well as a dehydrogenase. In our experiments the cortisol dehydrogenase activity was measured on the basis of NADPH formation. The detection of the reductase activity by fluorimetric way is practically impossible because the given NADPH would be oxidized by the NADPH-oxidase activity in the surface of the microsomes.

Hexose-6-phosphate dehydrogenase

We have estimated the hexose-6-phosphate activity by rapid filtration technique. The rapid filtration technique is a useful method for the detection of microsomal transport and intravesicular pools. Microsomes are incubated for a short time in the presence of radiolabelled molecules, then they are filtered with vacuum, then the uptake of radioactivity is detected with a scintillation counter.

Western blot

We have investigated the protein level expression by Western blot. Equal amounts of protein were loaded from liver and adipose tissue

microsomes. After polyacrylamide-gelelectrophoresis the proteins were blotted on nitrocellulose membrane. The protein contents of the bands were checked by Ponceau Red. The primer antibody was against the rat 11β-hydroxysteroid dehydrogenase protein.

Results

Despite the oxidizing environment in the lumen of the endoplasmic reticulum, the existence of a mostly reduced intraluminal pyridine nucleotide pool was proved, which is a new basis for the cooperation of 11β-hydroxysteroid dehydrogenase and hexose-6-phosphate dehydrogenase. The importance of this intraluminal pyridine nucleotide pool is that it can play an important role in the pathogenesis of the metabolic syndrome via alteration of the cofactor supply of the two enzymes.

Pyridine nucleotide pool in the endoplasmic reticulum microsomes

We have proved that the membrane of the endoplasmic reticulum is practically impermeable to the reduced, as well as the oxidized forms of pyridine nucleotides. Despite that neither the existence of a pyridine nucleotide transporter nor pyridine nucleotide synthesis was described, many indirect proofs supposed the existence of an intraluminal pyridine nucleotide pool.

The impermeability of the membrane was proved from inside, as well as from the outer surface of the vesicle. When cortisone was given to the microsomes a rapid formation of cortisol could be detected which disappeared with the permeabilization of the membrane. The phenomenon is a proof for the existence of an intraluminal pyridine nucleotide pool which amount permits the cortisone reduction, but the permeabilization of the membrane let this pool accessible for the superficial NADPH-oxidase activity of the membrane. So the intraluminal pyridine nucleotide concentration is not enough for cortisone reduction any more. The microsomes hold their intraluminal pyridine nucleotide pool during the preparation process.

The impermeability of the microsomal membrane was investigated both in high and physiological pyridine nucleotide concentrations with rapid sedimentation and light scattering technique.

The redox state of the intraluminal pyridine nucleotides

The intraluminal pyridine nucleotide pool mainly contents reduced pyridine nucleotides. We have proved this with two methods: the effect of membrane permeabilization on the redox state of intraluminal pyridine nucleotides was investigated. The membrane permeabilization cause a decrease in the amount of intraluminal reduced pyridine nucleotides. The difference of the fluorescent spectra of the microsomes before and after the permeabilization is similar to the spectrum of NADPH.

We have investigated the effect of oxidizing compounds on the redox state of the intraluminal pyridine nucleotide pool. We have used for these experiments the substrates of 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase.

Cortisone caused a decrease in the amount of reduced pyridine nucleotides in our fluorimetric experiments. The difference of the microsomal spectra before and after cortisone was given was similar to the spectrum of NADPH. Cortisone, as well as metyrapone, a synthetic substrate of 11β-hydroxysteroid dehydrogenase type 1, oxidize the intraluminal pyridine nucleotide pool in a concentration dependent way.

Reducing agents caused just a slight reduction of intraluminal pyridine nucleotides, which also prove the mainly reduce state of intraluminal pyridine nucleotides. However, the oxidizing effect of cortisone could be reversed both with cortisol and glucose-6-phosphate.

The concentration of the intraluminal pyridine nucleotide pool

The intraluminal reduced pyridine nucleotide content was calculated ca. 0.4 mM, the oxidized form exists in a concentration about 0.05 mM on the basis of our fluorimetric results. The ratio of reduced and oxidized pyridine nucleotides is approx. 8:1, which means a -376 mV calculated intraluminal redox potential.

The cooperation between 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase is based on a common pyridine nucleotide pool.

Our results showed that glucose-6-phosphate, the substrate of hexose-6-phosphate dehydrogenase supports the cortisone reductase activity of 11β-hydroxysteroid dehydrogenase type 1. Cortisone stimulates the hexose-6-phosphate dehydrogenase activity, which proves that the two enzymes use a common intraluminal pyridine nucleotide pool.

The reverting effect of glucose-6-phosphate on the oxidizing effect of cortisone is also a proof for the existence of a commonly used pyridine nucleotide pool. The preventing effect of the inhibition of glucose-6-phosphate transporter on the effect of glucose-6-phosphate provides an opportunity for therapeutic intervention in the treatment of metabolic syndrome.

The role of 11β-hydroxysteroid dehydrogenase type 1 in the development of different phenotypes of the metabolic syndrome- enzyme activity and expression in Zucker and Goto-Kakizaki rat liver and adipose tissue

According to the literature, our results showed that the in the liver of the fat type 2 diabetes/ metabolic syndrome model Zucker rat both the expression and enzyme activity was decreased. The expression and the enzyme activity in the adipose tissue of the animal were increased. The decreased hepatic 11β-hydroxysteroid dehydrogenase expression and activity can be a compensatory effect due to the developed metabolic syndrome to decrease the hepatic glucose production and blood glucose level via decreased hepatic cortisol production. The increased expression and activity of 11β-hydroxysteroid dehydrogenase type 1 in the adipose tissue of the obese Zucker rat causes abdominal-visceral obesity just like in Cushing syndrome and hypercortisolism.

In the liver of the type 2 diabetes model Goto-Kakizaki rat the expression and enzyme activity of 11β-hydroxysteroid dehydrogenase type 1 were increased, but in the adipose tissue of the animal were decreased, compare to the control. The decreased expression and enzyme activity in the adipose tissue may be responsible for the lean phenotype of the animal. The hepatic enzyme may be responsible for the development of insulin resistance and type two diabetes via the elevation of hepatic cortisol emission and glucose production due to the enhancement of the expression of the enzymes of gluconeogenesis and glycogenolysis

Conclusions

1. The cooperation between 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase is based on a common pyridine nucleotide pool.

2. The pyridine nucleotide pool is isolated; it is not accessible for the microsomal NADPH oxidase.

3. The microsomal pyridine nucleotide pool mainly consists of NADPH and $NADP^+$ because two enzymes (11β-hydroxysteroid dehydrogenase and hexose-6-phosphate dehydrogenase) using NADP(H) have an influence of its redox state.

4. Oxidizing compounds, cortisone and metyrapone oxidize the intraluminal NADPH pool. Reducing compounds, cortisol and glucose-6-phosphate hardly influence the redox state of the intraluminal pyridine nucleotide pool, however, they can revert the oxidizing effect of cortisone

5. Other proof for the common use of the pyridine nucleotide pool is that glucose-6-phosphate causes measurable cortisol production and cortisone supports hexose-6-phosphate dehydrogenase activity in the microsomes.

6. The 11β-hydroxysteroid dehydrogenase type 1 influences the phenotype of the metabolic syndrome besides its metabolic effect. In the obese Zucker rat the expression and enzyme activity of 11β-hydroxysteroid dehydrogenase type 1 is decreased in liver but increased in adipose tissue. The elevated enzyme activity and expression may be responsible for the lean phenotype of the animal.

7. In the case of the Goto-Kakizaki rat the expression and activity of 11β-hydroxysteroid dehydrogenase type 1 was increased in liver but decreased in adipose tissue. In Goto-Kakizaki rat the hepatic enzyme may be responsible for the metabolic abnormalities of type 2 diabetes.

Publications

The dissertation is based on the following publications:

Czegle I, Piccirella S, Senesi S, Csala M, Mandl J, Banhegyi G, Fulceri R, Benedetti A. (2006) 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. Mol Cell Endocrinol, 248 (1-2):24-5. **(IF= 2,786)**

Piccirella S*, **Czegle I***, Lizak B*, Margittai E*, Senesi S, Papp E, Csala M, Fulceri R, Csermely P, Mandl J, Benedetti A, Banhegyi G. (2006) Uncoupled redox systems in the lumen of the endoplasmic reticulum. Pyridine nucleotides stay reduced in an oxidative environment. J Biol Chem, 281 (8):4671-7. (****These authors contributed equally to this work***) **(IF=5,854)**

Other publication

Lizak B, **Czegle I**, Csala M, Benedetti A, Mandl J, Banhegyi G. (2006) Translocon pores in the endoplasmic reticulum are permeable to small anions. Am J Physiol Cell Physiol, 291 (3):511-7 **(IF=3,942)**