SUMMARY

Changes of energy metabolism of hepatocytes:

The effect of oxidative stress and triiodothyronine

Liver is a vital organ performing numerous essential functions. Due to its position in the blood circulation, liver is the first organ incessantly exposed to a great number of toxic substances. Respiratory chain located in mitochondria is a frequent target of toxic action of these substances. There are various mechanisms that participate in hepatocyte damage, nevertheless the most important mechanism of hepatotoxic effect is oxidative stress induced by increased production of free radicals. Impact of oxidative stress on hepatocytes is very complex and still not fully elucidated.

The aim of my thesis was to investigate the effect of oxidative stress on energy metabolism of rat hepatocytes using isolated hepatocytes and isolated mitochondria. We evaluated the effect of oxidative stress on the activity of mitochondrial enzymes and function of mitochondrial permeability transition pore (MPTP), respectively. Opening of this pore induces activation of apoptotic and necrotic processes. Our results document selective action of oxidative stress on the activity of various mitochondrial enzymes. Tert-butylhydroperoxide (t-BHP) causes significant inhibition of NADH-dependent substrates, while oxidation of flavoprotein-dependent substrate - succinate is not impaired. We also found substantial differences in the inhibition of various NADH-dependent substrates tested.

To evaluate function of MPTP we introduced a new method, which was not published yet.
This method converts classical swelling curves depicting decrease of absorbance in mitochondrial suspension to derivation form. Such conversion enables better evaluation of kinetics of mitochondrial swelling; we can obtain data about maximal velocity of the swelling process and time required
to reach this maximal rate. Using this method we assessed interaction of different factors that affect
MPTP opening. We obtained more accurate data about mutual interactions of calcium and phosphate on the swelling rate and about potentiating effect of t-BHP and triiodothyronine. Using
Cyclosporine A – a specific inhibitor of MPTP we confirmed that all described activating effects on mitochondrial swelling are connected to the opening of MPTP.