ABSTRACT

Cytochrome b_5 (CYB5) is heme protein capable of reduction of cytochromes P450 (CYP) or some other enzymes. However, his regulative capability was also observed by his apo form, i.e. in absence of heme prosthetic group in the active center. CYB5 can accept electron from cytochrome b_5 reductase (CYB5R) or from cytochrome P450 reductase (CYPOR). CYPOR by itself is reduced by NADPH and is also able to forward electron to CYP independently of CYB5. CYB5R on the other hand is reduced by NADH.

Efficiency of CYB5 to accept and forward an electron was studied *in vitro* with five different substrates – testosterone, Sudan I, aristolochic acid I (AAI), ellipticine and vandetanib. These substrates were chosen considering their characteristic reactions, which are catalyzed by their respective isoforms of CYP. The experiments with these substrates were carried out in the medium with recombinant CYPs prepared in insect cells or *E. coli* or in the medium with hepatic microsomes isolated from different organisms. Rats, from which the majority of these microsomes was isolated, were premedicated by different CYP inducers. The experiments were carried out in medium with NADH or NADPH in order to assess the capability of CYB5 to reduce CYP independently of CYPOR. The capability of CYB5 and CYB5R to act as a source of electrons for metabolism of testosterone (CYP3A4) and of Sudan I (CYP1A1) was assessed by this procedure. The formation of metabolites was also observed in medium with NADH considering the substrates ellipticine and vandetanib. In contrast, there was no metabolite formation observed in medium with NADH considering the substrate AAI. In the medium with NADPH, the CYB5 could stimulate activity of CYP considering the metabolism of testosterone (CYP3A4), Sudan I (CYP1A1) and vandetanib (CYP1A2). Contrarily, inhibitory effect of CYB5 in the medium with NADPH was observed considering the metabolism of vandetanib by CYP1A1. Higher activity of CYP in the medium with NADH in comparison to NADPH was not observed in any of undertaken experiments. The effect of CYB5 on activity of CYP dependent on the respective isoform of CYP as well as the respective substrate was proven in the proposed diploma thesis.

(In Czech)

Keywords: cytochrome b_5, cytochromes P450, benzo[*a*]pyrene, ellipticine, vandetanib, aristolochic acid, Sudan I