SUMMARY

Placenta is a unique organ which ensures a number of vital functions necessary for normal course of pregnancy and development of a new individual. In addition to its main function of oxygen supply and nutrient and waste product exchange, placenta also serves as an endocrine, metabolic and protective organ. Placenta is considered to be one of the physiological barriers of the organism which regulates transport of both endogenous and exogenous compounds between two compartments - maternal and fetal blood circulations.

Up to recently, the placental barrier was supposed to be formed only by cellular layers which separate maternal and fetal blood - syncytiotrophoblast and fetal capillary endothelium. However, it has been demonstrated that the activity of placental efflux transport proteins and metabolic enzymes contributes considerably to the protective function of placental barrier. Efflux transporters are membrane proteins which actively (along with consumption of ATP) "pump" a diversity of substrates out of the cell. It has been shown that the kinetics of transport of various substances across the placenta is affected predominantly by two transporters: P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP). Compared to these transporters, placental biotransformation enzymes are considered to be a minor, but an important part of active placental barrier. Several placental enzymes were suggested to have an impact on the kinetics of transplacental passage of various molecules. These are in particular: cytochrome P450 enzymes, conjugation enzymes of phase II. metabolism and enzymes involved in biotransformation of steroid molecules.

In this work both components of the active placental barrier - transport and metabolic - were studied. From the group of efflux transporters we focused on BCRP transporter whose expression and activity was studied using a model of the rat placenta *in-vivo* and *in-situ*. The presence of rat BCRP mRNA and protein were confirmed in both rat placental trophoblast cell line HRP-1 and the rat placenta at the end of pregnancy. Simultaneously, we analyzed the expression of P-gp which was detected in the rat placenta but not in HRP-1 cell line. Activity of BCRP *in-vitro* was confirmed in accumulation studies with fluorescently labeled substrate of BCRP- BODIPY FL prazosine. In consistence with the results of expression studies no activity of P-gp was observed in the HRP-1 cell line. Furthermore, we investigated the impact of BCRP activity on the pharmacokinetics of drug transport across the placenta using dually perfused rat placenta. Transport of a model substrate of BCRP - cimetidine - was studied in both materno-fetal and feto-maternal directions. To verify the specifity of BCRP-mediated transport two BCRP

inhibitors GF120918 and fumitremorgin C were used. Our results demonstrated that BCRP plays two distinct roles in the kinetics of placental transport of drugs: 1) reduces the passage of drugs from the mother to the fetus and 2) actively accelerates the excretion of the drug already present in the fetal blood.

In the following two studies we investigated the expression and activity of the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) which acts as a placental barrier to endogenous glucocorticoids (GC). In the first work we explored the role of placental 11β-HSD in the metabolism of GC in the course of rat pregnancy and the relationship between placental and fetal 11β-HSD activities and their effect on the ratio of active and inactive forms of GC in the fetal circulation. At first the expression and activity profiles of placental 11β-HSD type 1 and 2 during 13. and 21. day of pregnancy were studied. Expression of both types of 11β-HSD decreased at the end of pregnancy, however with different profiles. Dramatic decrease of mRNA levels of 11β-HSD1 was observed between 13. and 14. day of pregnancy followed by smaller reduction towards term. On the contrary, 11β-HSD2 was more abundantly expressed and decreased slowly from 13. to 21. day of gravidity. Decay of placental 11β-HSD2 in the last third of pregnancy was further confirmed on protein level. The decrease in 11β-HSD1 expression was followed by the drop in its activity. In contrast, an increase in NAD^+-dependent dehydrogenase activity of 11β-HSD2 was found in placental homogenates. These observations suggest the existence of an unknown posttranslational regulation mechanism which affects the activity of placental 11β-HSD2. The results from the functional studies with dithiotreitol *in-situ* revealed that this mechanism is presumably distinct from the process of activation and deactivation of 11β-HSD2 by reversible dimerization. Furthermore, the levels of corticosterone and 11-dehydrocorticosterone in the fetus were investigated and correlated with the activity of placental and fetal 11β-HSD. The results suggest that the activity of fetal 11β-HSD participates considerably on the regulation of GC levels in fetal blood, particularly at the end of pregnancy.

In the following study we examined the impact of antenatal GC administration on expression and conversion capacity of placental 11β-HSD type 2. Again rat placenta was chosen as an experimental model. Synthetic GC (dexamethasone and betamethasone) were administered to pregnant rats in two doses (low or high) during 16. and 21. days of pregnancy. The expression of 11β-HSD in term placentas was analyzed by real-time RT-PCR and Western blotting. Conversion capacity was assayed by dually perfused rat placenta *in-situ* with corticosterone as a

model substrate of 11β-HSD2. Our results showed that although the impact of antenatal steroid therapy on expression of 11β-HSD2 is negligible or, in the case of dexamethasone, limited to transcriptional level, conversion capacity of this enzyme is considerably decreased. The alteration in placental GC barrier was apparent not only at high doses of GC but also at low doses of dexamethasone. These observations suggest that synthetic steroids modulate the activity of 11β-HSD2 on the post-translational level. Furthermore, these results highlight the importance of functional analysis in the investigation of the effects which various exogenous compounds could have on the activity of placental enzymes.