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*Original Research*

# Expression of Constitutive and Inducible Cytochromes P450 in Fetal and Newborn Rat Liver

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## Abstract

The developing organism lacks many of the cytochrome P450 isoforms detected in the adult, or they are expressed at very low levels. It remains controversial whether P450 gene regulatory mechanisms are present prenatally. As a result, the catalytic function of P450s in fetal tissues has been questioned. The aim of our study was to evaluate CYP: 1A1, 1A2, 2B1/2, 2E1, 3A1 and 3A2 expression in livers of 18- and 20-days-old fetuses, and newborns from untreated and β-naphthoflavone-, phenobarbital-, dexamethasone- or ethanol-treated Sprague-Dawley rats. CYP expressions were evaluated at both transcriptional (RT-PCR) and protein (Western blotting) levels. CYP mRNA expressions were detected on day 18 of gestation. CYP: 1A1, 2B1/2 and 3A1 proteins were found on day 18; CYP2E1 protein – on day 20; 1A2 and 3A2 protein – in newborn livers. Studied P450s demonstrated a very low expression in animal tissues before and just after birth but, in most cases, they were inducible. It is concluded that the inductory mechanisms of CYP: 1A1, 2B1/2, 3A1/2 and 2E1 but not CYP1A2, are functional in fetal liver at transcriptional or translational levels. The effects of metabolic activation of CYP1A2 substrates may be reduced in fetuses.

**Keywords:** cytochrome P450, CYP1A1, CYP1A2, CYP2B1/2, CYP2E1, CYP3A1, CYP3A2, age, liver.

**Abbreviations:** AHH – aryl hydrocarbon hydroxylase; BNF – β-naphthoflavone; b.w. – body weight; Dex – dexamethasone; Et – ethanol; i.p. – intraperitoneally; 3-MC – 3-methylcholanthrene; PB – phenobarbital; PROD – pentoxyrezorufin deethylase; SD rats – Sprague-Dawley rats; TCDD – 2.3.7.8-tetrachlorodibenzo-p-dioxin.

## Introduction

Cytochromes P450 are the main element of the monooxygenase system, which participates in the I$^{st}$ phase of xenobiotics biotransformation and may lead to metabolic activation of substrates, increasing their toxic and carcinogenic properties. Constitutive and induced changes of P450 expressions underlie their catalytic activities. They determine individual variability of xenobiotic metabolism as well as personal sensitivity to metabolites' action. In fetal liver, they may be responsible for both cell exposure to active metabolites and the effectiveness of xenobiotic metabolism [1]. The developing organism lacks some of the cytochrome P450 isoforms detected in the adult or they are expressed at very low levels. It remains controversial which P450 gene regulatory mechanisms are pres-

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ent prenatally. As a result, the catalytic function of P450s in fetal tissues has been questioned. [2-5].

Furthermore, cytochrome P450 expression in pregnant mother organs, including placenta, can be a risk factor for a developing fetus. In rat placenta the expression of CYP3A1 protein was detected before the 19th day of pregnancy, CYP1A1 between the 19th and 21st days of pregnancy and CYP2E1 – 2 days before delivery. In human placenta, a few CYP isoforms were detected on the level of mRNA. The protein expression and its catalytic activity was confirmed for CYP1A1 and CYP2E1 [6]. CYP1A1-dependent activities were higher in placentas taken from young mothers, which indicates that CYP1A1 functions are age-dependent.

Expression of many P450s, including CYP: 1A1/2, 2B1/2, 2E1, 2C6, 3A2 and 4A1 and their participation in metabolism of xenobiotics was significantly diminished in the liver of pregnant women and female rats. At this time, progesteron and its metabolites as well as endogenous nitrogen oxide may have inhibitory effects on the hepatic expression of P450 [7]. In rats, pregnancy had no effect on CYP: 2C12, 2D1 and 3A1 protein synthesis [8].

Low levels of P450 expression and monooxygenase activities during pregnancy limit the production of reactive metabolites both in the placenta and fetal liver, reducing the risk connected with their influence on developing organism [5, 9]. On the other hand, the low biotransforming potential of enzymes involved in activation of prodrugs during pregnancy may significantly increase their half life or decrease their pharmacological effect. It may also lead to the accumulation of toxic hydrophobic compounds in the tissues of mother and fetus.

The aim of the study was to evaluate CYP: 1A1, 1A2, 2B1/2, 2E1, 3A1, and 3A2 expressions in fetal and newborn rat livers from untreated and P450-inducer-treated animals.

## Material and Methods

### Animals

Females of Sprague-Dawley (SD) rats were divided into: one control group (K) and four inducer-treated ones (BNF, PB, Dex, Et), as follows:

(1) Control: pregnant female rats kept under standard conditions;

(2) BNF: pregnant females treated with β-naphthoflavone at a daily i.p. dose of 50 mg/kg b.w. for 2 days before decapitation;

(3) PB: pregnant females treated with phenobarbital at a daily i.p. dose of 80 mg/kg b.w. for 2 days before decapitation;

(4) Dex: pregnant females treated with dexamethasone at a daily i.p. dose of 30 mg/kg b.w. for 2 days before decapitation;

(5) Et: pregnant females given 5% ethanol (6 g/kg b.w.) diluted in isocaloric diet for 21 days before decapitation.

The experiments were carried out on three age groups, as follows:

F18: 18-day-old fetuses;

F20: 20-day-old fetuses;

N1: Newborns.

CYP expressions were studied in normal liver (K) and induced ones (BNF, PB, Dex, Et) at both transcriptional (RT-PCR) and protein (Western blotting) levels.

### RT-PCR

Total cellular RNA was isolated from 30 mg of rat liver tissue by acid guanidinium thiocyanate/phenol/chloroform extraction [10] using commercially available kit (Trizol TM). RNA was reverse transcribed into cDNA. RT reaction was carried out for 1h at 42°C. The PCR reaction mixture contained primers specific for rat CYP: 1A1, 1A2, 2B1/2, 2E1, 3A1, 3A2 and GAPDH

Table 1. List of primers used in PCR reactions.

| Gen | Sequence of primers | Hybridization temperature (°C) | Product size (bp) | References |
|---|---|---|---|---|
| CYP1A1 | F) 5'-GAT GCT GAG GAC CAG GAA ACC GC<br>R) 5'-CAG GAG GCT GGA CGA GAA TGC | 64.2 | 679 | [11] |
| CYP1A2 | F) 5'-CTG CAG AAA ACA GTC CAG GA<br>R) 5'-GAG GGA TGA GAC CAC CGT TG | 57.4 | 139 | [12] |
| CYP2B1/2 | F) 5'-CCA AGC CGT CCA CGA GAC TT<br>R) 5'-TTG GGA AGC AGG TAC CCT C | 56 | 380/404 | [11] |
| CYP2E1 | F) 5'-GGA TGT GAC TGA CTG TCT CC<br>R) 5'-TGG GGT AGG TTG GAA GGG AC | 54 | 447 | [12] |
| CYP3A1 | F) 5'-CCG CCT GGA TTC TGT GCA GA<br>R) 5'-TGG GAG GTG CCT TAT TGG GC | 62.9 | 203 | [12] |
| CYP3A2 | F) 5'-TTG ATC CGT TGC TCT TGT CA<br>R) 5'-GGC CAG GAA ATA CAA GAC AA | 54 | 323 | [13] |
| GAPDH | F) 5'-GTG AAC GGA TTT GGC CGT ATC G<br>R) 5'-ATC ACG CCA CAG CTT TCC AGA GG | 66 | 543 | [14] |

(shown in Table 1). Amplification was carried out by 26 repeated cycles at 94°C for 1 min, 54-66°C (gradient temperature) for 1.5 min and 72°C for 1 min on Programmable Thermal Controller PTC-200 (MJ Research, Inc., Watertown, MA).

An aliquot of each reaction mixture (15 µl) was subjected to electrophoresis on 2% agarose gels. To standarize the protocol the products of GAPDH amplification were always run on the same gel as studied P450 isoforms. Gels were stained with ethidium bromide and analyzed with One D-scan software (Scanalytics).

### Western Blotting

In the microsomal fraction isolated according to Dallner [15] the protein content by Lowry et al. [16] and the expression of particular P450 proteins by Western blot immunoassay were determined. Microsomal samples (5-70 µg of protein) were subjected to polyacrylamide gel (8%) electrophoresis in the presence of sodium dodecyl sulphate as described by Laemmli [17]. The electrophoresed proteins were blotted electrophoretically onto PVDF membrane (Milipore) and stained immunochemically. CYP: 1A1, 2B1/2, 2E1, 3A1 and 3A2 proteins were identified with rabbit anti-rat polyclonal antibodies (Chemicon). CYP1A2 protein was detected using sheep anti-rat CYP1A2 polyclonal antibody. The binding of primary antibodies was detected with goat anti-rabbit or rabbit anti-sheep secondary antibody conjugated with alkaline phosphatase (Chemicon). The reaction was developed with BCIP/NBT liquid substrate system (Sigma) according to manufacturer's instructions. The molecular weight and intensities of stained bands were analyzed with One D-scan software (Scanalytics).

### Statistical Analysis

Rat liver RNA and liver microsomes were prepared from three individual livers or pools of 15-20 livers for RT-PCR and Western blot analysis, respectively. Gels obtained from RT-PCR and immunoblots were quantified by densitometry, and the results were expressed on graphs as Integrated Optical Density (IOD). Statistical significance was estimated with Student's t-test ($p<0.05$).

## Results

### CYP1A1

In 18- and 20-day-old fetuses the constitutive expression of CYP1A1 mRNA was very low; however, it raised 5.5 times in newborns ($p<0.01$, comparing to F20 group). Induction with BNF resulted in increase of CYP1A1 mRNA in all studied age groups; however, this was statistically significant in group F20 only (Fig. 1A).

We showed the presence of CYP1A1 in the microsomal fraction of the liver from 18- and 20-day-old fetuses (after loading 70 µg of protein per slot) as well as from newborns (Fig.1B). The expression of CYP1A1 protein raised after BNF induction (13-, 18- and 3.7-times in F18, F20 and N1 group, respectively) but the observed increase was statistically significant in the F20 group only ($p<0.05$).

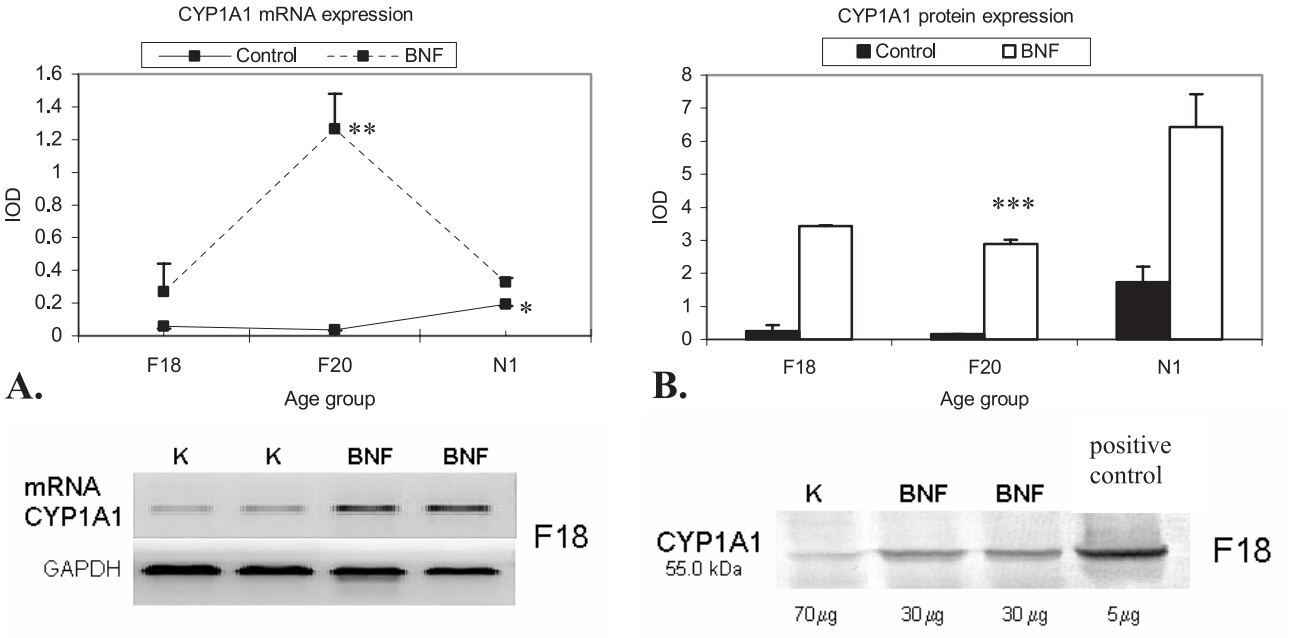


Fig. 1. Constitutive and BNF-inducible expression of CYP1A1 in fetal and newborn rat livers. A – CYP1A1 mRNA (n = 3); B – CYP1A1 protein (n = 3 pools). * – $p<0.01$ *vs* F18 and F20; ** – $p<0.01$ *vs* Control;. *** – $p<0.05$ *vs* Control; Positive control – liver microsomes isolated from BNF-induced adult rat.

## CYP1A2

Very low constitutive expression of CYP1A2 mRNA in the fetal groups (F18, F20) increased in newborns (Fig. 2A). In the latest group the inducible effect of BNF on the expression of CYP1A2 mRNA was evident (3 times increase) although statistically not significant.

The presence of CYP1A2 in the fetal liver was not detectable even after loading 70 µg of protein per well. The band corresponding to CYP1A2 showed in presence of microsomal proteins isolated from newborn livers (Fig. 2B). Intraperitoneal injection of BNF into the mothers did not result in the induction of CYP1A2 expression in the fetuses. The induction of CYP1A2 protein synthesis observed in newborns (1.6x) was statistically not significant.

## CYP2B1/2

Low constitutive expression of CYP2B1/2 mRNA was confirmed in both F18 and F20 fetal groups (Fig. 3A). In N1 group the expression increased 8 times ($p<0.01$). The effect of PB on the content of CYP2B1/2 mRNA was evident, although statistically not significant, in all studied age groups. The observed differences in mRNA content between rats induced with Dex and controls, were small and insignificant.

After immunoblotting of hepatic microsomal proteins isolated from 18- and 20-day-old fetuses only a faint band corresponding to CYP2B1/2 protein showed up (Fig. 3B). In comparison with fetuses, there was a more than 80-fold increase in the content of CYP2B1/2 protein in the livers of newborns. Induction with PB was evident on the protein level, both in fetuses (5x) and newborns (2.3x), although the observed changes were statistically not significant. 20-day-old fetuses responded to Dex induction with increased, 26-fold expression of CYP2B1/2 protein. However, this inductory effect was lost just after delivery.

## CYP2E1

The presence of CYP2E1 mRNA in the livers of 18- and 20-day-old fetuses (Fig. 4A) and increase of its content in the newborns ($p<0.05$) was shown. Et induced CYP2E1 mRNA expression significantly both in fetuses (F18) and newborns.

CYP2E1 protein was detected in the F20 group after loading 70 µg of protein per well (Fig. 4B) and its expression raised 90-fold in group N1. In 20-day-old fetuses the level of CYP2E1 protein increased 1.5-fold and in newborns it increased 2.5-fold ($p<0.001$) after induction with Et.

## CYP3A1

In all studied age groups the constitutive expression of CYP3A1 mRNA was detectable (Fig. 5A). In newborns

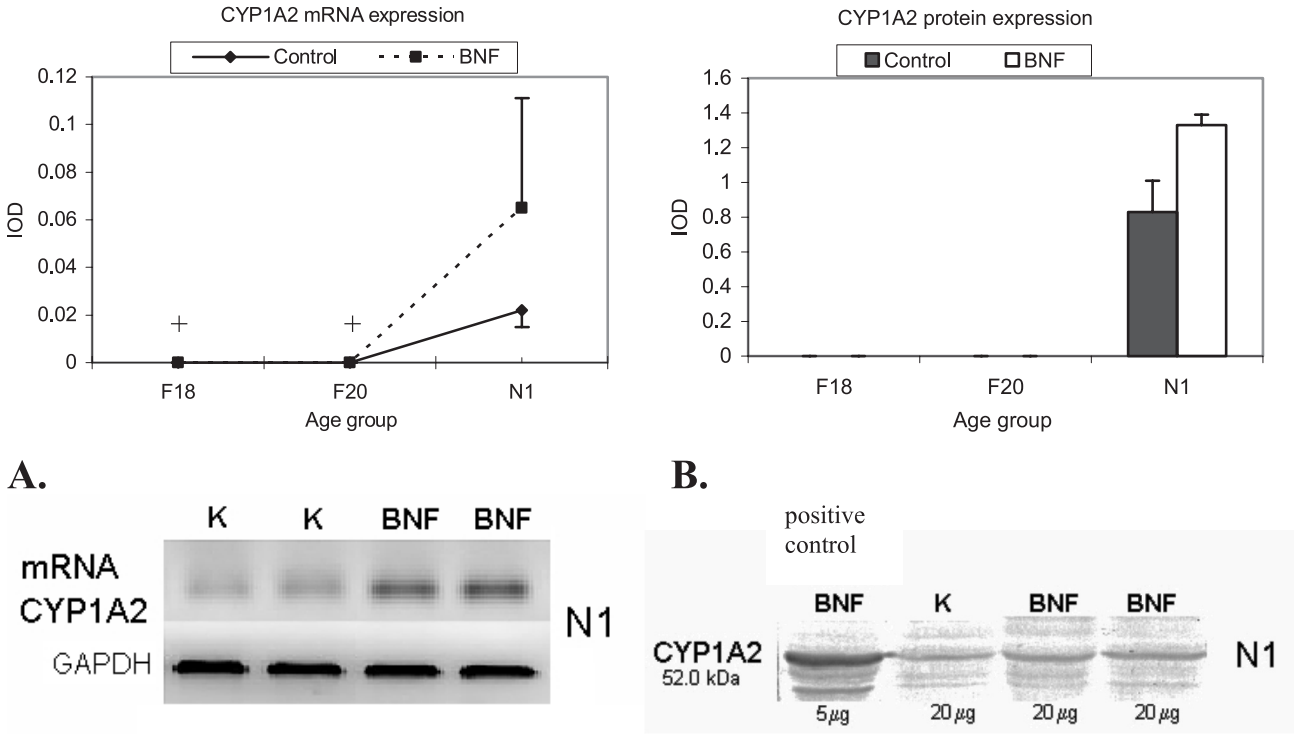


Fig. 2. Constitutive and BNF-inducible expression of CYP1A2 in fetal and newborn rat livers. A – CYP1A2 mRNA (n = 3); B – CYP1A2 protein (n = 3 pools). + – detectable, but very low expression level both in K and BNF group; Positive control – liver microsomes isolated from BNF-induced adult rat.

the expression was over 6-fold higher than in 20-day-old fetuses ($p<0.01$). Both in fetuses and newborns the changes in CYP3A1 mRNA expression after stimulation with PB were statistically not significant. Dex stimulated the transcription of CYP3A1 mRNA from the 20th day of fetal life.

Hepatic CYP3A1 protein was present in all studied age groups (Fig. 5B). Between the 18th day of fetal life and the 1st day of postnatal life the amount of CYP3A1 protein increased 25-fold. PB had no statistically significant effect on the amount of CYP3A1 protein in the microsomal fraction isolated from fetal livers, but it stimulated CYP3A1 three-fold. Induction by Dex was statistically significant in group F20, where a 14-fold increase in the amount of CYP3A1 protein was noted.

## CYP3A2

Constitutive expression of CYP3A2 mRNA was confirmed in F18, F20 and N1 groups (Fig. 6A). Changes in CYP3A2 mRNA expression after PB induction were noted in all age groups, but they were statistically significant only in the group of newborns. After Dex significant induction was noted in the F20 group only ($p<0.01$).

On the level of translation the result of immunoblotting unequivocally confirming constitutive expression of CYP3A2 in hepatic microsomes was noted in newborns (Fig. 6B). The inducible effect of PB was noted both in fetuses (although very weak) and newborns ($p<0.05$). The inducible effect of Dex was small in 18-day-old fetuses, ten times higher in group F20 and 36 times higher in group N1 (if compared to F18).

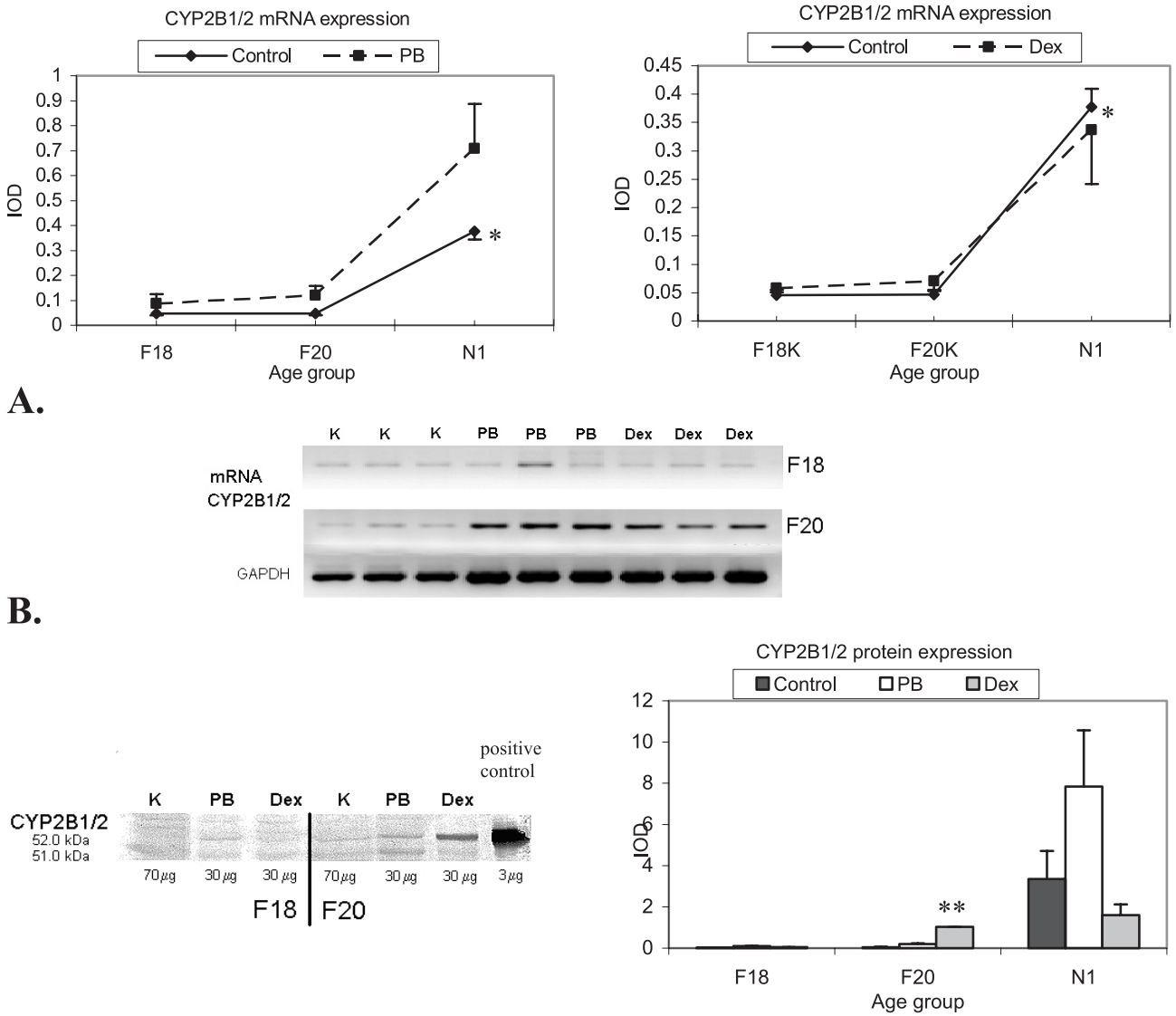


Fig. 3. Constitutive and PB or Dex-inducible expression of CYP2B1/2 in fetal and newborn rat livers. A – CYP2B1/2 mRNA (n = 3); B – CYP2B1/2 protein (n = 3 pools). * – $p<0.01$ *vs* F20; ** – $p<0.05$ *vs* Control; Positive control – liver microsomes isolated from PB-induced adult rat.

## Discussion

Early studies on rats suggested a lack of both constitutive and induced expression of mRNA and many CYP proteins in the fetal liver [4, 18-20]. However, it was shown that mechanisms of CYP1A1 and CYP2B1/2 induction in rat liver are efficient 1 day before the end of pregnancy. CYP1A1/2 synthesis and adequate monooxygenase activities were induced by polychlorinated biphenyls or a sigle dose of i.p. delivered 3-methylcholanthrene (3-MC), 40 mg/kg b.w. [21-23]. Using the immunoblotting method we showed that on the 21st day of pregnancy constitutive expression of CYP1A1 and 1A2 is not present in fetal rat liver. At the same time BNF, a noncarcinogenous inducer whose effect is similar to 3-MC [24], induced expression of CYP1A1 protein, although it did not stimulate synthesis of CYP1A2 [19, 25]. The expression of CYP1A2 protein appeared later than CYP1A1, despite having the same regulatory mechanism. In the next experiment we detected weak but clear bands corresponding to CYP1A2 in liver microsomes of 21-day-old fetuses, both control and taken from mothers exposed to cigarette smoke (after loading 65 µg of microsomal proteins per slot). We did not ascertain the inductive effect of smoke on 1A2 expression, which indicates that even if constitutive expression of 1A2 proteins was activated one day before birth, the CYP1A2 inductory mechanism was still not fully developed at this time [25].

Measurements of hepatic aryl hydrocarbon hydroxylase (AHH) activity in fetal rats showed that CYP1A1 synthesis can take place before the 21st day of pregnancy. On the 17th day of pregnancy a 100-fold induction of AHH was observed after applying a single dose (2.5 µg/kg b.w.) of TCDD (2.3.7.8-tetrachlorodibenzo-p-dioxin). A single dose of 3 µg TCDD/kg b.w. applied to females *per os* caused an 11-fold induction of AHH on the 16th day [26]. This study showed that constitutive expression of CYP1A1 mRNA is present in the fetal liver of rats at least since the 18th day of fetal life and is accompanied by a slight expression of 1A1 protein. The expression of CYP1A1 in 18- and 20-day-old fetuses was susceptible to induction by BNF, both on the transcriptional and translational levels, although statistically significant differences were noted only in the F20 group. These observations agree with studies showing that after applying 3-MC to pregnant mice and the induction of CYP1a1, the Ah receptor – involved in the regulation of CYP1A genes – is functional in early embryogenesis [27].

The above results also proove that CYP1A1, which in postnatal life is an isoform characteristic for extrahepatic organs, can play a significant physiological role in the fetal liver. The importance of developmentaly-dependent CYP1A1 gene activation is not limited only to initiation of the metabolism of xenobiotics, but it is also tied to morpho- and embryogenesis, and the cell cycle control [28].

In the presented study we did not show the presence of 1A2 protein in the fetal liver on the 18th and 20th day of pregnancy; however, we were able to confirm the expression of CYP1A2 mRNA. The inductive effect of BNF on

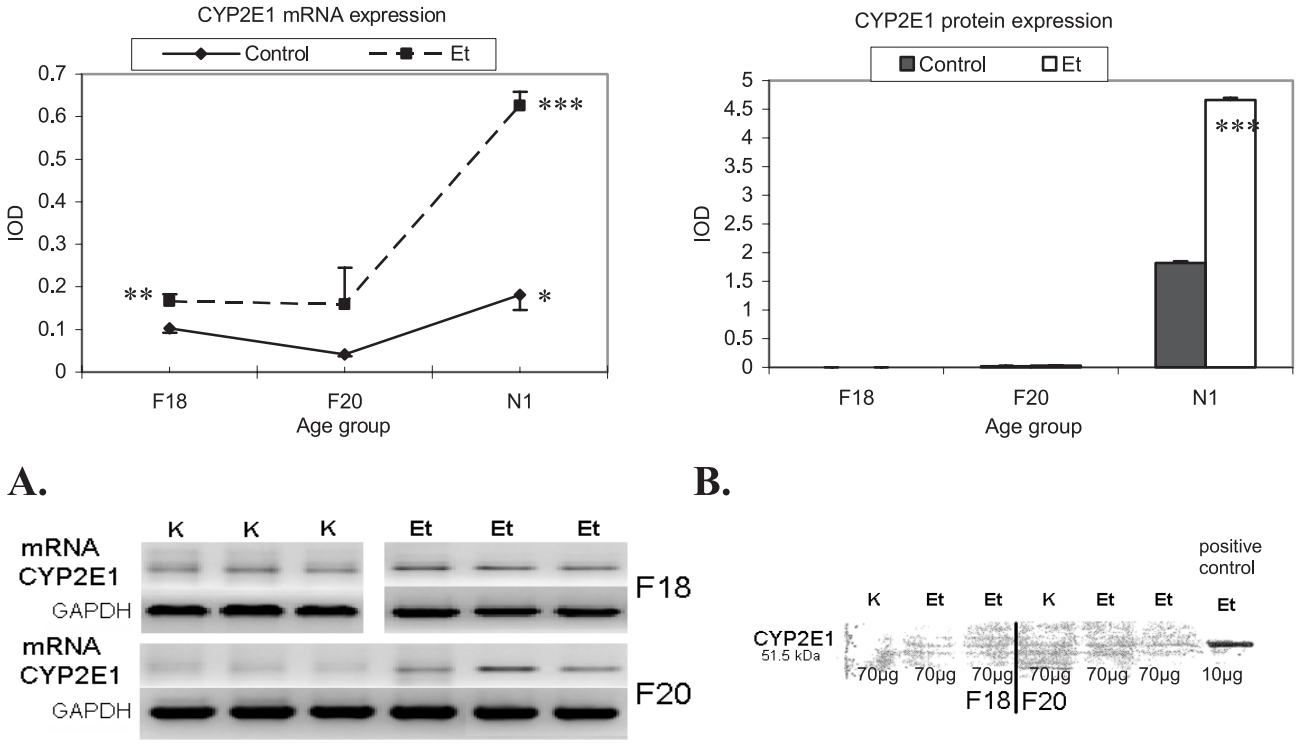


Fig. 4. Constitutive and Et-inducible expression of CYP2E1 in fetal and newborn rat livers. A – CYP2E1 mRNA (n = 3); B – CYP2E1 protein (n = 3 pools). * – p<0.05 *vs* F20; ** – p<0.01 *vs* Control; *** – p<0.001 *vs* Control; Positive control – liver microsomes isolated from Et-induced adult rat.

CYP1A2 mRNA appeared in group N1. The 3-fold rise in the mRNA expression level accompanied by a slight rise in 1A2 protein suggests that mechanisms of induction in the perinatal period were activated. It cannot be finally excluded, that constitutive expression of CYP1A2 protein is activated in the fetal liver 1 day before birth, while the induction mechanism develops slightly longer and is efficient in newborns.

CYP2B isoforms involved in the catabolism of nicotine to cotinine and, in newborns, responsible for CYP2B1/2 dependent activity of pentoxyrezorufin deethylase (PROD), were found in fetal livers of mice and hamsters. PROD activity was not detected in rat liver homogenate until the 7th day of postnatal life. However, PB applied i.p. to pregnant rat females induced CYP2B1/2 mRNA in fetal livers on the 15th and 17th days of pregnancy [3, 29, 30]. In the presented experiment, constitutive and induced expression of CYP2B1/2 mRNA was registered in fetal livers on the 18th and 20th day of pregnancy. The constitutive expression of mRNA was accompanied by the 2B1/2 protein. PB induced protein expression similarly in both age groups, while Dex showed a stronger inductive effect that was statistically significant in the 20-day-old group. It seems that, in rats, isoforms of the 2B family are both constitutively and inductively expressed from the 18th day of fetal life. In our previous studies on 21-day-old fetuses, by means of immunoblotting method, we detected proteins that corresponded to the CYP2B1/2, after induction with PB as well as with high doses of cigarette smoke [31].

Since the constitutive expression of mRNA, apoprotein or CYP2E catalytic activity in fetal rats, hamsters [3] and rabbit [32] liver was not shown in early studies, it was suggested that CYP2E1 and CYP2E2 genes are activated in the first hours (or days) after birth in a species-specific way. Activation of the rat 2E1 gene was noticed 6 hours after delivery [5, 9, 33]. Nevertheless, it was suggested that CYP2E1 mRNA can be present in the fetal rat liver at the end of pregnancy, but its expression is diminished by stress during delivery. A stimulating effect of some

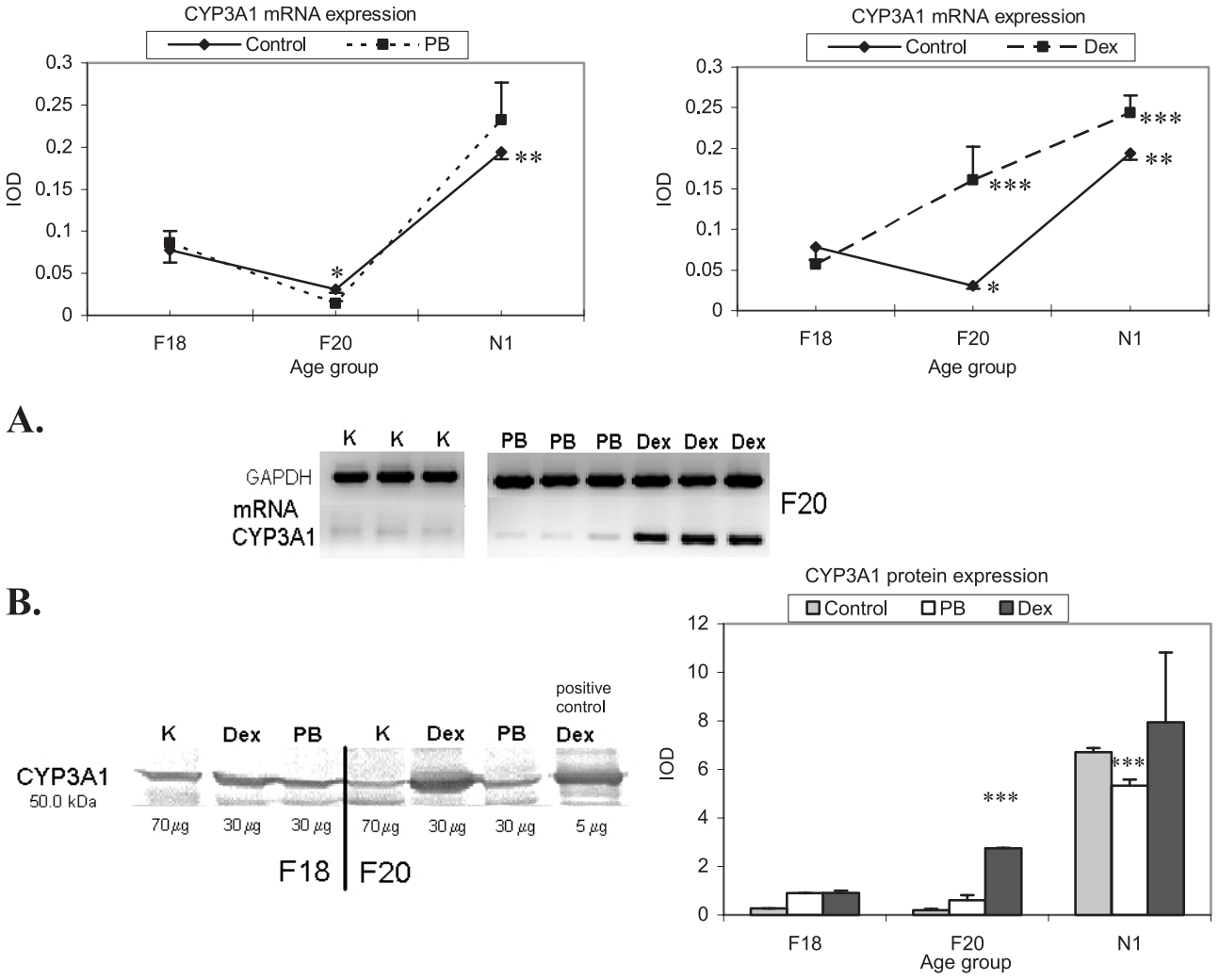


Fig. 5. Constitutive and PB or Dex-inducible expression of CYP3A1 in fetal and newborn rat livers. A – CYP3A1 mRNA (n = 3); B – CYP3A1 protein (n = 3 pools). * – $p<0.05$ *vs* F18; ** – $p<0.01$ *vs* F18 and F20; *** – $p<0.05$ *vs* Control; Positive control – liver microsomes isolated from Dex-induced adult rat.

CYP2E1 inducers in the fetal liver was confirmed, i.e. acetone – in rats, Et – in hamsters [3, 19, 34]. In our earlier studies both constitutive and Et-induced expression of CYP2E1 was noticed 1 day before birth. CYP2E1 was not induced by cigarette smoke, which indicates that on the 21st day of fetal life only some of the 2E1 inductory mechanisms are functional [19, 25].

It was also documented that microsomes isolated from fetal rat livers on the 20th day of pregnancy have the ability to metabolize carbon tetrachloride, aniline and $N_1$N-dimethylnitrosoamine. Oxidation of these substrates corresponded with higher levels of 2E1 protein and a rise of CYP2E1 mRNA level. The fetal isoform 2E1 induced by Et on the 20th day of pregnancy showed different catalityc activity in comparison with the protein induced in adult animals [9].

In our study CYP2E1 mRNA transcription in the fetal liver was an active process and it was significantly induced by Et from the 18th day of fetal life. It did not correlate with the activation of 2E1 protein synthesis: the constitutive 2E1 protein was measurable in 20-day-old fetuses but post inductive, statistically significant changes in 2E1 concentration were noted in newborns. The lack of correlation between CYP2E1 levels and the metabolism of chlorzoxazon [9] suggested that in 20-day-old fetus, the liver enzyme is unable to metabolize this substrate. However, taking into consideration the cited earlier studies [20, 25] and a 1.5-fold induction of CYP2E1 protein in group F20, the mechanism of CYP2E1 translation can be susceptible to induction by Et starting from the 20th-21st days of pregnancy, while induction by other substances i.e. presented in cigarette smoke, definitely later – during the first few days after birth. Since CYP2E1 takes part in the metabolism of endogenous substances like acetone or arachidonic and lauric acids, the fact that it is present in the prenatal period should not be a surprise. It can be assumed that the inducible effect of xenobiotics on fetal CYP2E1 is a marker of its natural protective function dur-

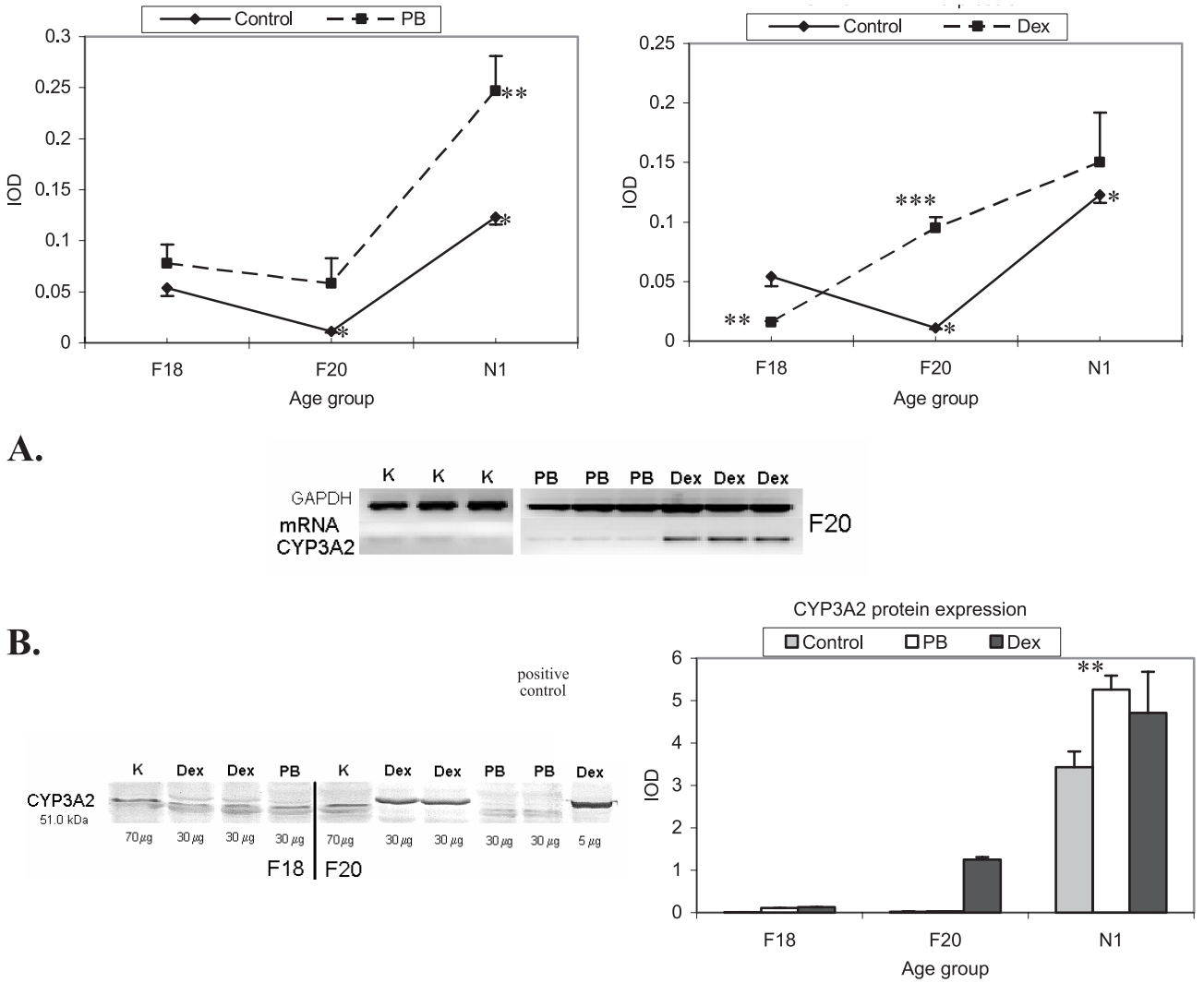


Fig. 6. Constitutive and PB or Dex-inducible expression of CYP3A2 in fetal and newborn rat livers. A – CYP3A2 mRNA (n = 3); B – CYP3A2 protein (n = 3 pools). * – p<0.01 *vs* F18 and/or F20; ** – p<0.05 *vs* Control; *** – p<0.01 *vs* Control; Positive control – liver microsomes isolated from PB-induced adult rat.

ing prenatal development. It is even suggested that the Et induction of CYP2E1 in the fetal liver is higher than in the mother's liver, which can be due to a different hormonal regulation of 2E1 expression or larger accumulation of xenobiotics in the fetal liver [9, 35].

In newborn rat livers and in the early postnatal life, Et, which is taken in with mother's milk, raised the CYP2E1 levels and the oxidation of its substrates. Et concentration in the rat's mammary gland was 10% higher than in the blood [5]. This compound slows down oxytocine release and milk secretion, which explains the slower growth of newborns. Moreover, starvation induces CYP2E1, increasing its mRNA expression. It is possible that the induction of this isoform in fetuses and newborns treated with Et is, for the most part, the effect of a higher level of CYP2E1 mRNA expression caused by feeding and hormonal interactions.

One of the P450 isoforms that metabolizes xenobiotics, which first appear in the liver during ontogenesis, is CYP3A1. Using the RT-PCR technique Omiecinski et al. [30, 36] identified CYP3A1 mRNA in livers of 15-day-old rat fetuses and showed their susceptibility to PB induction in a dose of 3 x 50 mg/kg b.w. We detected CYP3A1 mRNA and protein on the 18$^{th}$ day. The induction of CYP3A1 mRNA transcription by PB (2 x 80 mg/kg b.w.) in fetal groups turned out to be ineffective. Activation of 3A1 protein synthesis by PB (3-fold increase in 18- and 20-day-old fetuses), was statistically not significant. On the other hand, the results of immunohistochemistry and immunoblotting accomplished by Ejiri [37] prove that CYP3A1 proteins are susceptible to Dex induction in 17-day-old rat fetuses after one or four doses of the inducer. A single i.p. dose of 100 mg/kg b.w. applied to pregnant females induced expression of CYP3A1 more effectively than 4 doses of 25 mg/kg b.w. We showed a 3-fold rise in 3A1 protein concentrations, although statistically not significant, after Dex injection (2 x 30 mg/kg b.w.) on the 18$^{th}$ day of pregnancy. From the 20$^{th}$ day Dex controlled the mechanism of induction of both CYP3A1mRNA and protein, which resulted in a relevant rise of its expression.

In the case of CYP3A2 we observed a major time shift in activation of its constitutive mRNA and protein expression. CYP3A2 mRNA, like CYP3A1, was present in the fetal liver from the 18$^{th}$ day of pregnancy, while 3A2 protein appeared later – in the newborn group. Both CYP3A2 mRNA and protein were inducible from the 18$^{th}$ day of fetal life.

Cresteil [38] suggested that in humans, ontogenesis of P450 enzymes involves three main groups of cytochromes:

1. expressed in fetal liver and metabolizing endogenous substrates (CYP3A7);
2. expressed in early newborn life during the first hours after delivery (CYP2D6 and CYP2E1) and showing minimal expression in fetal liver;
3. expressed relatively late in newborn life (CYP1A2, CYP2C and CYP3A4). CYP1A2 is the last isoform expressed in developing human liver.

We showed that during ontogenesis of SD rats studied P450s demonstrated very low expression in animal tissues before birth but, in most cases they were inducible. P450 mRNA expressions were detectable from the 18$^{th}$ day of pregnancy. Hepatic P450 proteins appeared successively:
(1) CYP: 1A1, 2B1/2, 3A1 on the 18$^{th}$ day of pregnancy;
(2) CYP2E1 on the 20$^{th}$ day of pregnancy;
(3) CYP1A2 during the perinatal period and CYP3A2 in newborns.

Expression of 3A2 was susceptible to chemical induction already from the 18$^{th}$ day of fetal life; expression of CYP: 1A1, 2B1 and 3A1 – from the 18-20$^{th}$ day; expression of CYP2E1 from the 20$^{th}$ day, and expression of 1A2 from the first day of extrafetal life.

## Conclusions

Between the 18$^{th}$ and 20$^{th}$ days of fetal life the rat liver expresses constitutive CYP: 1A1, 2B1/2, 3A1 and 2E1 protein. The inductory mechanisms of CYP: 1A1, 2B1/2, 3A1/2 and 2E1, but not CYP1A2 are functional in fetal liver at transcriptional or translational levels. The effects of metabolic activation of CYP1A2 substrates may be reduced in fetuses.

## Acknowledgements

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