Gender-specific differences in cytochrome P450 in microsomes from post-pubescent pigs – implications for expression and activity

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Abstract

The gender-specific differences in cytochrome P450 (CYP) enzymes in liver has been studied both on protein expression and activity levels. The differences found in activity were not associated with protein amount as measured with Western blotting, indicating a direct inhibition of enzyme activities by some endogenous compounds, which are expressed differently in male and female pigs.

Introduction

In porcine liver the three isoforms of cytochrome P450 (CYP), *1A2*, *2A* and *2E1*, are involved in the Phase I metabolism of skatole. Skatole is associated with an off-odour and off-taste of pig meat. Thus the activity and expression of these enzymes are important for the regulation of skatole concentrations in adipose tissue. High concentrations of skatole in the adipose tissue are mainly found in male pig. This is partly due to the presence of steroids of testicular origin. The differences between genders have been reported in the pigs at body weight of 35-45 kg (Skaanild & Friis 1999) and in older pigs (Zamaratskaia et al, 2006). It was shown that at 90 kg body weight the activity of CYP2A were higher in female pig, while there were no differences between genders with respect to CYP2E1 activity. At 115 kg, the CYP2E1 activity differed, while there were no gender-related differences in CYP2A activity. The mechanisms behind this difference are not well-understood; however previous results has showed that gender related differences in CYP activity can be a result of the direct inhibition by steroids of testicular origin (Zamaratskaia et al, 2007; Rasmussen et al, 2010).

The aim of this study was to investigate gender-related differences in catalytic activities of CYP2E1, 1A2 and 2A in pigs at high slaughter weight. Furthermore, we investigated if enzyme expression could explain potential differences in the activities.

Method

Animals and sample preparation

Entire male and female pig were raised under similar conditions and slaughtered at the same age. All animals were crossbreeds between Landrace x Yorkshire sire and Duroc boars. Four entire male and six female pigs were raised under same conditions, fed *ad libitum* with a standard diet and slaughtered at the age of 164 days (approximately 120 kg). Liver samples were taken at slaughter and stored at – 80 °C.

Preparation of liver microsomes

Frozen liver tissue was minced and homogenized in a sucrose-containing buffer. After a 20 min centrifugation at 10.000 x g (4 °C), the supernatants were centrifuged at 100.000 x g for 60 min at 4 °C. The resulting pellets were used for western blotting and for enzymatic assays.

Western blotting

Total protein content of the microsomes was separated by SDS-PAGE. After electroblotting onto a PDVP membrane, the membrane was incubated with primary antibodies over night at 4 °C. Afterwards the membrane was incubated with Alexaflour448 attached secondary antibodies. The relative protein concentration was visualized by scanning on a molecular imager® FX (BioRad) scanner and quantified with Quantity One version. 4.5.2 (BioRad).

Specific Cytochrome P450 activity

The specific activity of CYP1A, CYP1A2, CYP2A and CYP2E1 was measured as ethoxyresorufin *O*-dealkylation (EROD), methoxyresorufin *O*-dealkylation (MROD), coumarin hydroxylation and *p*-nitrophenol hydroxylation, respectively. All measurements were done as previously described by Rasmussen *et al* (2010) and Zamaratskaia and Zlabek (2009). Briefly, 0.2-0.4 mg of total protein were incubated in appropriate buffer with 1 mM NADPH and one of the following substrates ethoxyresorufin, methoxyresorufin, coumarin or *p*-nitrophenol (Table 1).

Statistics

In Western blotting analysis, samples to be compared were run on the same gel with equal amounts in each lane. Protein concentration of microsomes from female pigs was expressed as mean ± SEM relative to the mean of samples from male pigs. Differences in relative protein concentrations and estimated enzyme activity were evaluated by Student's unpaired t-test.

Results

In the microsomes from female pigs the e activities of CYP2A and CYP1A2 were significantly higher than in male pigs. The activity of CYP2E1 was numerically 2 times higher in female pigs compared to male pigs. This increase was however not significant, due to a large variation in the male group.

There were no differences in the protein expression of CYP2E1, 1A2 and 2A6 between male and female pigs, besides a tendency to a slight decrease in expression of CYP2E1 in female pigs.

Discussion

The activities of CYP2A and CYP1A2 are significantly different in female and male pigs. However, those differences were not associated with protein expression as measured with Western blotting. That indicates that the cause of the difference in the activities could be due to some downstream regulation, for instance due to differences in concentrations of testicular steroids or other compounds in the circulation in female and male pigs.

It has been suggested that steroids are effective via gene regulation (nuclear receptors and transcription factors like CAR etc), but the results from this study indicate that the differences between genders may also be due to direct inhibition of CYP enzymes by some endogenous compounds. Those compounds can be testicular steroids, which were shown to have a potential to alter CYP2E1 and CYP2A directly (Zamaratskaia et al. 2007), possibly through a competitive mode of inhibition. The kinetics of steroids inhibition of CYP enzyme activities remain to be studied.

References

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Figure 1

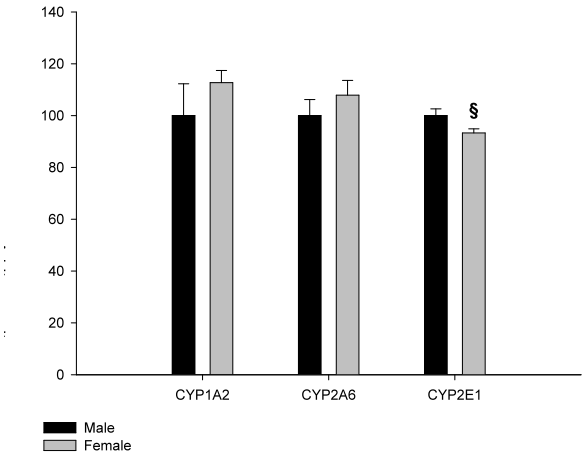

Figure 1: Expression of CYP450 isoforms in the microsomes from male and female pigs assessed by western blotting with specific antibodies. Values are expressed in arbitrary numbers relative to male pig (arbitrary set to 100). § $p < 0.1$.

Table 1

CYP enzyme-dependent activity in the microsomes from entire male or female pigs. Activities are expressed as pmol/min/mg protein

	Male	**Female**
CYP-dependent activity		
7-methoxyresorufin *O*-demethylation *(CYP1A2)*	8.2 ± 0.6	13.7 ± 1.7**
7-ethoxyresorufin *O*-deethylation *(CYP1A)*	38.1 ± 4.6	62.4 ± 6.0**
Coumarin 7-hydroxylation *(CYP2A)*	3.0 ± 1.1	22.7 ± 7.4*
p-nitrophenol hydroxylation *(CYP2E1)*	20.6 ± 9.1	41.0 ± 5.5

+. Values are given as mean activities with SEM. Levels of significance: ** $p < 0.01$, * $p < 0.05$.