EFFECTS OF SEX STEROIDS ON EXPRESSION OF ANDROSTENONE-METABOLISING ENZYMES IN ISOLATED PIG HEPATOCYTES

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Introduction

Boar taint is a meat quality defect which occurs due to an excessive accumulation of two major compounds, skatole and androstenone, in pig adipose tissue (Bonneau, 1982). One of the reasons for high levels of skatole and androstenone is a low rate of degradation of these compounds in pig liver (Squires and Lundstrom, 1997; Doran et al., 2004). The pig hepatic skatole metabolism has been extensively studied, whilst metabolism of androstenone has received less attention. Androstenone metabolism involves two stages: oxidative and conjugative. Oxidative stage is catalysed by the enzyme 3*β*-hydroxysteroid dehydrogenase (3*β*-HSD), which reduces a keto-group at the 3-position to a hydroxyl-group in the *β*-conformation (Doran et al., 2004; Sinclair et al., 2005). The second stage of androstenone metabolism is conjugation and involves sulfotransferases (SULTs). Sulfoconjugation increases the water solubility of steroids, facilitating their excretion (Sinclair et al. 2005). It has been previously established that pigs with high adipose tissue androstenone have a low hepatic 3*β*-HSD activity, mRNA and protein expression (Doran et al., 2004; Nicolau-Solano et al., 2006). The mechanism regulating expression of pig hepatic androstenone-metabolising enzymes is not clear. Since androstenone level is related to the hormonal status (Zamaratskaia et al., 2005), sex steroids might be one of the factors controlling expression of androstenone-metabolising enzymes, and hence the rate of androstenone degradation.

The present study investigated effects of three sex steroids; testosterone, androstenone and estrone sulphate, on expression of pig hepatic 3*β*-HSD and SUL2B1 using primary cultured pig hepatocytes as a model system.

Materials and Methods

The study was conducted on entire male pigs of a commercial cross-breed (75% Large White x 25% Landrace; n=4 for 3*β*-HSD and n=3 for SULT2B1). The pigs were reared under commercial conditions, fed the same standard diet and had an average carcass weight of 72.5 kg.

Hepatocytes were isolated and cultured as described previously (Doran et al., 2002) in the absence (control) or presence (treatment) of testosterone, androstenone or estrone sulphate. The hepatocytes were pre-incubated at 37 ºC for 24 h prior to the steroid treatment. The steroids were added to hepatocytes at the following final concentrations: 1, 10, 50, 100, 500 and 1000 nM, and the hepatocytes were further incubated for 24 h.

Expression of 3*β*-HSD and SULT2B1 proteins in primary hepatocytes was analysed by Western blotting. Proteins were separated on a SDS gel, electro-blotted onto nitrocellulose membrane, and probed with the appropriate primary and secondary antibodies. The primary antibodies against the porcine 3*β*-HSD were custom-made (see Nicolau-Solano et al., 2006 for details). SUL2B1 antibodies were purchased from Santa Cruz Biotechnology, Inc. (California, USA).The blots were developed using ECL reagent (Amersham, Bucks, UK) and the intensity of the corresponding bands was quantified using the ImageQuant program (Molecular Dynamics, UK).

Results and Discussion

Incubation with testosterone resulted in significant increase in expression of both 3*β*-HSD and SULT2B1 when compared to control cells (Table 1). Induction of 3β-HSD expression by testosterone observed in this study is consistent with reports on induction of 3β-HSD expression by steroids in rats (Naville et al., 1991).

Effect of testosterone on 3*β*-HSD and SULT2B1 expression was concentration-dependent. The levels of hormonal treatment used in the present study are comparable with steroid concentrations used by other researchers for cell culture experiments (Heggland et al. 1997; Sinclair et al., 2005). However, the concentration of testosterone required for activation of 3*β*-HSD expression was lower when

compared to that required for activation of SULT2B1 expression (10 nM and 500 nM respectively). The activatory effect of testosterone has been abolished at high (non-physiological) concentrations of the steroid, which might due to non-specific inhibition of enzyme expression. Such a biphasic response in enzyme expression is common for cell culture experiments (see for example Doran et al. 2002).

Table 1. Effect of testosterone, androstenone and estrone sulphate on expression of 3β-HSD and SULT2B1 proteins (arbitrary units) in cultured primary hepatocytes

Steroid	Added steroids (nM, final concentration)						
	0	1	10	50	100	500	1000
			3β-HSD				
Testosterone	100	104	137[d]	132	117	103	73
Androstenone	100	105	108	108	104	99	95
Estrone sulphate	100	106	98	114	116	121	103
			SULT2B1				
Testosterone	100	98	111	104	157[d]	196[d]	134
Androstenone	100	86	108	106	115	84	96
Estrone sulphate	100	65	56[d]	68	59[d]	58[d]	84

Treatments marked with "d" are significantly different from the corresponding controls (Dunnett's test at the 0.05 level, post hoc).

In contrast to testosterone, androstenone did not have a significant effect on 3β-HSD or SULT2B1 protein expression at the concentrations studied.

Incubation with estrone sulphate did not affect significantly expression of 3β-HSD protein but resulted in reduction of SULT2B1 protein level. The inhibitory effect of estrone sulphate on SULT2B1 protein expression was observed at the range of concentrations 10 to 500 nM.

Conclusions

The present study has established that expression of the androstenone metabolising enzymes 3β-HSD and SULT2B1 in isolated pig hepatocytes is under control of sex steroids testosterone and/or estrone sulphate but not androstenone. Further research is needed to investigate the relevance of these finding to regulation of androstenone metabolism *in vivo* and boar taint development.

References

1. Bonneau, M. (1982). Compounds responsible for boar taint, with special emphasis on androstenone: a review. *Livestock Production Science, 9,* 687-705.
2. Doran, E., Whittington, F.M., Wood, J.D. and McGivan, J.D. (2002) Cytochrome P450IIE1 (CYP2E1) is induced by skatole and this induction is blocked by androstenone in isolated pig hepatocytes. *Chemico-Biological Interactions, 140,* 81-92.
3. Doran, E., Whittington, F.M., Wood, J.D. and McGivan, J.D. (2004). Characterisation of androstenone metabolism in pig liver microsomes. *Chemico-Biological Interactions, 147*, 141-149.
4. Heggland, S.J., Signs, S.A., Stalvey, J.R. (1997). Testosterone decreases 3beta-hydroxysteroid dehydrogenase-isomerase messenger ribonucleic acid in cultured mouse Leydig cells by a strain-specific mechanism. J. Androl. 18, 646-655.
5. Naville, D., .Keeney, D.S., Jenkin, G., Murry, B.A., Head, J.R., Mason, J.I., (1991). Regulation of expression of male-specific rat liver microsomal 3 beta-hydroxysteroid dehydrogenase. *Molecular Endocrinology.5*, 1090-1100.
6. Nicolau-Solano, S.I., McGivan, J.D., Whittington, F.M., Nieuwhof, G.J., Wood, J.D. and Doran, O. (2006). Relationship between the expression of hepatic but not testicular 3β-hydroxysteroid dehydrogenase with androstenone deposition in pig adipose tissue. *Journal of Animal Science, 84,* 2809-2817.
7. Sinclair, P. A., Hancock, S., Gilmore, W.J. and Squires, E.J. (2005). Metabolism of the androstene steroids in primary cultured porcine hepatocytes. *Journal of Steroid Biochemistry and Molecular Biology ,96,* 79-86
8. Squires, E.J. and Lundstrom, K. (1997). Relationship between cytochrome P4502E1 in liver and levels of skatole and its metabolites in intact male pigs. *Journal of Animal Science, 75,* 2506-2511.
9. Zamaratskaia, G., Rydhmer, L., Chen, G., Madej, A., Andersson, H.K. and Lundstrom, K. (2005). Boar taint is related to endocrine and anatomic changes at puberty. *Reproduction of Domestic Animals, 40,* 500-506.