Inhibition of CYP2E1 expression by androstenone: relation to boar taint

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Boar taint

- Boar taint is an offensive odour in the meat of 5-10% of uncastrated male pigs.
- Is due to excessive accumulation in adipose tissue of the natural products skatole and androstenone.
- Can be eliminated by castration.
- An alternative to castration could be a genetic test.
Skatole and androstenone synthesis and degradation

Gut

↓

Skatole

Testis

↓

Androstenone

Liver

Skatole

CYP 2E1

Product

Adipose tissue

(Skatole, Androstenone)

Excreted

? Excreted

Product
CYP2E1 expression in pig liver

CYP2E1 protein

CYP2E1 mRNA

Backfat skatole (ug/g)

CYP2E1 protein (arbitrary units)

CYP2E1 mRNA (arbitrary units)
Effect of androstenone CYP2E1 protein expression in cell culture

[Graph showing the effect of androstenone on CYP2E1 protein expression. The graph compares control, +Skatole, and + Androstenone conditions at 0.5 mM, 0.2 mM, 0.5 mM, and 1 mM concentrations. Asterisks indicate significant differences.]
Objectives

- To investigate the molecular mechanism regulating CYP2E1 expression

- To investigate the mechanism regulating androstenone deposition
Investigation of the molecular mechanism regulating CYP2E1 expression

- To sequence the promoter of the pig CYP2E1 gene
- To identify regulatory elements in the pig CYP2E1 promoter
- To identify transcription factor(s) binding to these regulatory elements
- To investigate effect of androstenone on binding of the transcription factors to CYP2E1 promoter
CYP2E1 Promoter

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-850  CCCCCA GAAACAACCT  
-800  AACAGAAAGG TGAATGAAAA TAGTTTGGAG CTCTTATTTT AAATGAGAAT  
-750  GTCCACACAC ATTAGCACAG ATTTAAACCA ACACAGTAAA AATACTAATT  
-700  TTTTTTTTAG AATTTGACAA AATGACTCTA AACAGTAATT ATCCCTTAAA  
-650  TTTCTACAGT AAAATATAC CTTTTTTTGG TAGTAATCAG AGATGAACCTT  
-600  TTTTGAAATT TGTCAACTCT TTTCCCTTCT CTTTTCCTCC CCCACTGAAT  
-550  TTGCCAGTGTG ATTTCCCATA GTGGAGTGAAT TTCAGATAC TGAATTTCCC  
-500  TTCTCTGGCC CATGAGGCTG GCTGCTGATG ACTCAGTACC ACTGGGATGG  
-450  TCTAGACAGA CCTGCTCGGA GGCTGAGAGT TGCCACAGGA GATGGAGCAA  
-400  GACCGTCCGG ACATCATTGA TGTCGCCCTT CATAAATCCT ACCCCAAAAC  
-350  AACCCATGTA AATATGACAC TCTGTCCAAA CCAAGGTAAA GGAGAGGACA  
-300  GTTCCCCCAAC CTATGTTCCT TGCTCGGGAT TTGGTGAGCT AAATCGTGTG  
-250  ACATGTAAAA CTGACATTGG TGCAGGTGTC AGCAGCCAGT GTTGGCAGAG  
-200  CCCAGGCTAG AGGAAATGGA TGTCTTGGAGT GAGTTCTAAG GGGTAACCAC  
-150  CTCAGGGATC AGCCTTTGAA CTGATAGCCA ACAGCAGCTA ATAATAAACC  
-100  TATATCTTGG GCTGGAGGAA AAGGAAGGTG GCATTGGTTG GCTGGTCACC  
-50   CTCCTTCTCA AGGATGCC TATTTCCAC ACAGCAGCTA ATG  
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Identification of the regulatory elements in CYP2E1 promoter

- Generation of promoter fragments by PCR
- Ligation of the fragments into pGL-basic vector containing luciferase cDNA (generation of constructs)
- Transfection of hepatoma cells with the constructs
- Measuring promoter activity of the constructs as induction of luciferase activity
Promoter Activity of Plasmid Constructs

![Bar chart showing relative promoter activity for various constructs]

-98/+27
-128/+27
-269/+27
-278/+27
-313/+27
-815/+27
Empty Vector

Relative promoter activity
Identification of proteins binding to CYP2E1 regulatory elements

1. Electrophoretic Mobility Shift Assay

- Shifted bands due to specific liver nuclear extract protein binding
- Control probe

Nuclear extract proteins bind to:
- Oligonucleotides corresponding to the binding sites for HNF-1 (lines 2, 3)
- A sequence defined as TGTTCTGACCTCTCTGGG (lines 4-5).
Identification of proteins binding to CYP2E1 regulatory elements

2. Gel supershift assay

TGTTCTGACCTCTCTGGG sequence was identified as the transcription factor **COUP-TF1**.
Effect of androstenone on CYP2E1 promoter activity and COUP-TF1 binding

Promoter Activity

COUP-TF1 binding
Transcription factors HNF-1 and COUP-TF1 are required for activation of CYP2E1 promoter.

Androstenone represses CYP2E1 activity via inhibition of binding COUP-TF1 (but not HNF-1).
Objectives

1. To investigate the molecular mechanism regulating CYP2E1 expression

2. To investigate the relationship between hepatic androstenone metabolism and androstenone accumulation in adipose tissue.
Androstenone metabolism in pigs' liver

- The reaction requires **NADH**

- The major product of the reaction is **3-beta-androstenol**

- The reaction can be inhibited by a specific **HSD inhibitor trilostane**
Androstenone level in backfat

Rate of androstenone metabolism in liver

- Androstenone level in Meishan and Large White pig breeds.
  - Meishan: 1.4 ug/g backfat
  - Large White: 1.2 ug/g backfat

- Rate of androstenone metabolism in liver:
  - Large White: increasing over time
  - Meishan: relatively stable
HSD expression in pig liver

[Graph showing HSD mRNA expression in ng for Large White and Meishan pig breeds.]

Large White

Meishan

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Summary

- HNF-1 and COUP-TF1 activate CYP2E1 promoter
- Androstenone is metabolised via HSD in pig liver
- Low expression of HSD in liver is related to a high androstenone accumulation in adipose tissue
- Androstenone represses CYP2E1 promoter activity via inhibition of binding of COUP-TF1
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