ABSTRACT

A genetic deficiency in the renal formation of 20-HETE, reported to reduce sodium transport in the TALH, contributes to the development of hypertension in Dahl S (S) rats. Therefore, we hypothesized that increased expression of CYP450A4 protein and renal formation of 20-HETE attenuates the rise in MAP in S rats. We created a transgenic rat in which the CYP4A1 gene responsible for the production of 20-HETE was introduced into the S genetic background. We found the production of 20-HETE in the outer medulla was similar in S and CYP4A transgenic rats on standard (0.5%NaCl) chow (8 ± 103 pmol/mg/min). After 28 days on 8%NaCl diet, renal medullary 20-HETE production increased markedly and was 3-fold higher in CYP4A1 transgenic animals compared to S rats (127±2x2 vs 40±16 pmol/mg/min). MAP was significantly lower in the CYP4A1 (n=10) transgenic rats as compared to S rats (139±5 vs 177±10 mmHg, n=8, p<0.05). Protein excretion was significantly lower in CYP4A1 transgenic rats relative to S rats (175±22 vs 39±22 mg/day, p<0.05) and the degree of renal injury was greatly reduced. These results indicate that the renal medullary production of 20-HETE is elevated on a high salt diet and attenuation of the expression of CYP4A1 gene in the S genetic background increases the renal production of 20-HETE, improves proteinuria and opposes the development of hypertension. 1T32HL103524-01, AHA11POST752002S, HL36275, HL29587.

BACKGROUND

• The Dahl S rat, an animal model of salt sensitive hypertension, has an impaired pressure natriuretic response that is associated with a renal deficiency in 20-HETE production.
• Transfer of chromosome 5, containing the CYP4 gene that produce 20-HETE, from the Brown Norway rat onto the S genetic background in SS.BNS consomic strain increases the renal production of 20-HETE, attenuates the development of the hypertension, reduces urinary protein excretion, and markedly improves the renal pressure natriuretic response.

HYPOTHESIS

Increased expression of CYP450 protein and upregulation of the renal formation of 20-HETE in transgenic Dahl S rats has anti-hypertensive and renoprotective effects.

METHODS

Generation of CYP4A1 Transgenic Rats

Figure 1. Transposon plasmid construction and expression of functionally active CYP4A1 protein. (A) A full length CYP4A1 CDNA was cut by EcoRI from Lentiviral vector plasmid pshCAG(CYP4A1) and ligated into a transposon vector pT2CAG-eGFP, in which eGFP was cut out using EcoRI. The orientation of the insertion was confirmed by digestion with PstI and PCR by using primers PstR1 and PstR2. (B) Expression of functionally active CYP4 expression after transfection with the CYP4A1 transposon plasmid in Hela cells. LVU1A was used as a positive control from CYP4A1 transgenic transduced Hela cells.

Identification of CYP4A1 Transgenic Rats

Figure 2. Expression of GFP. Microinjection of 5ngul of pT2CAG-eGFP and 10 ngul SBI1 transposase mRNA in 0.5X TS buffer into the pronucleus of one cell embryos. After microinjection, the embryos were incubated in KSOM medium. After 24 hours media was changed to RCM2W or HEPES/FPA. The 2-cell-stage embryos were transferred into the recalcified medium and cultured in vitro until blastocyst (OD) was visible. (C) Promoter region of the mouse CYP4A1 gene and the transcriptional start site are shown.

RESULTS

Increased expression of CYP450 protein and upregulation of the renal formation of 20-HETE in transgenic Dahl S rats has anti-hypertensive and renoprotective effects.

Figure 3. Conformation of genotyping using transgene specific primers for CYP4A1 and the LTR. DNA was extracted from the tails. The following primers were used to detect and amplify CYP4A1 and the LTR found in the transgenic: TTAH1F 5’CCCGCAGTACATACCAAGCCG 3’ (HETE); TTAH1R 5’GGACTTCGTGTTAGAAGAAAC 3’ (CYP4A1) and the LTR. DNA was extracted from the tails. The following primers were used to detect and amplify CYP4A1 and the LTR found in the transgenic: TTAH1F 5’CCCGCAGTACATACCAAGCCG 3’ (HETE); TTAH1R 5’GGACTTCGTGTTAGAAGAAAC 3’ (CYP4A1) and the LTR.

Figure 4. CYP4A1 protein expression in the outer medulla of CYP4A1 transgenic rats fed an 8%NaCl diet for 28 days. The degree of glomerular injury was assessed in Masson’s trichrome stained sections (5x10). Scoring on a 4-scale: 0 representing no injury, 1 indicating loss of 50% of glomerular capillary area, and 4 representing the complete loss of capillaries. Fibrosis determined by the percentage of blue staining. (p<0.05)

Figure 5. Mean arterial pressure (MAP) and proteinuria in CYP4A1 transgenic rats after 28 days on a high salt diet. Increased CYP4A1 expression reduced glomerular injury and fibrosis in CYP4A1 transgenic rats fed a high salt diet.

Figure 6. Glomerular injury score of Dahl S and CYP4A1 transgenic rats after 28 days on an 8%NaCl diet (p<0.05).

RESULTS

Dahl S rats have an altered renal pressure natriuresis when compared to CYP4A1 transgenic rats

Figure 7. Renal pressure natriuresis relationship in Dahl S and CYP4A1 transgenic Dahl S rats. Controls measurements on 0.3% NaCl diet. Urinary sodium excretion measured on 0.14% of an 8% NaCl diet. Dahl S rats display a salt sensitive phenotype, while transgenic rats are protected after 21 days of a HS diet (p<0.05).

SUMMARY

• Introgenesis of the CYP4A1 gene into the Dahl S genetic background increases renal outer medullary CYP4A1 protein and renal 20-HETE production.
• MAP is significantly attenuated and there is a correction of the altered pressure natriuresis relationship in CYP4A1 transgenic rats after 28 days on an 8% NaCl diet.
• Proteinuria, degree of glomerular injury and renal fibrosis are significantly reduced in CYP4A1 transgenic rats when compared to Dahl S rats.

CONCLUSION

Increased renal CYP4A1 protein expression and 20-HETE production slows the development of hypertension and progression of renal injury in the Dahl S rat.

ACKNOWLEDGEMENTS

This work was supported in part by NIH grant 1T32HL105324-01, HL36275, HL29587 and AHA11POST7520052.

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