Upregulation of Renal Medullary 20-HETE Production Opposes the Development of Hypertension in Sleeping Beauty Transposon CYP4A1 Transgenic Dahl S rats

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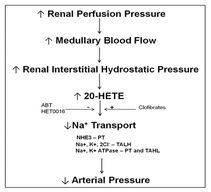
ABSTRACT

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 CYP4504A 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 renal outer medulla was similar in S and CYP4A transgenic rats on standard (0.3%NaCl) chow (8 ±4 vs 10±3 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±22 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 295±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 upregulation 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. 1T32HL105324-01, AHA11POST7520052, HL36279, 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 genes that produce 20-HETE, from the Brown Norway rat onto the S genetic background in SS.BN5 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

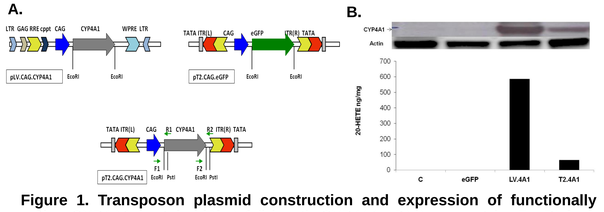


Figure 1. Transposon plasmid construction and expression of functionally active CYP4A1 protein. (A) A full length CYP4A1 cDNA was cut by EcoRI from Lentiviral vector plasmid pLV.CAG.CYP4A1 and ligated into a transposon vector pT2.CAG.eGFP, in which eGFP was cut out using EcoRI. The orientation of the insertion was confirmed by digestion with PstI and PCR by using primers F1/R1 and F2/R2. **(B)** Expression of functionally active CYP4A1 protein after transfection with the CYP4A1 transposon plasmid in Hela cells. LV.4A1 was used as a positive control from CYP4A1 lentiviral transduced Hela cells.

Identification of CYP4A1 Transgenic Rats

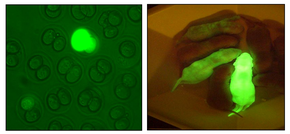


Figure 2. Expression of GFP. Microinjection of 3ng/ul of pT2.CAG.eGFP and/or CYP4A1 DNA and 10 ng/ul SB11 transposase mRNA in 0.1X TE buffer into the pronuclei of one cell embryos. After microinjection the oocytes were incubated in KSOM medium. After 24 hours media was changed to RECM2 w/ HEPE&PVA. The 2-cell oocytes were transferred into the oviducts of pseudopregnant foster Dahl SS females and generated eGFP and/or CYP4A1 transgenic rats.

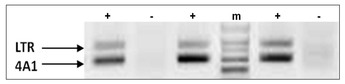


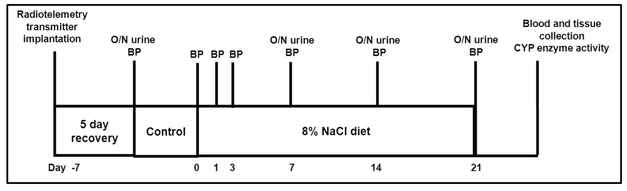
Figure 3. Conformation of genotyping using transgene specific primers for CYP4A1 and the LTR. DNA was extracted from pup tails. The following primers were used to detect and amplify CYP4A1 and the LTR found in the transgene:

T24A1F1:5'-TCGGGCGATCAGATCCAAAGGCC-3';
T24A1R1:5'-GCCATTGTGGCTGAAGGCACA-3';
T2LTRF1:5'-ACTGTATCACAATTCCAGTGGG-3';
T2LTRR1:5'GGAAAGTCCCATAAGGTCATGT-3'.

Transgenic animals were positive for both the CYP4A1 and LTR while the wild type animals did not express a product with these primer pairs.

Experimental Design

Studies performed on 9 week old Dahl S and CYP4A1 transgenic Dahl S rats. All experimental procedures were in accordance with NIH and IACUC at UMMC. Dahl S and CYP4A1 transgenic animals were maintained on 0.3% NaCl (Teklad) diet from weaning until placed on 8% NaCl (Teklad) diet at 9 weeks of age for 28 days.



RESULTS

CYP4A1 expression is increased in the outer medulla of CYP4A1 transgenic rats

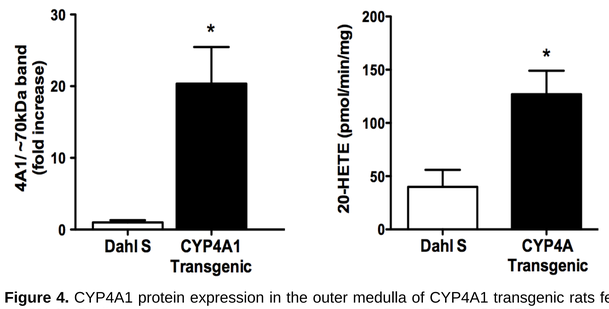


Figure 4. CYP4A1 protein expression in the outer medulla of CYP4A1 transgenic rats fed an 8% NaCl diet for 28 days is 20 times higher than Dahl S rats. 20-HETE production (pg/mg/min) from microsomes isolated from renal outer medulla CYP4A1 transgenic rats is significantly higher when compared to Dahl S rats. Data expressed as mean±SEM. *p<0.05.

RESULTS

Increases in 20-HETE attenuate the rise in MAP and proteinuria in CYP4A1 transgenic rats after 28 days on a high salt diet

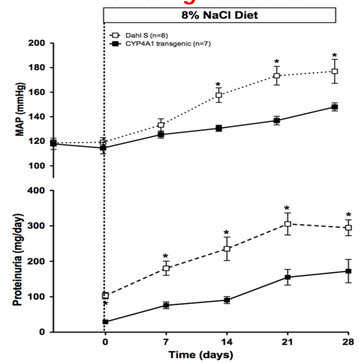


Figure 5. Mean arterial pressure (MAP) and urinary protein excretion in Dahl S and CYP4A1 transgenic rats fed an 8%NaCl diet for 28 days. Mean arterial pressure was measured by radiotelemetry through the femoral artery. Urinary protein excretion was measured by an overnight urine collection and analyzed by Bradford assay. Data expressed as mean±SEM. *p<0.05.

Increased CYP4A1 expression reduced glomerular injury and fibrosis in CYP4A1 transgenic rats fed a high salt diet

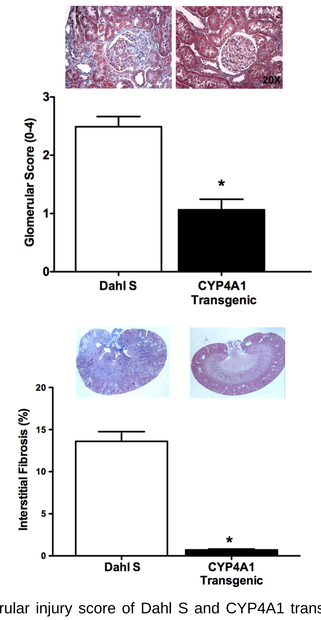


Figure 6. Glomerular injury score of Dahl S and CYP4A1 transgenic rats after 28 days on an 8% NaCl diet (*p<0.05). The degree of glomerular injury was assessed in Masson's trichrome stained sections (3µm). Scoring on a 0-4 scale, with 0 representing no injury, 2 indicating loss of 50% of glomerular capillary area, and 4 representing the complete loss of capillaries. Fibrosis determined by the percentage of blue staining. (*p<0.05)

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

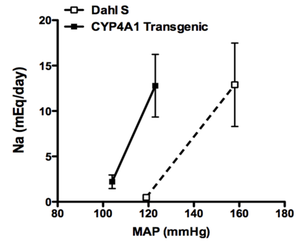


Figure 7. Renal pressure natriuresis relationship in Dahl S and CYP4A1 transgenic Dahl S rats. Control measurements on 0.3% NaCl diet. Urinary sodium excretion measured on day 14 of an 8% NaCl diet. Dahl S rats display a salt sensitive phenotype, while transgenic rats are protected after 21 days of a HS diet (*p<0.05).

SUMMARY

•Introgression 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

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