Online-Only Supplemental Materials

Renal Medullary 11β-Hydroxysteroid Dehydrogenase Type 1 in Dahl Salt-Sensitive Hypertension

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EXPANDED MATERIALS AND METHODS

Animals. The consomic SS-13^{BN} rat was generated by introgressing chromosome 13 from Brown Norway rats into the genome of SS rats through selective breeding based on genetic marker analyses. Inbred SS and SS-13^{BN} rats were maintained by brother-sister mating.

LC-MS/MS analysis of corticosterone and 11-dehydrocorticosterone. Samples, with an internal standard added, were de-proteinized with an acetonitrile crash and extracted with CH_2Cl_2. Extracted samples were resolved with a reversed-phase analytical column combined with a pre-column filter. Tandem mass spectrometry analysis was performed with an API 4000 Q-trap^{TM} tandem mass spectrometer (Applied Biosystems, Foster City, CA), operating with an electospray ionization source in the positive mode. Corticosterone, 11-dehydrocorticosterone, Corticosterone-d_8, and cortisol were monitored by ion pairs of 347.3/121.2, 345.2/121.2, 355.2/125.1, and 363.1/121.1, respectively. Plasma corticosterone was measured with an enzyme immunoassay (Cayman Chemical, Ann Arbor, MI).

Oligonucleotide sequences used in real-time PCR. Taqman primers and probes were designed using Primer Expression 2.0 (Applied Biosystems). Rat 11β-HSD1, forward CTCCTCCATGGCTGGGAAA, reverse AAGAACCCATCCAGAGCAAACTT, probe FAM-ACCCAACCTCTGATTGCTTCCTACTCTGCA-TAMRA; rat 11β-HSD2, forward CTCTCCTCTGCTTCATGAGACCAT, reverse CACGCTAAACTCTCCATCCATCT, probe FAM-CCTACCAGGCACGACCCTTCAGC-TAMRA; rat mineralocorticoid receptor, forward GTCCAGACACCCACGAGAAAG, reverse CGGGCCAGTCACACCATT, probe FAM-

TGCTCACGACGTTCCTTTCCCTAAGACA-TAMRA; rat glucocorticoid receptor, forward ATCTTCAGAACAGCAAAATCGAAA, reverse CAGCGGAAAACTCCAAATCC, and probe FAM-CCACAGACCAAAGCACCTTTGACCTCTTG-TAMRA. Real-time RT-PCR was performed using an ABI Prism 7900HT Sequence Detection System.

shRNA expression construct. Chemically synthesized shRNA oligonucleotides targeting rat 11β-HSD1, or control shRNA oligonucleotides containing two nucleotide substitutions, were annealed and ligated between the *Bam* HI and *Xho* I sites of pRNAT-U6.2/Lenti (Genscript, Piscataway, NJ). The plasmids were propagated in competent cells and extracted and purified with an EndoFree Plasmid Maxi kit (Qiagen, Valencia, CA). The shRNA insertions were verified by DNA sequencing.

Chronic measurement of arterial blood pressure. Arterial blood pressure in conscious, freely moving rats was monitored using indwelling femoral arterial catheters implanted a week before the recording began. Arterial blood pressure was recorded 3 hours daily from 9AM to noon using an online data collection and analysis system. Blood pressure measurements were taken at 500 Hz. MAP was calculated using an integral method that considered all data points within a pulse.

Plasma and urine collection. Plasma samples were collected from conscious, freely moving rats through a chronically implanted arterial catheter, and urine samples collected using metabolic cages. Plasma samples were collected consistently at about 10:30AM. Urine samples were collected over 24 hours.