OFFLINE SUPPLEMENT

Hsd11b2 HAPLOINSUFFICIENCY IN MICE CAUSES SALT-SENSITIVITY OF BLOOD PRESSURE

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Genotyping
Mice were obtained from heterozygote crosses of a congenic Hsd11b2-C57BL/6J line\textsuperscript{1}. For genotyping, genomic DNA, isolated from ear biopsies, was digested by \textit{BamHI} and separated on 0.8% agarose gel for Southern analysis using a \textit{EcoRI} hybridization probe subcloned from the first intron of the \textit{Hsd11b2} gene. The wild-type allele was represented by a 10kb (9899bp) restriction fragment. The targeted allele was represented by 2 kb (1850 bp) restriction fragment, resulting from the insertion of a \textit{BamHI} site at the 5’ end of the Neo cassette, upstream of the genomic \textit{XbaI} site. A sample blot, below, shows the three genotypes, \textit{Hsd11b2}\textsuperscript{+/−} (het), \textit{Hsd11b2}\textsuperscript{+/+} (WT) and \textit{Hsd11b2}\textsuperscript{−/−} (null).

Telemetry studies: Blood pressure was measured by radiotelemetry using a device (Model TA 11PAC10, Data Sciences, UK) implanted under isofluorane anesthesia. After recovery from surgery, blood pressure was monitored until circadian variation was restored and mean pressure stabilized (all within 7 days). Mice were single
housed throughout and allowed free access to water and a control chow (0.25% Na by weight; RM1 diet, Special Diet Services, UK). After 7 days, baseline blood pressure was recorded over a three-day period and mice then fed a high sodium diet (2.5% Na by weight; Diet 829504, SDS, UK) over a 19-day recording period. For each mouse, a daily average mean arterial blood pressure was taken and used to calculate the mean daily value per genotype.

**Inhibitor studies:** In addition to control (untreated) experiments, mice received one of 3 co-treatments:

i) Spironolactone pellets (Silastic, a gift from Dow-Corning, USA) were implanted subcutaneously under isofluorane anesthesia, five days before feeding 2.5% sodium diet. Two pellets were implanted, each containing 30mg of spironolactone. *In vitro* studies confirmed that spironolactone release from the matrix is at a constant rate over the experimental period. The concentration of canrenone in terminal plasma was measured by mass-spectrometry (Clinical Research Facility, University of Edinburgh) and was ~75 nmol/l, similar to a previous study in which the drug exerted a hypotensive effect.

ii) Dexamethasone was administered in the drinking water (1µg/ml in 0.1% ethanol) throughout the period of high sodium feeding. Plasma corticosterone was measured in samples taken from conscious, unrestrained animals at 6:30pm.

iii) RU38486 pellets were implanted five days before giving the high sodium diet. Two pellets were implanted, each containing 30mg and the concentration of RU38486 in terminal plasma, measured by mass spectrometry was ~100nmol/l.

**Analysis:** Sodium and potassium concentrations were measured by ISE or flame photometry. Osmolality was measured by freezing-point depression. Corticosterone,
deoxycorticosterone and aldosterone concentrations were measured by ELISA or RIA, as described\textsuperscript{4,5}. For urinary excretion, an average rate was measured during the baseline period and during the adaptive (days 1, 2 and 3) and plateau (days 11, 13, & 15) phases of the response to high sodium diet. Urinary albumin concentration was measured using a commercial kit (Olympus Diagnostics) as described\textsuperscript{6}

REFERENCES


