Supporting Information

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RT-PCR for AC3 and G_{olf}. RNA was isolated from C57BL/6 mouse kidneys by using the Qiagen RNeasy Protocol, and DNase-treated. RNA (2 μg) was reverse transcribed (RT) using SuperScript II (Invitrogen), or subjected to Mock RT (MRT; in which case 1 μL of water was added instead of 1 μL SuperScript II). In all cases, MRT reactions yielded no bands by PCR. As an additional control for genomic DNA, the primers used for AC3 and G_{olf} spanned introns (AC3—fwd: TTGGCAGGCTTTCTTTGTCT, rev: TCTGCAAACAGGATGCTGAC; 462 bp. G_{olf}—fwd: TACCAGCTGATCGACTGTGC, rev: TGGCATACTCCGGGAAATAG; 445 bp.). PCRs for AC3 and G_{olf} were performed using Taq polymerase (Roche) and the products were TOPO-cloned (Invitrogen) and sequenced.

Western Blot for AC3 and G_{olf}. In brief, kidneys were minced in lysis buffer (10 mM Tris·HCl, pH 7.4/250 mM sucrose/1 mM EDTA) containing protease inhibitors (Roche Complete Tablets, 11 697 498 001), then homogenized. Lysates were then centrifuged (10 min at 800 $\times$ *g* at 4° C), and the supernatant was centrifuged again (90 min at 100,000 $\times$ *g* at 4° C). The pellet was then resuspended in lysis buffer. Protein concentration was determined using the Bradford Protein Assay (Bio-Rad). Samples were resolved by SDS/PAGE and transferred to nitrocellulose. The resultant membranes were then blocked and subsequently incubated with antibodies against either AC3 or G_{olf} (Santa Cruz Biotechnology, sc-588 and sc-385). In control experiments, antibodies were preincubated with an excess of competing peptide. Blots were then probed with a peroxidase-conjugated anti-rabbit secondary antibody, and visualized by chemiluminescence (Amersham Biosciences).

Immunohistochemistry. Mouse kidneys (C57BL/6) were perfusion-fixed in 4% periodate-lysine-paraformaldehyde (PLP) and cryosections were obtained. Sections were washed 3 times in TBS, incubated for 5 min in TBS with 1% SDS, and washed in TBS again. After blocking in Goat Serum Dilution Buffer [GSDB; 10% goat serum, 1% Triton X-100, and 10 mM glycine in PBS supplemented with 100 μM $CaCl_2$ and 1 mM $MgCl_2$ (PBS++)], sections were incubated with antibodies for either AC3 or G_{olf} (rabbit antibodies, used at 1:200 and 1:100, respectively) diluted in GSBD. In control experiments, antibodies were preincubated with an excess of their competing peptides. Sections were then washed in TBS-High Salt (TBS-HS; containing 2.5% NaCl and 0.1% BSA), incubated with anti-rabbit fluorescent antibodies (rhodamine or FITC) for 1 h at room temperature, and washed again in TBS-HS followed by TBS. In other experiments, sections were double stained for Na^+-K^+-ATPase [α5 antibody, mouse (1)]. Alternatively, serial sections were stained for AC3 or G_{olf} and NCC (kind gift from David Ellison, Oregon Health and Science University, Portland, OR), AC3 or G_{olf} and calbindin, and for AC3 and G_{olf}. NADPH diaphorase staining was performed as described in Unit 10.6 of *Current Protocols in Toxicology* (2). COX-2 antibody was obtained from Cayman Chemical. To quantitate COX-2 staining, the section was scanned in a systematic pattern and a picture was taken of every macula densa which stained for COX-2. Staining intensity of each COX-2-positive MD was quantitated using Image J, and the mean of the intensities for each animal (3 $AC3^{+/+}$ and 4 $AC3^{-/-}$) were used for statistical analysis (*t* test, $P < 0.05$ considered significant).

In Vivo Studies. In one group of animals, plasma samples were collected by supraorbital puncture for measurement of plasma electrolytes and pH balance. In a separate group of mice, plasma samples were obtained by cardiac puncture for HPLC measurement of plasma creatinine. The number of glomeruli was assayed by systematically scanning kidney sections on a light microscope in a prearranged scanning pattern and tallying the number of glomeruli per cortical field of view.

Measurement of cardiovascular parameters and activity by telemetry was done in 3 mice of each genotype by using standard methodology available from a Yale core facility (3). After a recovery period following surgery, telemetry measurements were continually recorded for 5 consecutive days. The values in Table 2 represent the mean values for day (6:30 a.m.–6:30 p.m.) and night (6:30 p.m.–6:30 a.m.), defined by the light schedule of the room where the mice were housed. Activity data were analyzed as the percentage of time that there was an activity count >0 (4).

Renal function studies in anesthetized male mice were performed as described previously (5). In brief, $AC3^{-/-}$ animals, $AC3^{+/+}$ littermates, or wild-type mice of the same strain were anesthetized by i.p. injection of 100 to 120 mg of Inactin [5-ethyl-5-(L-methylpropyl)-2-thiobarbituric acid (BYK-Guiden Pharmazeutika)] per kilogram of body weight. After tracheotomy, the left jugular vein was cannulated for i.v. infusion, a carotid artery was catheterized for monitoring mean arterial pressure (MAP) and blood collection, and the bladder was catheterized for collection of urine. Initially, 0.3% body weight of isotonic saline was given intravenously to replace surgical fluid losses. Subsequently, a priming dose of 5 μCi of [^{3}H]methoxyinulin (New England Nuclear) was given in 0.05 mL isotonic saline followed by a maintenance infusion of 0.9% NaCl and 4 mM of KCl containing 10 μCi/mL of [^{3}H]inulin (infusion rate: 0.41 mL/h). An equilibration period of 60 min was followed by two 30 min collection periods. Blood samples for the measurement of plasma inulin were taken in the middle of each urine collection period. Na^+ and K^+ concentrations were measured by flame photometry (type 480 flame photometer, Corning Medical and Scientific). Significant differences were determined using the unpaired *t* test. Details of the method to determine GFR in conscious mice have been published recently (6). FITC-inulin was measured using a fluorescence spectrometer.

Determination of tubuloglomerular feedback was performed as described in detail previously (7). Briefly, anesthetized mice (inactin/ketamine) were given a saline infusion through a jugular vein catheter, and blood pressure was monitored by a femoral artery catheter. The left kidney was exposed, placed in a lucite cup and covered with mineral oil. A beveled micropipette (diameter 7–8 μm) was inserted into a randomly selected proximal segment, and subsequent segments of the same nephron were identified by downstream flow of stained perfusate (FDC green). Tubular stop-flow pressure proximal to an obstructing wax block was measured as an index of glomerular capillary pressure while flow along the downstream nephron was controlled through a perfusion pipette in the late proximal segment.

Plasma renin concentrations were measured as previously described (8–11); in brief, red cells and plasma were separated by centrifugation and then frozen and stored at −20 °C until the assay was performed. Plasma renin concentration (PRC) was determined using an RIA (Gammacoat, DiaSorin) to detect the generation of angiotensin (ANG) I after addition of excess

substrate for a 1 h incubation period at 37 °C. In these experiments an amino acid peptide was used as renin substrate (renin substrate tetradecapeptide, American Peptide). Incubation of the substrate without plasma did not yield detectable angiotensin I levels. As an additional control, background ANG I levels were assayed in a plasma aliquot to which no substrate was added.

Detection of Renal ORs. RNA was prepared from wild-type C57BL/6 mouse kidneys by using TriZol reagent, and was subsequently cleaned using an RNeasy cleanup protocol (Qiagen, catalog no. 74104). PCR (Taq polymerase, Roche) using dOR primers on reverse transcribed (RT; SuperScript II, Invitrogen, 2 μg RNA) mouse kidney RNA yielded a 389 bp product which was TOPO-cloned and sequenced to identify individual ORs.

ORs expressed in the kidney were identified through the initial dOR screen, as well as through a directed screen making use of primers directed against a selected subset of specific ORs. Following the initial dOR screen, we subsequently used primers specific for each OR identified to confirm whether the OR was truly present in the kidney by performing RT-PCR. Primer sequences are available upon request. Because ORs do not contain introns within the coding region, we performed a Mock RT control (water was added to the RT reaction instead of reverse transcriptase enzyme) for every PCR as a negative control. These PCR products were TOPO-cloned (Invitrogen) and sequenced to confirm their identity.

To determine whether any of these renal ORs may be expressed in the macula densa, we then isolated RNA from the macula densa cell line (described above) by using the RNeasy RNA Isolation Kit (Qiagen) followed by the RNeasy cleanup protocol. Following reverse transcription (as described), PCR was performed using primers specific for the ORs confirmed to be found in kidney. Mock RT reactions were performed in parallel, and any resultant RT-PCR bands were TOPO-cloned and sequenced. For Olfr90, primers encompassing the full-length sequence (as well as appropriate cloning sites) were used for this experiment.

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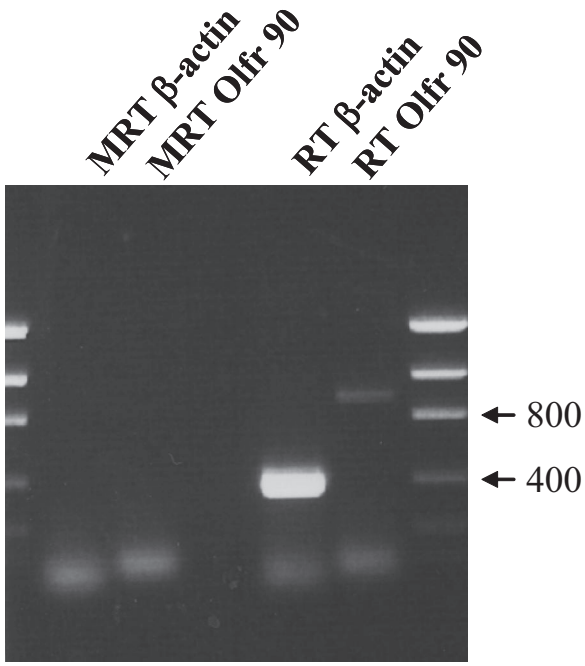

Fig. S1. RT-PCR demonstrating the expression of Olfr90 in a macula densa cell line. Mock reverse transcriptase-PCR (MRT) and RT-PCRs were performed for both β-actin and Olfr90. The resultant band for Olfr90 encompassed the entire coding sequence, and was cloned and sequenced and found to be identical to previously published sequences for Olfr90. The expected sizes for β-actin and Olfr90 were 353 and 957 nt, respectively.