Development of a selective small-molecule inhibitor of Kir1.1, the Renal Outer Medullary Potassium Channel. Gautam Bhave, Brian A. Chauder, Wen Liu, Eric S. Dawson, Rishin Kadakia, Thuy, T. Nguyen, L. Michelle Lewis, Jens Meiler, C. David Weaver, Lisa M. Satlin, Craig W. Lindsley, and Jerod S. Denton. *Molecular Pharmacology.*

SUPPLEMENTAL METHODS

Animals. Pathogen-free Sprague-Dawley (SD) rats of either sex (~3 wk old; Taconic Farms, Inc., Germantown, NY) were housed in the animal care facility at the Mount Sinai School of Medicine (Center for Comparative Medicine). All animals were allowed free access to tap water and standard rat chow. Animals were euthanized in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Animal protocols were approved by the IACUC Committee at the Mount Sinai School of Medicine as appropriate.

Microperfusion of Isolated Rat Cortical Collecting Ducts. Kidneys were removed via a midline incision, and single tubules dissected freehand in cold (4^oC) Ringer's solution containing (in mM): 135 NaCl, 2.5 K_2HPO_4, 2.0 $CaCl_2$, 1.2 $MgSO_4$, 4.0 lactate, 6.0 L-alanine, 5.0 HEPES, and 5.5 D-glucose, pH 7.4, 290±2 mOsm/kg, as previously described (Liu et al., 2003). A single tubule was studied from each animal. Isolated collecting ducts (CD) were microperfused *in vitro* as previously described (Liu et al., 2003; Woda et al., 2003). Briefly, each isolated tubule was immediately transferred to a temperature and O_2/CO_2-controlled specimen chamber, mounted on concentric glass pipettes, and perfused and bathed at 37^oC with Burg's perfusate containing (in mM): 120 NaCl, 25 $NaHCO_3$, 2.5 K_2HPO_4, 2.0 $CaCl_2$, 1.2 $MgSO_4$, 4.0 Na lactate, 1.0 Na_3 citrate, 6.0 L-alanine, and 5.5 D-glucose, pH 7.4, 290 ± 2 mOsm/kg (Liu et al., 2003). During

the 30 min equilibration period and thereafter, the perfusion chamber was continuously suffused with a gas mixture of 95% O_2-5% CO_2 to maintain pH of the Burg's solution at 7.4 at 37°C. The bathing solution was exchanged continuously at a rate of 10 ml/hr using a syringe pump (Razel, Stamford, CT).

Transport measurements were performed in the absence of transepithelial osmotic gradients and thus water transport was assumed to be zero. Three to four samples of tubular fluid were collected under water-saturated light mineral oil by timed filling of a calibrated 15 nl volumetric constriction pipette at each perfusion rate. To determine the concentrations of K^+ and Na^+ delivered to the tubular lumen, ouabain (500 μM) was added to the bath at the conclusion of each experiment to inhibit active transport, and an additional three to four samples of tubular fluid were obtained for analysis. The cation concentrations of perfusate and collected tubular fluid were determined by helium glow photometry and the rates of net transport (Jx, in pmol·min^{-1}·mm^{-1} tubular length) were calculated using standard flux equations, as previously described (Estilo et al., 2008). The calculated ion fluxes were averaged to obtain a single mean rate of ion transport for the CCD under each condition.

Measurements of net cation transport in each CD were performed in the absence and then presence of 10 μM VU591 added to the luminal perfusate. The inhibitor was present for at least 10 min before tubular fluid samples were obtained for experimental measurements. Subsequent samples of tubular fluid were collected in the continuous presence of the inhibitor.

REFERENCES

Estilo G, Liu W, Pastor-Soler N, Mitchell P, Carattino MD, Kleyman TR and Satlin LM (2008) Effect of aldosterone on BK channel expression in mammalian cortical collecting duct. *Am J Physiol Renal Physiol* **295**(3):F780-788.

Liu W, Xu S, Woda C, Kim P, Weinbaum S and Satlin LM (2003) Effect of flow and stretch on the $[Ca^{2+}]_i$ response of principal and intercalated cells in cortical collecting duct. *Am J Physiol Renal Physiol* **285**(5):F998-F1012.

Woda CB, Miyawaki N, Ramalakshmi S, Ramkumar M, Rojas R, Zavilowitz B, Kleyman TR and Satlin LM (2003) Ontogeny of flow-stimulated potassium secretion in rabbit cortical collecting duct: functional and molecular aspects. *Am J Physiol Renal Physiol* **285**(4):F629-639.