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Sujay V Kharade<sup>a</sup>, Daniel R Swale<sup>b</sup> & Jerod S Denton<sup>ac</sup>

<sup>a</sup> Department of Anesthesiology; Vanderbilt University Medical Center; Nashville, TN USA

<sup>b</sup> Department of Entomology; Louisiana State University; Baton Rouge, LA USA

<sup>c</sup> Department of Pharmacology; Vanderbilt University Medical Center; Nashville, TN USA

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## ROMK (Kir1.1) pharmacology comes of age

Sujay V Kharade<sup>1</sup>, Daniel R Swale<sup>2</sup>, and Jerod S Denton<sup>1,3,\*</sup>

<sup>1</sup>Department of Anesthesiology; Vanderbilt University Medical Center; Nashville, TN USA; <sup>2</sup>Department of Entomology; Louisiana State University; Baton Rouge, LA USA; <sup>3</sup>Department of Pharmacology; Vanderbilt University Medical Center; Nashville, TN USA

The Renal Outer Medullary K<sup>+</sup> channel, ROMK (Kir1.1), is broadly expressed in the nephron, where it plays fundamental roles in the regulation of extracellular fluid volume and blood pressure.<sup>1</sup> In the thick ascending limb (TAL) of Henle's loop, ROMK-mediated K<sup>+</sup> secretion across the luminal membrane provides K<sup>+</sup> ions needed to maintain the catalytic activity of the Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> co-transporter, NKCC2, which accounts for approximately 25% of Na<sup>+</sup> absorbed along the nephron. In collecting duct, ROMK constitutes the major pathway for regulated K<sup>+</sup> excretion and helps generate a favorable electrochemical gradient for Na<sup>+</sup> reabsorption through the Epithelial Na<sup>+</sup> Channel, ENaC. Thus, ROMK activity is important for the function of 2 independent diuretic targets – NKCC2 and ENaC – and has therefore been postulated as a novel diuretic target.

Genetic validation of ROMK as a diuretic target came from 2 major observations. First, autosomal recessive loss-of-function mutations in the gene encoding ROMK, *KCNJ1*, cause antenatal (type II) Bartter syndrome, a severe salt and water wasting renal tubulopathy characterized by polyuria, low blood pressure, and hypokalemic metabolic alkalosis.<sup>2</sup> Second, incomplete loss of ROMK function in heterozygous carriers of *KCNJ1* mutations lowers blood pressure without causing Bartter's syndrome.<sup>3</sup> Taken together, these physiological and genetic data supported the notion that a partial antagonist of ROMK could lower blood pressure efficaciously and safely.

The major drug discovery efforts for ROMK have been made by our group at Vanderbilt and Merck Research Laboratories. In 2009, we published the first-in-

class small-molecule ROMK inhibitor, termed VU590, which was discovered in a high-throughput screen of approximately 225,000 compounds from the NIH Molecular Libraries Small-Molecule Repository.<sup>4</sup> VU590 inhibits ROMK with an IC<sub>50</sub> of approximately 220 nM, but also inhibits another member of the inward rectifier K<sup>+</sup> (Kir) channel family: Kir7.1 (IC<sub>50</sub> ~8 μM). The second ROMK inhibitor to be published by our group, termed VU591, inhibits ROMK with an IC<sub>50</sub> of approximately 300 nM, and is selective for ROMK over more than 70 other ion channels, transporters, and receptors.<sup>5</sup>

ROMK is a relatively simple homo-tetrameric membrane protein containing 8 membrane-spanning domains (2 per subunit), a modest extracellular domain, and larger cytoplasmic domain. There are no voltage-sensing domains, inactivation loops, or regulatory subunits. Given its relatively simple architecture and considerable amino acid identity (30–45%) with other Kir channels, the development of a highly selective, nanomolar affinity inhibitor raised important questions about the physicochemical nature of the VU591 binding site.

We knew from patch clamp experiments that VU591 exhibits a voltage-dependent 'knock-off' at potentials more negative than the equilibrium potential for K<sup>+</sup>. This suggested that the VU591 binding site was somewhere in the ion-conduction pathway, possibly in the membrane-spanning pore where the voltage gradient is steepest.<sup>5</sup> To test this hypothesis, a combination of molecular modeling and in silico ligand docking was used to guide site-directed mutational analysis with patch clamp electrophysiology. In an effort to

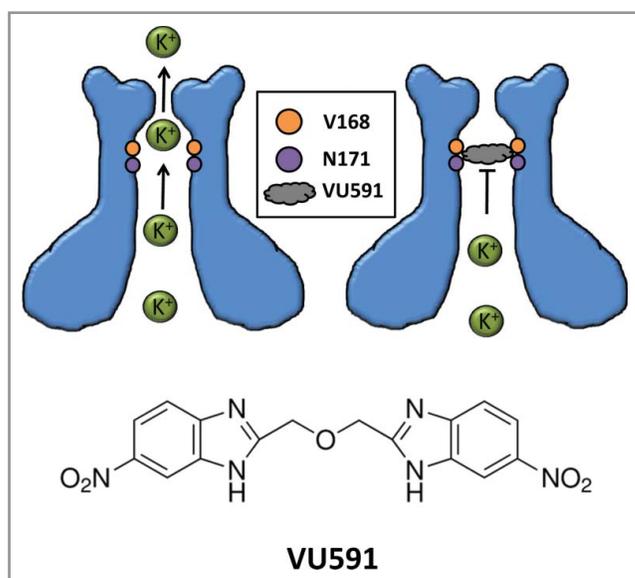
\*Correspondence to: Jerod S Denton; Email: jerod.s.denton@vanderbilt.edu

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**Figure 1:** Chemical structure of VU591 along with a simplified cartoon depicting the ion conduction pathway in ROMK channel in the absence (left side) or presence (right side) of blocking VU591. Residues Asn171 and Val168 comprise the VU591 binding site.

recapitulate the flexibility of ROMK not revealed in static X-ray structures, an ensemble of 10 ROMK homology models was constructed based on crystal structures of Kir2.2 and Kir3.2 solved in the presence of different ligands, ions and functionally important mutations. Similarly, 200 low-energy conformations of VU591 were used for a total of 10,000 docking simulations within the ROMK channel pore.<sup>6</sup>

The docking studies implicated 2 regions of the pore that could participate in energetically favorable interactions with VU591. Scanning mutagenesis analysis ruled out the 'lower' site, but identified 2 'upper' site residues—Val168 and Asn171—as being critical for VU591-dependent block of ROMK (Fig. 1). Mutation of either residue alone led to significant reductions in VU591 sensitivity, whereas double mutations were additive, suggesting that both residues contribute to the VU591 binding site. Mutation of Asn171 to the corresponding residues present in most other Kir channels—namely, Asp or Glu—caused a complete

loss of VU591 sensitivity. This raises the possibility that the selectivity of VU591 is almost exclusively due to the identity of the residue at this position. Consistent with this idea, mutation of Asp172 at the corresponding position in Kir2.1 to Asn, conferred partial VU591 sensitivity to Kir2.1.<sup>6</sup>

Merck Research Laboratories identified a compound in a high-throughput screen of approximately 1.5 million compounds that bears some structural similarities to VU591. Extensive medicinal chemistry led to the development of a potent ( $IC_{50} \sim 50$  nM), highly selective, and *in vivo* active ROMK inhibitor termed compound A that does not resemble VU591. Interestingly, but perhaps not surprisingly, compound A requires Asn171 to block ROMK. Furthermore, as predicted from human genetics data, compound A induces diuresis and lowers blood pressure in rats.<sup>7</sup> This confirms the long-standing hypothesis that ROMK does indeed represent a novel-mechanism diuretic target.

Despite their structural simplicity and apparent paucity of druggable binding

sites in inward rectifier  $K^+$  channels, progress over the last several years has demonstrated that it is indeed possible to develop experimentally useful small-molecule modulators. We believe this is a worthwhile effort because emerging data suggests Kir channels may represent drug targets for myriad diseases. The development of computational modeling techniques<sup>6</sup> should enable *in silico* screening for channel-specific blockers at a fraction of the cost of HTS.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### References

1. Welling PA, Ho K. A comprehensive guide to the ROMK potassium channel: form and function in health and disease, *Am J Physiol Renal Physiol* (2009); 297 F849-863; PMID:19458126; <http://dx.doi.org/10.1152/ajprenal.00181.2009>
2. Simon DB, Karet FE, Rodriguez-Soriano J, Hamdan JH, DiPietro A, Trachtman H, Sanjad SA, Lifton RP. Genetic heterogeneity of Bartter's syndrome revealed by mutations in the  $K^+$  channel, ROMK, *Nat Genet* (1996); 14:152-156; PMID:8841184; <http://dx.doi.org/10.1038/ng1096-152>
3. Ji W, Foo JN, O'Roak BJ, Zhao H, Larson MG, Simon DB, Newton-Cheh C, State MW, Levy D, Lifton RP. Rare independent mutations in renal salt handling genes contribute to blood pressure variation, *Nat Genet* (2008); 40:592-599; PMID:18391953; <http://dx.doi.org/10.1038/ng.118>
4. Lewis LM, Bhawe G, Chauder BA, Banerjee S, Lornsen KA, Redha R, Fallen K, Lindsley CW, Weaver CD, Denton JS. High-throughput screening reveals a small-molecule inhibitor of the renal outer medullary potassium channel and Kir7.1, *Mol Pharmacol* (2009); 76:1094-1103; PMID:19706730; <http://dx.doi.org/10.1124/mol.109.059840>
5. Bhawe G, Chauder BA, Liu W, Dawson ES, Kadakia R, Nguyen TT, Lewis LM, Meiler J, Weaver CD, Satlin LM, Lindsley CW, Denton JS. Development of a selective small-molecule inhibitor of Kir1.1, the renal outer medullary potassium channel, *Mol Pharmacol* (2011); 79:42-50; PMID:20926757; <http://dx.doi.org/10.1124/mol.110.066928>
6. Swale DR, Sheehan JH, Banerjee S, Husni AS, Nguyen TT, Meiler J, Denton JS. Computational and functional analyses of a small-molecule binding site in ROMK, *Biophys J* (2015); 108:1094-1103; PMID:25762321; <http://dx.doi.org/10.1016/j.bpj.2015.01.022>
7. Garcia ML, Priest BT, Alonso-Galicia M, Zhou X, Felix JP, Brochu RM, Bailey T, Thomas-Fowlkes B, Liu J, Swensen A, et al. Pharmacologic inhibition of the renal outer medullary potassium channel causes diuresis and natriuresis in the absence of kaliuresis, *J Pharmacol Exp Ther* (2013); 348:153-164; PMID:24142912; <http://dx.doi.org/10.1124/jpet.113.208603>