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The pelvis–kidney junction contains HCN3, a hyperpolarization-activated cation channel that triggers ureter peristalsis

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Peristaltic waves of the ureteric smooth muscles move urine down from the kidney, a process that is commonly defective in congenital diseases. To study the mechanisms that control the initiation and direction of contractions, we used video microscopy and optical mapping techniques and found that electrical and contractile waves began in a region where the renal pelvis joined the connective tissue core of the kidney. Separation of this pelvis–kidney junction from more distal urinary tract segments prevented downstream peristalsis, indicating that it housed the trigger for peristalsis. Moreover, cells in the pelvis–kidney junction were found to express isoform 3 of the hyperpolarization-activated cation on channel family known to be required for initiating electrical activity in the brain and heart. Immunocytochemical and real-time PCR analyses found that hyperpolarization-activated cation-3 is expressed at the pelvis–kidney junction where electrical excitation and contractile waves originate. Inhibition of this channel caused a loss of electrical activity at the pelvis–kidney junction and randomized the origin of electrical activity in the urinary tract, thus markedly perturbing contractions. Collectively, our study demonstrates that hyperpolarization-activated cation-3 channels play a fundamental role in coordinating proximal-to-distal peristalsis of the upper urinary tract. This provides insight into the genetic causes of common inherited urinary tract disorders such as reflux and obstruction.

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The excretory and regulatory functions of the kidney are dependent on filtration of blood at the glomerulus; reabsorption of solutes and fluids required for homeostasis by the renal tubules; and the movement of excess fluids, solutes, and metabolic wastes into the bladder for storage prior to excretion.[1,2] This latter process, the transport of urine from the kidney to the bladder, is controlled, in large part, by coordinated contractions of the smooth-muscle coat investing the renal pelvis and ureter.[3] Congenital defects in this peristaltic process are common and can cause a wide spectrum of pathologies ranging from obstruction and permanent, pressure-induced renal damage to more subtle conditions such as persistent infection due to reflux of urine from the bladder back into the kidney.[4–7] Despite the high incidence of acquired and congenital defects that impair upper urinary tract peristalsis, the molecular mechanisms that control the initiation site and unidirectional nature of this process remain poorly understood.

In animals with uni-papillary kidneys, such as the mouse, the smooth-muscle coat investing the upper urinary tract begins where the renal pelvis joins the connective tissue core of the kidney (Figure 1).[8] It is extremely thin at this site, the pelvis–kidney junction (PKJ), but gradually thickens toward the ureteral–pelvic junction. Although cells along the entire length of the smooth-muscle coat surrounding the renal pelvis and ureter are excitable, under normal conditions contractions always initiate at the PKJ and move distally, in a coordinated, wave-like manner, down the ureter. These coordinated contractile waves are myogenic, occurring in the absence of nervous input, and current hypotheses predict that they are triggered by a specialized cell population at the PKJ.[9–11] Specifically, large numbers of atypical smooth-muscle cells are found at the PKJ adjacent to the smooth muscle and when isolated, they exhibit spontaneous depolarizations that are greater in frequency than the pelvic smooth-muscle cells. Moreover, atypical smooth-muscle-cell depolarizations precede contractile activity in tissue strips taken from the wall of the renal pelvis. Thus, it is likely that presence of spontaneous electrical activity at the PKJ localizes the origin of upper urinary tract peristalsis and coordinates downstream contractile activity. However, the molecular

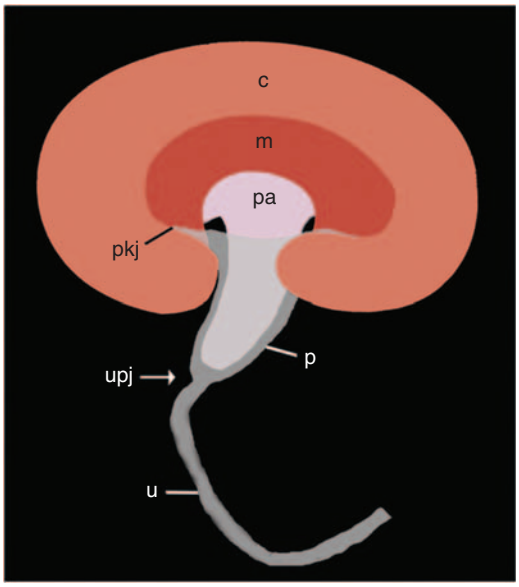

Figure 1 | The upper urinary tract is composed of the kidney and ureter. Blood is filtered in the kidney by the glomeruli and plasma ultrafiltrate is modified as it passes through the tubular nephron segments and collecting ducts located in the cortical (c) and medullary (m) zones of the organ. The collecting ducts coalesce into several large ducts in the papilla (pa), and urine flows from these ducts into the renal pelvis (p). The renal pelvis, a funnel shaped structure between the renal tubules and the ureter (u), joins the connective tissue core of the kidney at the pelvis–kidney junction (pkj). Both the renal pelvis and the ureter have a water impermeable epithelium and smooth muscle coat, which thickens at the ureter–pelvis junction (upj) and functions to carry urine, in relatively unmodified form, from the kidney to the bladder.

mechanisms underlying spontaneous depolarizations at the PKJ have yet to be established and a definitive relationship between this electrical activity and peristalsis remains unclear.

We used a candidate gene approach to identify ion channels that play a role in initiating urinary tract peristalsis at the PKJ. Specifically, we hypothesized that spontaneous membrane depolarizations observed at the PKJ may be controlled, in part, by members of the hyperpolarization-activated cation (HCN) ion channel family. HCN channels have been shown to be required for spontaneous membrane depolarizations exhibited by pacemaker cells of the heart and for spontaneous depolarizations that underlie autorhythmic electrical activity in the brain.[12–16] To test this hypothesis we devised a novel explant system to analyze contractile and electrical activity in the murine urinary tract from the PKJ through to the distal ureter. Reverse transcription-PCR (RT-PCR) and immunohistochemical techniques were used to identify HCN-family members expressed in the urinary tract and strikingly, a high level of HCN3 expression was observed at the PKJ. Most importantly, inhibition of HCN channel activity disrupted coordinated, proximal-to-distal contractions of the upper urinary tract smooth-muscle coat, causing it to twitch along its entire length. Optical mapping experiments showed that HCN channel inhibition caused a loss of electrical activity at the PKJ and randomized the site where spontaneous membrane depolarizations initiate along the urinary tract. Thus, we have shown, for the first time, that inhibition of HCN channel activity results in loss of electrical activity at the PKJ, and perturbs both the origin and propagation of peristaltic waves along the upper urinary tract. In addition, these results provide new insight into possible genetic causes of urine reflux and abnormal ureteral peristalsis.

RESULTS

Unidirectional waves of contractile and electrical activity initiate at the PKJ

We devised an explant system to directly and continuously analyze waves of contractile and electrical activity from the PKJ through to the distal ureter. Briefly, ureters with adjoining kidneys were isolated from adult male mice and the PKJ and renal pelvis were exposed by bisecting the kidneys along their sagittal plane (Figure 2b and c). Direct video-microscopic examination of explants prepared in this manner showed that contractile waves always initiated at the PKJ and then moved distally through the renal pelvis and then down the ureter ($n = 25$) (Figure 2d and e, and Supplementary Movie S1). Contractile waves occurred 4–9 times/min in the explanted urinary tracts, similar to frequencies reported *in vivo*.[17] To analyze electrical activity in explants, changes in membrane potential were detected using the voltage-sensitive dye RH237.[18] Explants were loaded with the dye and changes in the intensity of fluorescence signals across the upper urinary tract were recorded over time. Results of these studies showed that spontaneous electrical excitation initiates at the PKJ and propagates distally in a coordinated wave-like manner ($n = 15$) (Figure 3). Collectively, these video-microscopic and optical mapping data demonstrate that both electrical and contractile activity initiate at the PKJ.

Proximal urinary tract tissues containing the PKJ are required for peristalsis

We next asked whether the PKJ was required to initiate contractile waves that propagate distally down the renal pelvis and the ureter. Urinary tract explants were prepared as described above and then further dissected to sequentially remove portions of the urinary tract as shown in Figure 4 ($n = 4$). Initial dissections removed major portions of the kidney, including the papilla and the outer regions of the cortex and medulla (Figure 4a, illustration; Figure 4a, representative sample; Supplementary Movie S2). Real-time video microscopy of these explants showed that the upper urinary tract continued to contract in a proximal-to-distal wave, initiating at the PKJ ($n = 4$) (Figure 4d–f). These data showed that kidney tissues, including cortex, medulla, and renal papillae, were not required for initiating peristalsis.

To test whether the PKJ was required for triggering the observed proximal-to-distal contractions, we separated the