Human Podocytes Possess a Stretch-Sensitive, Ca$^{2+}$-Activated K$^+$ Channel: Potential Implications for the Control of Glomerular Filtration

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Abstract. Podocytes express many proteins characteristic of smooth muscle, such as actin and myosin. They also express receptors to several vasoactive agents, including acetylcholine and angiotensin II; these phenotypic properties suggest that podocytes are not static entities but may respond to physiologic stimuli. The electrophysiologic properties of a conditionally immortalized human podocyte cell line that expresses the specific podocyte proteins nephrin, podocin, and synaptopodin were examined by patch clamp. Channels that were highly K$^+$-selective and had a conductance of 224 ± 11.5 pS in symmetrical 150 mM K$^+$ solutions were identified. Channel activity was Ca$^{2+}$- and voltage-dependent, being increased with an increase in Ca$^{2+}$ or depolarization, and inhibited by penitrem A. The conductance and voltage- and Ca$^{2+}$-dependence suggest that this is the large-conductance calcium-activated K$^+$ channel, BK (KCNMA1)—this was supported by reverse transcription–PCR experiments that showed the presence of the BK encoding mRNA, along with expression of KCNMB subunit types 3 and 4. In sections of human glomeruli, immunocytochemistry revealed that BK co-localizes with the podocyte-specific protein nephrin, indicating that these channels are present in native human podocytes. In whole-cell experiments, penitrem A inhibited outward currents to the same extent as tetra-ethyl ammonium (TEA) but did not affect the membrane potential. Channel activity was also increased by applying suction to the patch pipette or by dilution of the bathing medium, indicating that these channels are stretch sensitive. Thus, these channels do not contribute to the resting membrane potential but are activated by a rise in intracellular Ca$^{2+}$, membrane depolarization, cell swelling, or membrane stretch. By implication, these results suggest that podocytes may be able to respond to changes in the glomerular capillary pressure and modulate the GFR.

A key function of the mammalian kidney is the ability to filter selectively the plasma, allowing water and small molecules to pass freely into the filtrate while retaining larger molecules such as plasma proteins. This filtration takes place in the glomerulus, a specialized knot of blood vessels whose wall forms the filtration barrier and is composed of three layers: the endothelium, glomerular basement membrane, and visceral epithelial cells or podocytes. Podocytes have finger-like projections, called pedicels, or foot processes, that interdigitate with those from neighboring cells and that are separated by a glycoprotein network forming slit pores of ~40 nM in width (1). Although the role of these slit pores in filtration is conjectural, there is clear evidence that changes in podocyte morphology are associated with changes in the integrity of the glomerular filter; for example, the loss of podocytes and progressive nephropathy of diabetes (2). Importantly, congenital forms of nephrotic syndrome are associated with mutations of podocyte-specific genes (1). The limited ability of the podocyte to repair and/or regenerate is believed to be crucial in progressive renal disease (3).

The physiologic function of the podocyte remains unknown. Its location within the glomerulus, surrounding the glomerular capillaries, means that it will be exposed to the transmural hydrostatic pressure favoring glomerular filtration. Podocytes contain actin and myosin, suggestive of contractile ability; if coupled with mechanosensitivity, these proteins would allow podocytes to react to changes in capillary diameter and thus maintain the patency of Bowman’s space in the face of variations in perfusion pressure. Mature podocytes express several types of receptor and second messenger systems (1). Receptors include muscarinic, angiotensin II, prostaglandin E$_2$, and atrial natriuretic peptide, which are targeted onto Ca$^{2+}$, phospholipase C/inositol 1,4,5-triphosphate (PLC/IP$_3$), cAMP, and cyclic guanosine monophosphate signal cascades. Thus, the evidence suggests that, far from being passive entities, simply providing passive support for the capillary endothelium and slit pores, podocytes have the relevant cellular machinery to allow rapid responses to external stimuli.

Remarkably little is known of podocyte electrophysiology,
previously because of the difficulty of isolation and the loss of phenotype in tissue culture (4). To date, published work has been limited to measurements of the membrane potential and whole-cell conductance in rodent podocytes and their response to vasoactive agonists (5). The membrane potential lies between −38 and −64 mV (6,7), suggesting moderate K⁺ selectivity but also the presence of other conductances, such as Cl⁻ or nonselective cation channels.

In this article, we present the first recordings of single ion channel activity from podocytes. To overcome the problem of dedifferentiation associated with primary podocyte culture, we used a recently developed conditionally immortalized human podocyte cell line that expresses the specific podocyte proteins nephrin, podocin, and synaptopodin (4).

Materials and Methods
Cell Culture
All experiments were carried out on an immortalized monoclonal cell line generated from outgrowths of explanted glomeruli of human kidney that had been subsequently transfected with the temperature-sensitive SV40 gene construct (4). The incorporation of this construct confers temperature sensitivity on cell development, such that at a permissive temperature of 33°C, the cells remain in an undifferentiated proliferative state, whereas raising the temperature to 37°C results in growth arrest and differentiation to the parental podocyte phenotype. Undifferentiated podocyte cultures were maintained at 33°C in plastic culture flasks that contained RPMI 1640 medium with penicillin, streptomycin, ITS (insulin, transferrin, selenite), and 10% FCS. Once cells had reached 70 to 80% confluence, they were transferred to an incubator set at 37°C and left for at least 14 d before use, when full differentiation had taken place (4). On the day before a patch-clamp experiment, adherent cells were dispersed by brief incubation in Ca²⁺-free medium, followed by gentle trituration, seeded onto sterile glass coverslips, and maintained at 37°C.

Electrophysiology
A small section of coverslip was transferred to a perfusion chamber (Model RC-22; Warner Instruments) situated on the stage of an inverted microscope (Nikon Diaphot) and standard patch-clamp techniques were used to measure channel activity in cell-attached and inside-out configurations. All experiments were performed at room temperature. The bath perfusion system was a gravity feed system inside-out configurations. Typical recordings with Ringer solution in the pipette resulted in a large increase in channel activity. Openings in the hypotonic shock experiments, the bath solution results in a large increase in channel activity. Openings are upward in all traces.

Reverse Transcription–PCR
Mature podocyte cultures, 14 d after thermoswitching, were rinsed with RNase-free PBS and dispersed using trypsin-EDTA, and the cells were pelleted by brief centrifugation. The resulting pellet was resuspended in Tri-Reagent (Sigma), and total RNA was purified by chloroform extraction and precipitation with isopropanol. Approximately 1 µg of total RNA was reverse-transcribed using oligo-dT primers and Superscript reverse transcriptase, according to the manufacturer’s instructions (Invitrogen). Reverse transcription (RT) products were subject to 30 to 40 cycles of PCR with primers directed toward the α and β subunits of BK channels (KCNA1, KCNB1–4) and SLACK (KCNT1). Primer sequences were as follows (5’ to 3’): KCNMA1 sense AACCAGCCCATATGAGTTTG and antisense GGATGGGATGGAGTGAACAG; β1 sense AGAGACACGAGCCCTTTGCC and antisense CGGTCAACGAGGAACTGG; β2 sense CAAAAGGAAAAACGTCACACG and antisense GAAGTGAATGGAGACAC; β3 sense GAGGAGCCGACCCTGATG and antisense CACCACTAGCAGTGTCAGGAAG; β4 sense TCTATCTCGGGCTCTCTGCTG and antisense TGCTGGGAACTAATGCTCATC; SLACK sense AGATCCAGCCTGAGCATCG and antisense GAAACACGGGGATGAACA. To control for genomic DNA contamination, primers were designed to span intron sequences, and RT were performed in the absence of Super- script. The integrity of total RNA and efficacy of RT were confirmed by amplification by β-actin with sense GCATTGTTACCACTGGGACG and antisense TGGAAAGTGGGACATGGGC primers. The identity of PCR products was confirmed by agarose gel electrophore-
sis and automated fluorescence sequencing (Lark Laboratories). RT-PCR was performed in duplicate on total RNA from three different batches of podocytes.

**Immunocytochemistry**

Frozen sections (5 μM) were mounted on polylsine-coated microscope slides and fixed with 2% paraformaldehyde and 4% sucrose in PBS for 10 min, then permeabilized with 0.3% Triton X-100 (Sigma) in PBS for 10 min. Sections were washed with 4% FCS in PBS for 5 min. Nonspecific binding sites were blocked with 4% FCS + 0.1% Tween 20 (Sigma) in PBS for 30 min. Primary antibodies and FITC- or TEXAS red–conjugated secondary antibodies (purchased from Biochem-Pharm, Salzburg, Austria) were applied at 1:100 and 1:200 dilutions in the blocking solution, respectively (4). Sections were washed with 4% FCS in PBS for 3 × 10 min after each antibody. Coverslips were mounted with 10 μl of Vectashield (Vector Laboratories, Burlingame, CA). Images were obtained using a Spot 2 slider digital camera (Diagnostic Instruments Inc., Sterling Heights MI) on a Leica DM-IRB photomicroscope (Wetzlar, Germany) and processed with Adobe Photoshop 6.0 software.

Results are presented as mean ± the SEM, with the number of observations, n. Statistical analysis was performed using t test (Excel, Microsoft), and graphs were plotted in Origin (Microcal).

**Results**

In the cell-attached configuration, with Ringer solution in the pipette and a high-K⁺ bath solution, BK channel activity was evident upon strong depolarization (Figure 1, left). Under these circumstances, the cell membrane potential will be close to 0 mV, and there will be a chemical driving force favoring K⁺ movement from the cell to the pipette; however, in the absence of an imposed potential, no channel activity was observed. Similarly, in a separate series of experiments, with the more physiologic Ringer solution in the bath and either Ringer or a K⁺-rich solution in the pipette, channel activity was evident only with applied depolarizations in excess of 80 mV. Excision of the patch caused the immediate appearance of channel activity (Figure 1, right)—in this inside-out configuration, the cytosolic face of the channel is exposed to the bath solution that contains 1 mM Ca²⁺.

The conductance and K⁺ selectivity of the channels is shown in Figure 2. The slope conductance in excised inside-out patches in symmetrical 150 mM K⁺ was 224 ± 11.5 pS (n = 6). For a K⁺-selective channel, lowering the bath perfusate K⁺ concentration should cause a rightward shift in the reversal potential, as was seen with bath K⁺ concentrations of 31.5 and 4.5 mM. The data were reasonably well-described by the GHK equation (dotted lines are best fits), which gave estimates of the PK/PNa ratio of 31.5 mM K⁺ of 100 (31.5 mM K⁺) and 21.5 (4.5 mM K⁺). Thus, the channels are highly K⁺ selective.

The Ca²⁺ and voltage dependence of the channels suggested in Figure 1 is shown more clearly in Figure 3. In excised inside-out patches in symmetrical 150 mM K⁺ was 224 ± 11.5 pS (n = 6). For a K⁺-selective channel, lowering the bath perfusate K⁺ concentration should cause a rightward shift in the reversal potential, as was seen with bath K⁺ concentrations of 31.5 and 4.5 mM. The data were reasonably well-described by the GHK equation (dotted lines are best fits), which gave estimates of the PK/PNa ratio of >100 (31.5 mM K⁺) and 21.5 (4.5 mM K⁺). Thus, the channels are highly K⁺ selective.

The Ca²⁺ and voltage dependence of the channels suggested in Figure 1 is shown more clearly in Figure 3. In excised patches, raising the Ca²⁺ concentration increases the open probability at any given potential. In addition, the open probability at any bath Ca²⁺ concentration increases with depolarization. The voltage dependence of channel open probability, Po, can be described by the Boltzmann function Po =
Po_max[(1/e - (V - V_0.5)zF/RT)], where Po_max is the maximum Po, V is the membrane potential, V_0.5 is the voltage at which Po is 0.5, z' is the effective valence, F is Faraday's constant, R is the gas constant, and T is the absolute temperature. The effective valence is an index of voltage sensitivity and was 1.91 ± 0.11 at 1 mM Ca^{2+} and 2.12 ± 0.10 at 1 μM Ca^{2+}.

BK channels are selectively inhibited by penitrem A (8). Inclusion of 100 nM penitrem A in the pipette solution gave a marked reduction in channel activity in cell-attached patches (Figure 4). In this series of experiments, the patch was depolarized by 160 mV to ensure that channel activity could be detected. For minimizing potential experimental artifacts, penitrem A was added to the pipette solution in alternate patches. All control patches showed BK channel activity, whereas channel activity was observed in only one of the seven patches that were exposed to penitrem A, and the mean channel activity was significantly reduced (Figure 4; NB, logarithmic scale of NPo).

To detect macroscopic BK currents, we performed both conventional and perforated patch whole-cell recordings. In both cases, we determined the sensitivity of the currents to the BK inhibitors tetra-ethyl ammonium (TEA) (5 mM) and penitrem A (100 nM) (8,9). In conventional whole-cell clamp, the cell becomes dialyzed with the pipette solution, which had a Ca^{2+} activity of 100 μM to maximize BK currents. The I/V curves (Figure 5) show a substantial outward current of 5.17 ± 0.7 pA/pF (539 ± 114 pA, n = 4) at 100 mV, which is inhibited to the same extent by TEA and penitrem A, with a voltage dependence and time course of activation characteristic of BK channels [e.g., see (10)]. To detect macroscopic BK currents at the resting intracellular Ca^{2+} activity, we used the perforated patch technique. The TEA- and penitrem A–sensitive currents at 100 mV were the same as those in the whole-cell recordings, indicating that maximal channel activity was achieved in both recording modes. Penitrem A had no effect on the membrane potential: the mean change was −0.2 mV ± 1.2 mV (n = 4), indicating that BK does not contribute to the resting membrane potential.

During the course of these experiments, it was noted that current noise arising from the patch increased during the formation of a seal, when suction is typically applied to the rear

![Control](image1)

![Penitrem A](image2)

![N.Po](image3)

*Figure 4. Inhibition of channels by penitrem A. (TOP) Typical cell-attached recordings of channel activity at a holding potential 160 mV positive to the resting membrane potential, where outward currents are readily seen in the absence of penitrem A (control). Inclusion of penitrem A in the pipette solution drastically reduces the occurrence of channel openings. (Bottom) Mean data of channel activity (N.Po) for experiments depicted in top panel (control, n = 5; penitrem A, n = 7). Bars = 1 SEM. Note that the N.Po axis is logarithmic.
of the pipette. Figure 6 shows the clear increase in channel activity when a negative pressure of 20 cmH2O is applied to cell-attached patches after the establishment of a Giga-seal. Such increased activity was completely reversible. Thus, these channels are stretch sensitive. Furthermore, cell swelling induced by hypotonic shock also increased channel activity in a reversible manner (Figure 7).

The two channels that could be responsible for these large-conductance, calcium-activated channel currents are BK (KCNMA1) and SLACK (KCNT1). To discriminate between these two gene product candidates, we performed RT-PCR of RNA derived from mature podocytes. We could detect no evidence of the expression of SLACK (Figure 8)—this was not due to inefficacy of the primers because the positive control brain tissue yielded products of appropriate size. However, KCNMA1 mRNA was present in both brain- and podocyte-derived RNA, suggestive of its expression in these cells. Finally, the properties of BK channels are influenced by the accessory β subunits (10). Once again, using brain RNA as positive control, we could detect evidence of β3 and β4 mRNA but not β1 or β2 mRNA in podocytes (Figure 9). In both of these sets of RT-PCR experiments, DNA product bands were absent in the negative-RT controls, indicating lack of contamination. The ability to detect all β subunits in brain RNA but only β3 and β4 products in podocyte RNA indicates the selective expression of these two β subunits in podocytes.

To address the question as to whether KCNMA1 is present in native human podocytes, we subjected human kidney sections to immunocytochemistry with antibodies to KCNMA1 and the podocyte-specific marker nephrin (Figure 10, A and B). Coexpression of KCNMA1 and nephrin is shown by the yellow regions of the overlay (Figure 10C and indicates that KCNMA1 is expressed in podocytes in human kidney.

**Discussion**

The glomerular podocytes form the earliest part of the nephron and are continuous, with the epithelial cells forming Bowman’s capsule. These specialized cells form a complex interdigitating network that envelops the glomerular capillary tuft and are an integral part of the filtration barrier. They are thought to be important in the determination of GFR and in the discrimination against the filtration of proteins. For years, it has been supposed that these cells may have contractile activity, i.e., that they are myogenic, and that they may be able to
respond to changes in the glomerular capillary pressure (11). To enable this, the cells would need to respond to deformation; the discovery of channels whose open probability is increased by membrane stretch, reported in this article, provides the first direct experimental evidence indicating that podocytes may be able to respond acutely to mechanical stress.

The data presented in this article clearly show the presence of BK channels in immortalized human podocytes and in native human glomerular tissue, where BK channels co-localize with the podocyte-specific marker nephrin. The large-conductance, high-K⁺ selectivity, Ca²⁺ and voltage dependence and sensitivity to penitrem A all are characteristic of BK channels (12). In addition, RT-PCR showed the presence of mRNA encoding the pore-forming α subunit (KCNMA1) and two accessory β subunits (KCNMB3 and KCNMB4). However, the lack of channel activity in the absence of an applied depolarization or negative pressure, together with the lack of effect of penitrem A on the membrane potential, indicates that BK channels probably play little role in determining the resting membrane potential of immortalized human podocytes. However, the current study shows that these channels are activated when the cell membrane is stretched, either by the application of negative pressure to the patch pipette or by an increase in intracellular pressure as a consequence of cell swelling.

The gating of BK channels is modulated by the coexpression of β subunits (10). Our RT-PCR data indicated the presence of β3 and β4 subunits. When coexpressed with the α subunit in a heterologous expression system, β4 caused a slight slowing of activation upon depolarization and increased the sensitivity to intracellular Ca²⁺. A consequence of β subunit expression is to confer estrogen sensitivity on BK (13). This is of potential interest because estrogen increases GFR independent of changes in BP, i.e., it increases Kf (14). By the same token, it has been postulated the contractile state of podocytes may alter the size of the filtration slits and thereby alter Kf and GFR (11).
This raises the possibility that the effects of estrogen on GFR are mediated by changes in podocyte contractility.

What is the likely physiologic role of BK channels in podocytes? BK channels are present in a wide variety of cell types (15). In excitable cells such as neurons and myocytes, BK channels provide a negative-feedback mechanism to control the membrane potential, resulting in relatively long periods of silence during otherwise intense activity, therefore avoiding overstimulation. In nonexcitable tissues, such as renal and colonic epithelia, they are involved in K secretion and volume regulation, whereas in exocrine glands, they play a role in stimulus-secretion coupling. Our own preliminary data on gene expression suggest a smooth muscle phenotype of podocytes (16). Vascular smooth muscle cells depolarize as the luminal pressure is increased (17), possibly as a consequence of activation of nonselective cation channels (18). Nonselective cation channel activation results in entry of Na\(^+\) and Ca\(^{2+}\), causing depolarization. At the same time, intracellular Ca\(^{2+}\) is raised, as a result both of Ca\(^{2+}\) influx (19) and release from intracellular stores (20). This increase in Ca\(^{2+}\) is accompanied by an increase in BK channel activity, which acts to moderate the degree of membrane depolarization (21); this is seen as a protective mechanism in limiting the degree of smooth muscle contraction such that the vessels remain patent. By analogy, one might hypothesize that the role of BK channels in podocytes is in the negative-feedback control of the membrane potential, thus moderating the degree of podocyte contraction in response to membrane stretch.

In conclusion, this article provides the first evidence of the expression and activity of BK channels in human podocytes. These channels do not contribute to the resting membrane potential but are activated by a rise in intracellular Ca\(^{2+}\), membrane depolarization, membrane stretch, and an increase in cell volume. The stretch sensitivity lends further support to the idea that podocytes may be myogenic, capable of responding to changes in the glomerular capillary pressure and potentially affecting the GFR.

Acknowledgments

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References