K\textsubscript{ATP} channel conductance of descending vasa recta pericytes

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Cao, Chunhua, Whaseon Lee-Kwon, Erik P. Silldorff, and Thomas L. Pallone. K\textsubscript{ATP} channel conductance of descending vasa recta pericytes. Am J Physiol Renal Physiol 289: F1235–F1245, 2005.—Using nystatin-perforated patch-clamp and whole cell recording, we tested the hypothesis that K\textsubscript{ATP} channels contribute to resting conductance of rat descending vasa recta (DVR) pericytes and are modulated by vasoconstrictors. The K\textsubscript{ATP} blocker glybenclamide (Glb; 10 \mu M) depolarized pericytes and inhibited outward currents of cells held at −40 mV. K\textsubscript{ATP} opens pinacidal (PnC; 10 \mu M) and P-1075 (1 \mu M) hyperpolarized pericytes and transiently augmented outward currents. All effects of PnC and P-1075 were fully reversed by Glb. Inward currents of pericytes held at −60 mV in symmetrical 140 mM K\textsuperscript{+} were markedly augmented by PnC and fully reversed by Glb. Ramp depolarizations in symmetrical K\textsuperscript{+}, performed in PnC and PnC + Glb, yielded a PnC-induced, Glb-sensitive K\textsubscript{ATP} difference current that lacked rectification and reversed at 0 mV. Immunostaining identified both KIR6.1, KIR6.2 inward rectifier subunits and the sulfonylurea receptor subtype 2B. ANG II (1 and 10 nM) and endothelin (ET)-1 potently reduced resting pericyte membrane conductance to K\textsuperscript{+} and inhibited the ability of the K\textsubscript{ATP} opener, pinacidal (PnC), to activate K\textsuperscript{+} currents. K\textsubscript{ATP} inhibition is specific to vasoconstrictors because vasopressin was less effective than ET-1 and ANG II to inhibit DVR pericyte K\textsubscript{ATP} channels.

METHODS

Isolation of DVR. Investigations involving animal use were performed according to protocols approved by the Institutional Animal Care and Use Committee of the University of Maryland. Kidneys were harvested from Sprague-Dawley rats (100–150 g; Harlan) that had been anesthetized by an intraperitoneal injection of ketamine/xylazine (80 mg/kg; 10 mg/kg). Tissue slices were stored at 4°C in a physiological saline solution (PSS; in mM: 155 NaCl, 5 KCl, 1 MgCl\textsubscript{2}, 1 CaCl\textsubscript{2}, 10 HEPES, and 10 glucose, pH 7.4). Small wedges of renal medulla were dissected and transferred to a perfusion chamber for patch-clamp recording. Initially, DVR were isolated from the enzyme-digested renal tissue and incubated at 37°C for 45 min, transferred to PSS, and stored at 4°C. At intervals, DVR were isolated from the enzyme-digested renal tissue and transferred to a perfusion chamber for patch-clamp recording.

Whole cell patch-clamp recording. Patch pipettes were made from borosilicate glass capillaries (PG5215–4, external diameter 1.5 mm, internal diameter 1.0 mm; World Precision Instruments, Sarasota, FL), using a two-stage vertical pipette puller (Narshige PP-830) and heat polished. For whole cell perforated patch-clamp recording, the pipette solution contained (in mM) 120 kaspamate, 20 KCl, 10 NaCl, 10 HEPES, pH 7.2, and nystatin (100 \mu g/ml with 0.1% DMSO) in ultrapure water. Patch pipettes were backfilled with a ligh-protected syringe through a 0.2-\mu m filter. Measurements were obtained as previously described (45, 46). Membrane potential was measured using a CV201AU headstage and Axopatch 200 amplifiers (Axon Instruments, Foster City, CA) in current clamp mode (I = 0) at a sampling rate of 10 Hz using 8- to 10-M\Omega pipettes. Whole cell current

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DVR PERICYTE K\textsubscript{ATP} CHANNELS

recording was performed with 6- to 8-M\textOmega{} pipettes. Whole cell currents were sampled at 10 kHz during 100-ms ramp depolarizations and at 10 Hz during continuous recordings. To calculate cell capacitance (C\textsubscript{m}), 10-mV pulses from a holding potential of \(-70 \text{ mV}\) were applied using the “membrane test” feature of Clampex (Axon Instruments). Some pericytes showed prolonged capacitance transients indicative of cell-to-cell coupling via gap junctions. Such cells were abandoned, and recordings were not obtained from them. To ensure that selected pericytes were uncoupled, we compared their capacitances to those of pericytes preexposed for at least 5 min to gap junction blockers (100 \mu{}M carbenoxolone, 1 mM heptanol). All data have been corrected for junction potentials (39, 45, 46).

These studies focused on the putative expression of K\textsubscript{ATP} channels in DVR pericytes. To generate electrochemical forces favoring a K\textsuperscript{+} outward current, the holding potential of the cell (V\textsubscript{h}) was increased above the K\textsuperscript{+} equilibrium potential (K\textsubscript{eq}) of our bath and electrode buffers (\(-89 \text{ mV}\); PSS and electrode solutions, see above) by shifting V\textsubscript{h} from \(-80 \text{ to } -40 \text{ mV}\). To generate conditions favoring a K\textsuperscript{+} inward current, V\textsubscript{h} was held at \(-60 \text{ to } -65 \text{ mV}\), whereas K\textsubscript{eq} was shifted from \(-89 \text{ to } 0 \text{ mV}\) by raising bath KCl concentration from 5 to 140 mM by isosmotic substitution of KCl for NaCl. In the former case, a holding potential of \(-40 \text{ mV}\) is close to the Cl\textsuperscript{−} equilibrium potential (Cl\textsubscript{eq}) of our buffers, minimizing contamination of recordings with Cl\textsuperscript{−} currents. In the latter case, V\textsubscript{h} = \(-60 \text{ mV}\) is below the threshold for global activation of the Ca\textsuperscript{2+}-dependent Cl\textsuperscript{−} current of DVR pericytes (46).

Chord conductance (G\textsubscript{k}) was calculated from the expression \(I_{K} = G_{K}(V - K_{eq})\) where I\textsubscript{K} is current carried by K\textsuperscript{+} ion, V is membrane potential, and K\textsubscript{eq} is the K\textsuperscript{+} equilibrium potential for the electrode and extracellular buffers.

Immunohistochemical staining of renal medulla and isolated DVR. Immunohistochemical staining of isolated DVR was performed as previously described (36). Hand-dissected DVR were placed on glass coverslips, fixed with 3% paraformaldehyde in 0.1 M cacodylate for 5 min at 25°C, and washed with PSS solution. The fixed DVR were permeabilized for 30 min with 0.1% Triton X-100 in PSS, and placed in 5% BSA blocking solution for 30 min at room temperature. Primary antibodies were incubated overnight at 4°C in blocking solution. The primary antibodies directed against inward rectifier K\textsuperscript{+} channels, K\textsubscript{ATP} subunits SUR1, SUR2B were obtained from Santa Cruz Biotechnology (CA). Antibodies were diluted 1:200 (SUR1, SUR2B) or 1:100 (sc11225, sc11228, sc5789, sc5793, respectively, 1:30 dilution) (36). Pericytes were identified with primary monoclonal antibody against α-smooth muscle actin (Sigma, diluted 1:500). DVR were subsequently washed three times with PSS and incubated with Alexa-conjugated donkey anti-goat or anti-mouse secondary antibodies (Molecular Probes, diluted 1:200). The vessels were again washed with PSS and then mounted in Vectashield (Vector Laboratories). Negative controls were performed by omitting the incubation with primary antibody. Immunofluorescent images were captured with a Zeiss LSM410 confocal fluorescence microscope. Images were captured at 512 × 512-pixel resolution with z-axis sectioning at 0.5-\mu{}m intervals.

Reagents. ANG II, ET-1, vasopressin, Pnc, glybenclamide (Glb), heptanol, carbenoxolone, nystatin, and other chemicals were from Sigma (St. Louis, MO). The K\textsubscript{ATP} channel activator, N-cyano-N\textsuperscript{−}(1,1-dimethylpropyl)-N\textsuperscript{−}3-pyridylguanidine (P-1075), was from Tocris Cookson (Ellisville, MO). Liberase Blenzyme 1 was from Sigma (St. Louis, MO). The KATP channel activator, N\textsuperscript{−}3-pyridylguanidine (P-1075), was from Tocris Cookson (Ellisville, MO). Liberase Blenzyme 1 was from Sigma (St. Louis, MO). The KATP channel activator, N\textsuperscript{−}3-pyridylguanidine (P-1075), was from Tocris Cookson (Ellisville, MO). Liberase Blenzyme 1 was from Sigma (St. Louis, MO). The KATP channel activator, N\textsuperscript{−}3-pyridylguanidine (P-1075), was from Tocris Cookson (Ellisville, MO). Liberase Blenzyme 1 was from Sigma (St. Louis, MO). The KATP channel activator, N\textsuperscript{−}3-pyridylguanidine (P-1075), was from Tocris Cookson (Ellisville, MO). Liberase Blenzyme 1 was from Sigma (St. Louis, MO). The KATP channel activator, N\textsuperscript{−}3-pyridylguanidine (P-1075), was from Tocris Cookson (Ellisville, MO). Liberase Blenzyme 1 was from Sigma (St. Louis, MO).

Statistics. Data in the text and figures are reported as means ± SE. The significance of differences was evaluated with SigmaStat 3.11 (Systat Software, Point Richmond, CA) using parametric or nonparametric tests as appropriate for the data. Comparisons between two groups were performed with Student’s t-test (paired or unpaired, as appropriate) or the Rank Sum Test (nonparametric). Comparisons between multiple groups employed one-way ANOVA, repeated-measures ANOVA, or repeated-measures ANOVA on ranks (nonparametric). Post hoc comparisons were performed using Tukey’s or Holm-Sidak tests. P < 0.05 was used to reject the null hypothesis.

RESULTS

Cell capacitance measurements in DVR pericytes. As previously described, we studied pericytes on the abluminal surface of isolated intact DVR (46). Table 1 provides measurements of C\textsubscript{m} from the pericytes we studied. We did not measure currents of cells that showed prolonged capacitance transients indicative of gap junction coupling. Peptide contractors such as ANG II and ET-1 have been shown to close gap junctions in other preparations (22). C\textsubscript{m} exposed to ANG II (10 nM) or ET-1 (10 nM) as part of these protocols was not significantly different from baseline (Table 1). Also, for comparison, the capacitance of pericytes exposed to high concentrations of gap junction blockers, carbenoxolone (100 mM) or heptanol (2 mM), is provided. Table 1 shows that pericytes selected for study were electrically isolated from the adjacent cells of this intact microvessel preparation.

Role of K\textsubscript{ATP} channels in the determination of pericyte membrane potential. DVR pericyte membrane potential, under conditions of nystatin-perforated patch recording, typically lies between −50 and −65 mV (45, 46, 53), implying contribution of K\textsuperscript{+} conductance to resting membrane potential. We first tested whether K\textsubscript{ATP} channels contribute to that K\textsuperscript{+} conductance by examining the ability of the K\textsubscript{ATP} channel blocker Glb (10 \mu{}M) to depolarize pericytes (Fig. 1). Glb depolarized most cells (for example, Fig. 1, A and B) and, on average, raised membrane potential from −60.4 ± 2.0 to −56.4 ± 2.8 mV (Fig. 1C, n = 10). Thus K\textsubscript{ATP} channels contribute to resting K\textsuperscript{+} conductance.

In separate series, we tested the ability of the K\textsubscript{ATP} channel opener, Pnc (10 \mu{}M) and P-1075 (1 \mu{}M), to hyperpolarize DVR pericytes. The selectivity of those reagents for K\textsubscript{ATP} channels was also verified by testing their sensitivity to Glb. Pnc reliably hyperpolarized DVR pericytes and the effect was completely reversed by Glb (Fig. 2, A and B). On average, Pnc lowered membrane potential from −59.8 ± 5.0 to −76.6 ± 3.0 mV (Fig. 2C, n = 6). The effect of Pnc was maximal at a concentration of 10 \mu{}M (Fig. 2D, n = 5) (69). The K\textsubscript{ATP} channel opener P-1075 also hyperpolarized DVR pericytes in a concentration-dependent manner and was reversed by Glb (Fig. 2E, n = 5). Taken together, the findings verify that K\textsubscript{ATP} channels are available to be activated and increase pericyte membrane conductance to K\textsuperscript{+}.

Participation of K\textsubscript{ATP} channels in K\textsuperscript{+} outward currents of DVR pericytes. We next tested whether outward currents, induced by clamping DVR pericytes to Cl\textsubscript{eq} (V\textsubscript{h} = −40 mV),

Table 1. Pericyte capacitance measurements

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Concentration</th>
<th>N</th>
<th>C\textsubscript{m} pF</th>
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<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td>70</td>
<td>12.1 ± 0.7</td>
</tr>
<tr>
<td>ANG II</td>
<td>10 nM</td>
<td>16</td>
<td>13.7 ± 0.6</td>
</tr>
<tr>
<td>ET-1</td>
<td>10 nM</td>
<td>12</td>
<td>11.3 ± 0.7</td>
</tr>
<tr>
<td>Carbenoxolone</td>
<td>100 \mu{}M</td>
<td>5</td>
<td>11.7 ± 2.1</td>
</tr>
<tr>
<td>Heptanol</td>
<td>2 mM</td>
<td>12</td>
<td>12.1 ± 0.8</td>
</tr>
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</table>

Values are means ± SE. ET-1, endothelin 1; C\textsubscript{m}, cell capacitance.
are carried by K⁺ efflux through K<sub>ATP</sub> channels. When holding potential was shifted from −80 to −40 mV (Fig. 3, A and B), whole cell current reversed sign from net inward to outward. The mean currents at −80 and −40 mV were −54 ± 9.8 and 115 ± 18 pA, respectively (Fig. 3C). Application of Glb (10 μM) reversibly lowered the mean outward current from 115 ± 18 to 65 ± 18 pA (n = 15). Because −40 mV is near Cl<sub>eq</sub>, those currents are largely carried by K⁺ ion. Glb reduced the chord conductance for K⁺ from 2.37 ± 0.39 to 1.34 ± 0.39 nS. Thus K<sub>ATP</sub> channels contribute ~43% of K⁺ conductance at −40 mV. In separate series, we measured the ability of the K<sub>ATP</sub> channel openers, Pnc and P-1075, to augment outward currents of pericytes held at V<sub>h</sub> = −40 mV. As illustrated in Fig. 4A, Pnc (10 μM) markedly enhanced outward current but its effect spontaneously reversed. Pnc increased the mean outward current from 102 ± 13 to a peak of 324 ± 37 pA (Fig. 4B, n = 24). To test specificity, we verified that preapplication of Glb prevented Pnc augmentation of outward current (Fig. 4, C and D). P-1075 (1 μM) had a similar effect, transiently increasing outward current from 142 ± 21 to 295 ± 33 pA (Fig. 4, E and F, n = 9). P-1075-induced currents were also completely blocked by Glb.

**Inward currents conducted by K<sub>ATP</sub> channels in DVR pericytes.** The protocols of the experiments reported in Figs. 3 and 4 examined Pnc, P-1075, and Glb effects on outward current. K<sub>ATP</sub> channels typically exhibit linear “ohmic” current-voltage (I-V) relationships with little rectification, i.e., they carry K⁺ outward or inward current with similar conductance (41, 51). To test for that behavior, DVR pericytes were held at −60 mV as external KCl was increased from 5 to 140 mM to create an electrochemical gradient favoring a K⁺ inward current. The ability of Pnc (10 μM) to augment the inward current and the ability of Glb (10 μM) to block the Pnc-augmented current were examined by sequentially introducing Pnc and then Pnc−Glb into the bath (Fig. 5, A–C). During the recording, at 5-s intervals, pericytes were subjected to ramp depolarizations

![Image](http://ajprenal.physiology.org/)

**Fig. 1. Effect of glybenclamide (Glb) on pericyte membrane potential.** A and B: sample tracings show the effect of Glb (10 μM) on descending vasa recta (DVR) pericyte membrane potential. The membrane potential (in mV) at the beginning of the recording is shown under the start of each trace. C: summary of membrane potential measurements from n = 10 cells before, during, and after exposure to Glb (*P < 0.05, control period vs. Glb exposure).

**Fig. 2. Glb reversal of pinacidil (Pnc) and P-1075 DVR pericyte membrane potential hyperpolarization.** A and B: sample tracings show the effect of Pnc (10 μM) on pericyte membrane potential. Pnc-induced hyperpolarization was reversed by Glb (10 μM). The membrane potential (in mV) at the beginning of the recording is shown under the start of each trace. C: summary of membrane potential measurements from n = 6 cells before Pnc, during Pnc, during Pnc + Glb, and after Glb washout (*P < 0.05, control period vs. Pnc; #P < 0.05, Pnc vs. Pnc + Glb). D and E: summary of the concentration dependence of Pnc- and P-1075-induced hyperpolarization. Results are means ± SE of n = 5 cells each (*P < 0.05, vs. 0 μM Pnc or P-1075).
from -60 to +40 mV over 100 ms while sampling at 10 kHz so that I-V characteristics could be repeatedly examined in Pnc and Pnc + Glb. An example is shown in Fig. 5, A and B. The I-V traces labeled as "1" and "2" in Fig. 5B correspond to ramp depolarizations in Pnc and Pnc + Glb, respectively (times 1 and 2, Fig. 5A). Their difference (1 - 2) provides the I-V characteristic of Glb-sensitive KATP current in symmetrical 140 mM KCl. At the holding potential (-60 mV), Pnc increased chord conductance from a baseline of 2.25 ± 0.32 to 6.71 ± 0.66 nS, i.e., overall conductance can be increased 198% by stimulating Pnc-sensitive KATP channels. The Glb-sensitive current calculated from ramp depolarizations had a mean conductance of 4.2 ± 0.74 nS for n = 10 cells (Fig. 5D). The current did not

Fig. 4. Effect of Pnc on outward currents in DVR pericytes. A: sample trace shows current recording from a DVR pericyte as V_h was shifted from -80 to -40 mV at the time of the arrow. I_m increased from -42 to 104 pA. The cell was subsequently exposed to Pnc (10 μM), via the bath, which stimulated a large, transient outward current. B: summary of the peak membrane current (I_m) induced by Pnc in n = 24 DVR pericytes held at -40 mV. Pnc induced a peak I_m of 324 ± 37 pA (P < 0.05, peak I_m vs. baseline). C: sample trace shows membrane current recording of a DVR pericyte as V_h was shifted from -80 to -40 mV at the time of the arrow. Subsequent addition of Glb (10 μM) lowered the holding current slightly and prevented Pnc from subsequently inducing an outward current (contrast with tracing in A). Repeat Pnc exposure after Glb washout induced a robust increase in outward current. D: summary of the change in peak outward current (∆I_m) observed in Pnc (n = 24) and Pnc + Glb (n = 9; *P < 0.05, Pnc vs. Pnc + Glb). E: sample trace shows membrane current recording from a DVR pericyte as holding potential (V_h) was shifted from -80 to -40 mV at the time of the arrow. That change in V_h increased membrane current (I_m) from -78 to 41 pA. The cell was subsequently exposed to P-1075 (1 μM), via the bath, which stimulated a large transient outward current. F: summary of peak outward current (I_m) induced by P-1075 in n = 9 cells. P-1075 induced a peak current of 295 ± 33 pA (P < 0.05, peak I_m vs. baseline).
rectify and it reversed near 0 mV as expected for conductance of K\textsubscript{ATP} channels in symmetrical K\textsuperscript{+}. Application of Glb with Pnc reduced conductance from 6.71 ± 0.66 to 1.47 ± 0.27 nS, a value that is less than the pre-Pnc baseline. The latter implies that ~35% of the resting conductance in symmetrical KCl at -60 mV is accounted for by Glb-sensitive K\textsubscript{ATP} channels. The concentration dependence of Pnc stimulation of inward current is shown in Fig. 5E (n = 4 to 10 cells at each Pnc concentration) and is similar to effects on membrane potential Fig. 2D.

Several features warrant attention. Raising external KCl from 5 to 140 mM induced a large inward current that rapidly waned. After completion of the waning phase, the inward current that persisted was markedly augmented by Pnc and fully reversed by Glb. The inhibition was reversible on Glb washout, showing that Pnc augmentation of the inward current does not wane on the time scale of these experiments (Fig. 5, A and C). The latter contrasts with the transient nature of Pnc-stimulated outward currents (Fig. 4A).

**Expression of Kir6.1, Kir6.2, SUR1, and SUR2B in DVR.**

The sensitivity of pericyte membrane current to K\textsubscript{ATP} channel openers (Pnc, P-1075) and the blocker, Glb (Figs. 1–4), coupled with linear I-V characteristics, and reversal potential consistent with K\textsuperscript{+} ion as the charge carrier (Fig. 5) provide strong evidence for robust K\textsubscript{ATP} expression in DVR pericytes. For further verification, we performed immunohistochemical staining of isolated DVR to identify Kir6.1x and SUR regulatory K\textsubscript{ATP} channel subunits. Microfluorescent images verified that DVR express both Kir6.1 and Kir6.2 subtypes (n = 4) and that they are present in both pericytes and endothelium (Fig. 6). Fluorescence was not observed when the primary antibody was omitted (not shown). Immunochemical staining for SUR subunits (n = 3) revealed predominant expression of SUR2B (Fig. 7).

**Inhibition of K\textsubscript{ATP} channels by vasoconstrictors.** In a recent communication, we showed that TEA and Ba\textsuperscript{2+}-sensitive K\textsuperscript{+} currents are blocked by ANG II (45). We extended those observations by determining whether K\textsubscript{ATP} channels partially carry the ANG II-sensitive K\textsuperscript{+} current and by measuring the concentration dependence of the ANG II effect. In addition, we tested whether two other DVR vasoconstrictors, ET-1 and AVP, can inhibit K\textsubscript{ATP} currents. To perform those experiments, we used the conditions illustrated in Figs. 3 and 4. The effects of the constrictors on baseline and Pnc (10 μM)-induced outward currents of pericytes held at -60 mV were measured.

Examples of records obtained after 10-min pretreatment with ANG II are provided in Fig. 8, A-C. ANG II, at 10 pM (Fig. 8A) and 100 pM (Fig. 8B), did not block Pnc stimulation of outward current. Pnc tended to induce oscillations in cells pretreated with 100 pM ANG II (Fig. 8B). At 1 nM (Fig. 8C) and 10 nM, ANG II completely prevented Pnc stimulation of outward current. The summary of the effects of ANG II
pretreatment on holding current and Pnc-induced outward currents is provided in Fig. 8, D and E, respectively.

In same context, AVP, at the vasoconstrictor concentration of 100 nM (63), was less effective than ANG II to block K⁺ current. Ten-minute pretreatment with AVP failed to lower holding current or reduce Pnc-stimulated outward current in some cells (Fig. 9A) but seemed to markedly affect it in others (Fig. 9B). On average, significant Pnc stimulation of outward current persisted after vasopressin pretreatment (Fig. 9C).

ET-1, at the maximal vasoconstrictor concentration of 10 nM (58), had potent effects to inhibit outward current. As illustrated in Fig. 10A, ET-1 promptly lowered holding current and rendered pericytes insensitive to subsequent application of Pnc (10 μM). Figure 10B shows the effect of pretreatment of a pericyte, held at −80 mV, with ET-1 (10 nM). ET-1 application was followed by repetitive, spiking, spontaneous, transient inward currents (STICs; Fig. 10Ba) similar to those previously reported after ANG II stimulation (46). Shifting Vh to −40 mV (near Cleq) reduced their amplitude, implying Cl⁻ efflux as the likely charge carrier of the STICs. At Vh = −40 mV, Pnc failed to elicit outward currents (Fig. 10Bb). The average holding and Pnc-augmented currents observed after ET-1 exposure are summarized in Fig. 10, C and D, along with the means ± SE of those measured in ANG II (10 nM) and vasopressin (100 nM). High concentrations of ANG II and ET-1, but not vasopressin, lowered pericyte holding current and eliminated Pnc-inducible outward current. Similarly, after ANG II (10 nM) or ET-1 (10 nM) pretreatment, the reduction in holding current at Vh = −40 mV was accompanied by a loss of sensitivity to Glb (10 μM) implying inhibition of basal activity of KATP channels (Fig. 10E).

Fig. 6. Immunochemical identification of KIR6.1 in isolated DVR. Hand-dissected rat DVR were fixed, permeabilized, and immunolabeled with anti-α-smooth muscle actin (A and C) and anti-KIR6.1 (B) or anti-KIR6.2 (D). The arrowheads point to pericyte cell bodies protruding from the abluminal surface. The bar is 8 μm. Both luminal endothelial cells and abluminal pericytes show KIR expression.

Fig. 7. Immunochemical identification of SUR1 and SUR2B in isolated DVR. Hand-dissected rat DVR were fixed, permeabilized, and immunolabeled with anti-α-smooth muscle actin (A and C) and anti-SUR1 (B) or anti-SUR2B antibody (D). The arrowheads point to pericyte cell bodies protruding from the abluminal surface. The bar is 8 μm. Minimal SUR1 staining is observed but luminal endothelial cells and abluminal pericytes show strong SUR2B expression.
Fig. 8. Inhibition of outward currents by ANG II. A-C: representative tracings show the effect of pretreating pericytes for 10 min with ANG II at 10 pM, 100 pM, and 1 nM, respectively, on holding current and Pnc (10 μM)-induced outward current. As in Figs. 3 and 4, holding potential was first increased, at the time indicated by the arrow, from −80 to −40 mV to favor a K⁺ outward current. Subsequently, Pnc was added to the bath. Pretreatment with ANG II at 10 pM did not affect Pnc-induced current, whereas 100 pM and 1 nM ANG II induced oscillations and eliminated Pnc-induced current, respectively. D: summary of holding current ($I_m$) at $V_h = −40$ mV in cells pretreated for 10 min with varying concentrations of ANG II ($n = 15, 6, 8, 4, 6$ at 0, 10 pM, 100 pM, 1 nM, 10 nM, respectively). ANG II reduced holding current at 1 and 10 nM ($P < 0.05$ vs. control lacking ANG II). E: summary of the current increase ($ΔI_m$) induced by Pnc at $V_h = −40$ mV in cells pretreated for 10 min with increasing concentrations of ANG II ($P < 0.05$ vs. control lacking ANG II).

Fig. 9. Inhibition of outward currents by vasopressin. A and B: representative tracings show the effect of pretreating pericytes for 10 min with arginine vasopressin (AVP; 100 nM) on holding current and Pnc (10 μM)-induced outward current. As in Figs. 3 and 4, DVR pericyte holding potential was first increased, at the time indicated by the arrow, from −80 to −40 mV to favor a K⁺ outward current. Subsequently, Pnc was added to the bath. Pretreatment with AVP had variable effects, sometimes showing little inhibition (A) and sometimes achieving complete inhibition (B). C: summary of the effect of Pnc on holding current ($I_m$) at $V_h = −40$ mV in $n = 22$ cells pretreated for 10 min with vasopressin ($P < 0.05$ control vs. Pnc). On average, Pnc effectively increased the outward current despite AVP pretreatment.
Fig. 10. Inhibition of outward currents by endothelin (ET)-1. A: representative trace in which DVR pericyte holding potential was first increased, at the time indicated by the arrow, from −80 to −40 mV to favor a K⁺ outward current. Subsequently, ET-1 (10 nM) was added to the bath leading to a prompt and sustained reduction of current. Following ET-1 treatment, Pnc failed to augment outward current. B: representative tracing in which a DVR pericyte was held at −80 mV during exposure to ET-1. A large transient inward current (Ba) was followed by repetitively spontaneous transient inward currents (STICs) the amplitude of which were nearly eliminated (Bb) by a shift of holding potential from −80 to −40 mV at the time shown by the arrow. Subsequent exposure to Pnc was without effect. C: summary of the membrane current (Im) of pericytes held at −40 mV. Bars show controls (n = 15) vs. cells pretreated with ANG II (10 nM, n = 6), ET-1 (10 nM, n = 6), or vasopressin (100 nM, n = 22; *P < 0.05 vs. control). D: summary of the change in membrane current (∆Im) induced by Pnc (10 μM) of pericytes held at −40 mV. Bars show controls (n = 22) and cells pretreated with ANG II (10 nM, n = 6), ET-1 (10 nM, n = 6), or AVP (100 nM, n = 15; *P < 0.05 vs. control). E: summary of the effect of Glb (10 μM) on membrane current of cells preexposed to ANG II (10 nM) or ET-1 (10 nM) for 10 min (n = 4 each). Data from Fig. 1 showing basal effects of Glb are reproduced for comparison (*P < 0.05 vs. baseline). After ANG II exposure, Glb yielded no further KATP channel inhibition.

DISCUSSION

DVR pericytes, like smooth muscle cells from many vascular beds, increase intracellular cytoplasmic Ca²⁺ through activation of voltage-gated Ca²⁺ channels. We previously showed that depolarization, whether mediated by contractile agonists or extracellular K⁺, raises pericyte cytoplasmic Ca²⁺ and induces contraction (48, 53, 68, 69). KATP channels are the tetrameric association of SUR-regulatory subunits and KIR6.1 or KIR6.2 pore-forming subunits. Despite their importance in the control of DVR vasoactivity and MBF, the classes of K⁺ channels responsible for control of membrane potential of DVR pericytes have not been explored. In this study, we verified KATP channel subunit expression and quantified the role of KATP channels in the maintenance of membrane potential and K⁺ conductance. Apart from effects on membrane potential (Figs. 1 and 2), evidence that KATP channels contribute to the conductance of DVR pericytes to K⁺ is provided by the observation that K⁺ outward currents are inhibited by Glb (Fig. 3) and transiently enhanced by K⁺ channel openers, Pnc and P-1075 (Fig. 4). On exposure to Glb, the resting conductance of pericytes held at −40 mV in 5 mM KCl, fell from 2.37 ± 0.39 to 1.34 ± 0.39 nS (Fig. 3). In symmetrical K⁺, at −60 mV, Glb induced a similar reduction of conductance from 2.25 ± 0.32 to 1.47 ± 0.27 nS (Fig. 5). Those values correspond to a 43 and 35% contribution of KATP channels to conductance under the respective experimental conditions. In contrast, stimulation with high concentration of the channel opener Pnc in symmetrical KCl increased K⁺ conductance by 4.46 to 6.71 ± 0.66 nS (Fig. 5). The Pnc-induced increase can be used to bracket the number of channels expressed on the surface of DVR pericytes. Assuming that Pnc stimulates open probability to near unity, and taking a high estimate for the average single channel conductance(s) of ~100 pS, channel number could be as low as 44 per cell. Alternately, if channel conductance is only 10 pS, 444 channels per cell would be present. Those channel numbers are similar to estimates summarized by Quayle et al. (51) for smooth muscle of other origin.

In this study, we quantified KATP currents by pharmacological maneuvers. Specificity of the agonists, Pnc and P-1075, for KATP channel activation is supported by their complete reversal by Glb, a reagent that is highly specific for KATP channels (41). Waning of currents after stimulation with KATP openers, similar to that observed in Fig. 4, has been observed in renin-secreting cells (54) and smooth muscle of the pig urethra (61). The mechanisms responsible for the spontaneous reduction of KATP current stimulated by openers are uncertain, but they are unlikely to be explained by run-down attributable to loss of intracellular signaling molecules. The latter problem is largely prevented during nystatin-perforated patch recording. Furthermore, Pnc activated durable inward currents in symmetrical K⁺ (Fig. 5).

Unlike currents mediated by KIR2.2.x and KIR4.4.x subclasses, KATP (KIR6.3.x) channel currents do not rectify (51). Using ramp
depolarizations, we confirmed that Pnc-activated, Glb-sensitive currents in DVR pericytes reverse at $K_{eq} = 0$ mV, as expected for selective transport of K$^+$ ion in symmetrical KCl. We also confirmed that the Glb-sensitive currents do not inwardly rectify (Fig. 5, B and D). Finally, $K_{ATP}$ channel expression was verified with immunohistochemical staining. Commercial antibodies against KIR6.1 and KIR6.2 identified targets in DVR pericytes and endothelia (Fig. 6). Antibody directed at SUR2B identified it as a predominant $K_{ATP}$ regulatory subunit in DVR (Fig. 7). Taken together, the prior and current evidence point to a role for $K_{ATP}$ channels to modulate DVR pericyte membrane potential, K$^+$ conductance, and contractility.

The hypothesis that metabolically induced reduction of ATP stimulates $K_{ATP}$ channels to favor vasodilation has received much support, including demonstration of vulnerability of murine knockout models to ischemic insult (57). In the renal outer medulla, oxygen is consumed to transport NaCl by the thick ascending limb of Henle, a nephron segment that is vulnerable to ischemic insult (4, 5). Given that oxygen tensions in the renal medulla may be as low as 10–25 mmHg (5) and that DVR are its sole blood supply, it is inviting to speculate that $K_{ATP}$ channel activation in response to metabolic demand is an important regulatory mechanism in DVR pericytes. Also, in view of the ability of Glb to depolarize DVR pericytes (Fig. 1) and reduce MBF (49, 55), the popularity of Glb and similar hypoglycemic agents in the treatment of diabetes raises concerns about their use in diabetic patients that undergo procedures, such as surgery and radiotransitory dye administration, which can precipitate acute renal failure.

$K_{ATP}$ channels are present in smooth muscle cells of various vascular beds. Along with voltage-dependent ($K_v$), Ca$^{2+}$-activated ($K_{Ca}$), and other classes of inward rectifier ($K_{IR}$) channels, they regulate membrane potential and vascular tone (3, 6, 18, 23, 25, 38). Evidence favors basal $K_{ATP}$ channel activity in mesenteric, coronary, and carotid arteries (12, 14, 24, 38). Other investigations in cerebral and pulmonary arteries have favored the interpretation that $K_{ATP}$ channels are largely dormant in the resting state but are available to be activated when vasodilation is required (31). In this study, we showed that the $K_{ATP}$ channel blocker Glb, and the activators, Pnc or P-1075, depolarize and hyperpolarize DVR pericytes, respectively (Figs. 1 and 2). Glb has also been described to depolarize smooth muscle cells from isolated mesenteric (31), coronary (12, 24), and carotid arteries (8). The conditions to which DVR pericytes are exposed are unique because osmolality and extracellular K$^+$ concentration in the renal medullary interstitium are higher than that of systemic plasma (20). Thus the intracellular and extracellular K$^+$ concentrations to which pericyte $K_{ATP}$ channels are exposed in vivo are uncertain and probably vary with the location of the parent vessel along the corticomedullary axis. Nonetheless, the current data make it clear that $K_{ATP}$ channels are expressed in DVR and contribute substantially to K$^+$ conductance. Their activation and inhibition are expected to favor hyperpolarization and depolarization, respectively, as a target for hormones and paracrine agents to mediate vasodilation and constriction.

$K_{ATP}$ channel regulation of vasoactivity in vascular beds, including the renal medulla, has been examined by testing the ability of Glb to lower blood flow. Gardiner and colleagues (14, 24) found that Glb infusion into rats induced mesenteric, skeletal muscle, and renal vasoconstriction. A similar effect was observed in splanchnic and portal circulations (24, 35) and the canine diaphragm (64). In the kidney, a role for modulation of arteriolar tone by $K_{ATP}$ channels has been repeatedly observed (30, 49, 55, 60). DVR, the subject of the current study, supply the renal medulla with blood flow. Tonic effects of $K_{ATP}$ channel activity to regulate MBF have been reported. Using laser-Doppler flowmetry, Parekh and Zou (49) reported that Glb reduced MBF by 19% while the $K_{ATP}$ opener lemakalim increased MBF by 27%. With different methods, Sadowski et al. (55) reported a 15% reduction of MBF by Glb.

$K_{ATP}$ channels are named for their characteristic inhibition by intracellular ATP (42) and activation by nucleotide diphosphates (NDPs). Reduction of intracellular ATP concentration increases $K_{ATP}$ open probability, an action that probably serves to hyperpolarize smooth muscle, reduce Ca$^{2+}$ entry by voltage-gated channels, dilate blood vessels, and increase the supply of oxygen to meet metabolic demands (3, 41, 51). In vivo, $K_{ATP}$ channel inhibition also increases resistance to blood flow in mesentery (56), renal cortex and medulla (32, 43, 55), and skeletal muscle (6). Conversely, endogenously released vasodilators inhibit vascular smooth contraction by activating $K_{ATP}$ channels (23, 40, 41, 59). Variation of intracellular ATP may not be the sole or primary modulator of $K_{ATP}$ channel activity. $K_{ATP}$ channels are the targets of a variety of signaling cascades. Endogenous vasodilators such as adenosine, β-adrenergic agonists, vasoactive intestinal peptide, adrenomedullin, and calcitonin gene-related peptide activate them, generally via protein kinase A-mediated phosphorylation (15, 23, 40, 51, 52, 59, 60). $K_{ATP}$ channels are also the target of vasoconstrictors that activate protein kinase C (PKC). Although reports of PKC studies in the vasculature have largely identified inhibition of channel activity by vasoconstrictors and PKC (17, 21, 26, 33, 34, 50, 62, 65–67), reports of PKC-mediated $K_{ATP}$ activation in ventricular myocytes and insulin-secreting islet cells also exist (51).

Within smooth muscle cells, constrictors can either inhibit K$^+$ channels or activate them as a braking mechanism that limits vasoconstriction (13, 41). In a prior study, we found that K$^+$ currents sensitive to the nonselective K$^+$ channel blockers, Ba$^{2+}$ and tetraethylammonium, were inhibited by 10 nM ANG II (45). In this study, we verified that $K_{ATP}$ channels are at least one of the subtypes so affected (Fig. 8). We extended our observations by determining the concentration range over which ANG II mediates $K_{ATP}$ inhibition and by examining parallel effects of AVP and ET-1. With regard to concentration dependence of ANG II, at 100 pM, oscillations were induced and there was partial inhibition of Pnc-stimulated currents. At 1 and 10 nM, ANG II fully blocked Pnc-induced $K_{ATP}$ currents. Thus the ANG II concentrations that block $K_{ATP}$ currents coincide well with those that induce DVR vasoconstriction, the EC$\text{S}_0$ of which is ~500 pM (44). ANG II inhibition of $K_{ATP}$ currents has been reported in smooth muscle of coronary and mesenteric arteries (16, 17, 26, 33).

The most potent constrictor of DVR studied to date is ET-1 (48, 58). Like ANG II, ET-1 promptly lowers whole cell K$^+$ currents and eliminates the ability of Pnc to activate $K_{ATP}$ currents (Fig. 10). ET-1 has also been described to inhibit $K_{ATP}$ channels in porcine pupil arteries and arterioles (21), rabbit coronary and pulmonary arteries (50), and cardiac myocytes (67), whereas in the pulmonary bed, endothelin type B receptor stimulation can be associated with $K_{ATP}$ channel activation and...
vasodilation (37). It is interesting to note that ANG II (10 nM) and ET-1 (10 nM) virtually abolish the outward current of pericytes held at −40 mV (Figs. 8 and 10). Because Glb did not have as potent an effect to lower outward currents (Fig. 3), we conclude that other classes of K⁺ channels probably also blocked ANG II and ET-1. Efflux of Cl⁻ is unlikely to be involved because the holding potential for those experiments (Figs. 8 and 10) was the equilibrium potential for Cl⁻ ion. The complete elimination of Pnc-inducible currents by ANG II and ET-1 also strongly favors the interpretation that KATP-mediated K⁺ current is potently blocked by those constrictors. The elimination of the ability of Glb to inhibit outward K⁺ current (Fig. 10E) also indicates suppression of basal KATP channel activity in the cells. A similar effect has been reported by Lahaye et al. (27). The potency of ANG II and ET-1 to depolarize pericytes, inhibit K⁺ channels, activate Ca²⁺-dependent Cl⁻ channels, and constrict DVR probably does not fully reflect the influences to which DVR are exposed. Dickhout and colleagues (10) showed that renal medulary vasoconstrictors such as ANG II and ET-1 can activate a variety of compensatory pathways including those on endothelia and nearby neurons to release paracrine vasodilators and influence vasoreactivity.

Compared with ET-1 and ANG II, AVP is a weak vasoconstrictor of isolated DVR (48, 63). Based on the current results, it seems possible that the lack of potency of arginine vasopressin to induce vasoconstriction is related to its inability to inhibit K⁺ channel activity in pericytes (Fig. 9). At picomolar concentrations, vasopressin exerts hydro-osmotic effects by activating collecting duct V₂ receptors. At nanomolar concentrations, it stimulates V₁ receptors to induce vasoconstriction. We previously showed that vasopressin-mediated constriction of isolated DVR maximizes at ~10 nM (63). In this study, we used 100 nM vasopressin to test whether it induces KATP channel inhibition as part of that action. The data showed a tendency toward partial inhibition of basal and Pnc-stimulated outward currents but neither achieved statistical significance (Fig. 10). Examination of vasopressin-mediated inhibition of KATP channels in other preparations has also produced variable results (11, 65, 66).

In summary, we showed that KATP channels are present in rat DVR pericytes and contribute substantially to resting K⁺ conductance. They mediate a substantial portion of the outward current observed in pericytes held at −40 mV. Both outward currents at −40 mV and inward currents in 140 mM external KCl are markedly augmented by KATP channel agonists, Pnc and P-1075. Glb-sensitive currents in DVR pericytes do not rectify, a characteristic expected for KATP channels. By immunocytochemistry, Kir6.1 and Kir6.2 pore-forming subunits are expressed along with the SUR2B. High concentrations of ANG II and ET-1 inhibit KATP channels as part of their action. The identification of robust KATP expression in DVR pericytes is consistent with prior reports of Glb-mediated reduction of renal MBF.

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