Inhibition of K⁺ conductance in descending vasa recta pericytes by ANG II

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Inhibition of K⁺ conductance in descending vasa recta pericytes by ANG II. Am J Physiol Renal Physiol 287: F1213–F1222, 2004. First published August 17, 2004; doi:10.1152/ajprenal.00241.2004.—We tested whether K⁺ channel inhibition accompanies ANG II-induced depolarization of descending vasa recta (DVR) pericytes. An increase in extracellular K⁺ concentration ([K⁺]o) from 5 to 100 mM depolarized resting pericytes but had no effect after prolonged (10 nM, 20 min) ANG II exposure. In contrast, reduction of extracellular Cl⁻ concentration ([Cl⁻]o) from 154 to 34 mM had a minor effect on resting membrane potential but strongly depolarized pericytes treated with ANG II. The K⁺ channel blockers BaCl₂ (0.1, 1 mM) and tetraethylammonium (TEA; 30 mM) depolarized resting pericytes but did not affect membrane potential of ANG II-treated pericytes. Pericyte whole cell currents were reduced by ANG II and nearly eliminated by combined ANG II exposure and the Cl⁻ channel blocker niflumic acid (100 μM). Augmentation of inward current induced by raising [K⁺]o from 5 to 50 mM was eliminated by preexposure to ANG II. TEA- and BaCl₂-sensitive outward currents, generated by depolarizing pericytes from −80 to −40 mV, were eliminated by ANG II. We conclude that ANG II depolarizes DVR pericytes by a combination of Cl⁻ channel activation and K⁺ channel inhibition.

Patch clamp; microcirculation; kidney; medulla

Blood flow to the medulla of the kidney is supplied by descending vasa recta (DVR). DVR are ≈15-μm-diameter arteriolar microvessels that arise predominantly from juxamedullary efferent arterioles to form outer medullary vascular bundles. The radial arrangement of DVR within the bundles leads to the conclusion that DVR participate in the transport processes essential to countercurrent exchange and to the control of blood flow distribution within the medulla (9, 24, 27). Pericytes are smooth muscle-like cells that surround DVR and impart contractile function. As such, the DVR pericyte is a key regulator of medullary perfusion. DVR are vasoactive and respond to a wide variety of systemic and paracrine vasoconstrictors and dilators (27).

A growing body of literature points to regulation of perfusion of the renal medulla in determination of diuretic state, blood pressure, sodium balance, and extracellular fluid volume (9, 24). Despite the importance of the DVR pericyte in this scheme, fundamental information concerning the channel architecture and signaling processes that govern its behavior has been lacking. To bridge that gap, we adapted electrophysiological methods to permit investigations of the control of membrane potential in pericytes of DVR explanted by microdissection. We found that ANG II constriction of DVR is presaged by activation of a 16.8-pS Ca²⁺-dependent Cl⁻ channel and an increase in Ca²⁺-activated, niflumic acid-sensitive Cl⁻ conductance. Using patch-clamp techniques with artificial buffers, we observed Cl⁻ channel activation that depolarizes membrane potential from resting values (−50 to −70 mV) toward the equilibrium potential of the Cl⁻ ion (26, 30, 38), an action that favors voltage-gated Ca²⁺ entry into the pericyte cytoplasm (39). In other smooth muscle cells, ANG II has also been shown to modulate the activity of K⁺ channels. ANG II activates K⁺ conductance in mesangial cells and inhibits K⁺ conductance in smooth muscle of the renal artery (6, 11, 32). ANG II activation of K⁺ channels could serve as a braking mechanism that serves to limit depolarization, cytoplasmic Ca²⁺ entry, and vasoconstriction (10, 18, 25). In contrast, K⁺ channel deactivation has been also been demonstrated in small arterioles of the renal and cerebral circulation, mediated by signaling through 20-hydroxyeicosatetraenoic acid (2, 23, 33). K⁺ channel inhibition would be predicted to favor smooth muscle depolarization and intensification of vasoconstriction.

In this study, we tested whether ANG II-induced depolarization of DVR pericytes is accompanied by modulation of pericyte K⁺ conductance. The results show that prolonged ANG II exposure renders pericyte membrane potential insensitive to extracellular K⁺ concentration ([K⁺]o) changes or K⁺ channel blockers. Similarly, whole cell K⁺ currents in DVR pericytes are reduced by prolonged ANG II stimulation. The data support the hypothesis that ANG II-induced depolarization of DVR pericytes occurs through combined stimulation of a Ca²⁺-activated Cl⁻ conductance and inhibition of K⁺ channels.

Methods

Whole cell patch-clamp recording. All 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 removed from Sprague-Dawley rats (70–150 g; Harlan), sliced, and stored at 4°C in a physiological saline solution (PSS; in mM: 145 NaCl, 5 KCl, 1 MgCl₂, 1 CaCl₂, 10 HEPES, and 10 glucose, pH 7.4) at room temperature. As previously described, membrane potential and whole cell currents were monitored by patch-clamp recording from pericyte cell bodies on isolated vessels at room temperature (26, 30, 38, 39). Small wedges of renal medulla were separated from kidney slices by dissection and transferred to Blendzyme 1 (Roche) at 0.27 mg/ml in high-glucose DMEM media (Invitrogen), 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 by microdissection and transferred to a perfusion chamber on the stage of an inverted microscope (Nikon Diaphot). Patch pipettes were made from borosilicate glass capillaries (PGS2151-4, 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 K aspartate, 20 KCl, 10 NaCl, and 10 HEPES, pH 7.2, as well as nystatin (100 µg/ml with 0.1% DMSO) in ultrapure water. Nystatin was dissolved in DMSO, and the excess was discarded daily. Nystatin stock was dispensed into the K aspartate pipette solution at 37°C by vigorous vortexing for 1 min and protected from light. To clear any slight remaining nystatin precipitate, patch pipettes were backflushed from a syringe via a 0.2-µm filter.

Membrane potential was measured using a CV201AU headstage and Axopatch 200 amplifier (Axon Instruments, Foster City, CA) in current-clamp mode (I = 0) at a sampling rate of 10 Hz using pipettes of 8- to 10-MΩ resistance. Whole cell current recording in voltage-clamp mode was accomplished with 4- to 6-MΩ pipettes. Due to the small size of the pericytes, lower resistance pipettes proved difficult to use. Pipettes with nystatin-containing electrode solution were inserted into the bath under positive pressure, positioned near the cell with a piezoelectric manipulator (Burleigh PCS-5000), and the offset of the amplifier was adjusted to null the junction and electrode potentials. After gigaseal and nystatin pore formation, final access resistance was monitored and, if necessary, the amplifier was adjusted to null the junction and electrode potentials. The piezoelectric manipulator (Burleigh PCS-5000) was used to position the pipette tip near the cell and membrane potential was measured using a CV201AU headstage and Axopatch 200 amplifier (Axon Instruments, Foster City, CA) in current-clamp mode (I = 0) at a sampling rate of 10 Hz.

To examine the effects of [K+]o, or Cl– ion concentration ([Cl–]o), changes, either [Cl–]o, was lowered by substituting NaCl with Na gluconate or [K+]o, was raised by isosmotic substitution of NaCl with KCl. In those experiments, to avoid errors resulting from a change in the reference electrode-bath interface, a 3 M KCl-3% agar bridge was substituted for a Ag/AgCl wire as the reference electrode. The low-Cl– buffer contained (in mM) 130 Na gluconate, 25 NaCl, 5 KCl, 1 CaCl2, 1 MgCl2, 10 HEPES, and 10 glucose, pH 7.4, at room temperature. This buffer has a final Cl– concentration of 34 mM. As previously described, after accounting for Donnan effects, this yields a Cl– concentration that is approximately symmetrical across the cell membrane (17, 26). The high-K+ buffer contained (in mM) 50 NaCl, 100 KCl, 1 CaCl2, 1 MgCl2, 10 HEPES, and 10 glucose, pH 7.4, at room temperature. Junction potential and Donnan potential effects were corrected as previously described (26). The predicted offset was −2 mV and was neglected.

Reagents. ANG II, nystatin, nilfumic acid, and other chemicals were from Sigma (St. Louis, MO). Liberase Blendzyme 1 was from Roche Applied Science. Blendzyme was stored in 40-µl aliquots of 4.5 mg/ml in water and diluted into high-glucose DMEM on the day of the experiment. ANG II was stored as 10 m M aliquots in water, nilfumic acid was stored in 100 µM aliquots, and both were frozen at −20°C and diluted into PSS on the day of the experiment. Stock reagents were thawed once, and the excess was discarded each day.

Statistics. Data are given as means ± SE. The significance of differences between means was calculated using Student’s t-test (paired or unpaired, as appropriate) and analysis of variance. Some data sampled at 10 Hz were averaged 10 values at a time for display at 1 Hz. In figures that show averaged current or membrane potential records, the majority of error bars are suppressed to optimize graphic display of the data.

RESULTS

Effect of alterations in K+ and Cl– equilibrium potentials on pericyte membrane potential. The central hypothesis tested by this study is that, in addition to activation of pericyte Cl– conductance (26, 39), ANG II depolarizes pericytes by inhibiting conductance to K+. To examine the relationship between the membrane potential and conductance to K+ and Cl–, we compared the effects of altering the equilibrium potentials (Keq, Cleq) before and after ANG II treatment. [K+]o was raised from 5 to 100 mM (electrode K+, intracellular K+ concentration = 140 mM) by isosmotic substitution for Na+, a maneuver that changed Keq from −89 to −9 mV. Similarly, [Cl–]o was lowered from 154 to 34 mM by isosmotic substitution for Na gluconate (electrode Cl–, intracellular Cl– concentration = −34 mM), changing the Cleq from −43 to −3 mV. Bath K+ and Cl– concentration changes were for 2 and 3 min, respectively. The effects of [K+]o and [Cl–]o changes are shown in Fig. 1. Raising Keq from −89 to −9 mV before ANG II depolarized pericytes from resting level to about −10 mV.
Raising Cl<sub>eq</sub> to −3 mV was substantially less effective; pericyte membrane potential remained well below Cl<sub>eq</sub> = −3 mV, increasing to only −42 mV. Those results are largely as expected if resting membrane potential is predominantly determined by conductance to the K<sup>+</sup> ion. Before ANG II exposure, reduction of [Cl<sup>-</sup>]<sub>o</sub> to 34 mM led to rapid depolarization, followed by some tendency toward repolarization. The duration of exposure to low [Cl<sup>-</sup>]<sub>o</sub> (3 min) was too short to fully define the response, but an effect of changes in [Cl<sup>-</sup>]<sub>o</sub> on Cl<sup>-</sup> channel activity might exist.

After the effects of [K<sup>+</sup>]<sub>o</sub> and [Cl<sup>-</sup>]<sub>o</sub>, changes on resting potential were measured, pericytes were exposed to ANG II (10 nM). As previously reported (26), the membrane depolarized to about −30 mV. At intervals after exposure to ANG II, [K<sup>+</sup>]<sub>o</sub> and [Cl<sup>-</sup>]<sub>o</sub> were again changed to 100 and 34 mM, respectively. The magnitude of responses to the associated change in Keq and Cleq changed with the time period that elapsed since ANG II application. At 5, 15, and 25 min, raising [K<sup>+</sup>]<sub>o</sub> to 100 mM became progressively less effective to induce further depolarization toward Keq (Fig. 1A). In contrast, low [Cl<sup>-</sup>]<sub>o</sub> became progressively more effective to depolarize membrane potential toward Cleq (Fig. 1B). Time controls in which the effects of altering [K<sup>+</sup>]<sub>o</sub> and [Cl<sup>-</sup>]<sub>o</sub> were tested before and after 25 min of perforated patch formation revealed stable responses (data not shown), ruling out K<sup>+</sup> channel rundown as an explanation for the decline in response to raising [K<sup>+</sup>]<sub>o</sub> in Fig. 1A.

The experiments in Fig. 1 have two potential interpretations. First, after ANG II exposure, pericyte Cl<sup>-</sup> conductance might have progressively increased to become overwhelmingly large, making K<sup>+</sup> conductance insignificant by comparison. A second explanation is that pericyte K<sup>+</sup> conductance was inhibited so that pericyte membrane potential was determined by conductance to Cl<sup>-</sup> and other ions. Further experiments were performed to distinguish those possibilities.

Effect of K<sup>+</sup> channel blockers on pericyte membrane potential. We examined the effects of the nonspecific K<sup>+</sup> channel blockers Ba<sup>2+</sup> and tetraethylammonium (TEA) on membrane potential before and after ANG II exposure. Figure 2, A and B, shows the respective effects of 0.1 and 1.0 mM Ba<sup>2+</sup> on resting pericyte membrane potential. At both concentrations, Ba<sup>2+</sup> induced substantial depolarization. In contrast, after ANG II exposure (10 nM, 20 min), 1.0 mM Ba<sup>2+</sup> had no effect (Fig. 2C). Experiments with Ba<sup>2+</sup> are summarized in Fig. 2, D–F. We also tested the effect of 30 mM TEA on membrane potential (Fig. 3). TEA depolarized pericytes before (Fig. 3A) but not after 20 min of ANG II (Fig. 3B). Results with TEA are summarized in Fig. 3, C and D.

In two cells, ANG II induced membrane potential oscillations. Those results were excluded from the summaries shown.

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

**Fig. 2.** Effect of Ba<sup>2+</sup> on DVR pericyte membrane potential. A and B: sample tracings showing the effect of extracellular Ba<sup>2+</sup> (0.1, 1.0 mM) on resting membrane potential of DVR pericytes. The membrane potential (in mV) at the beginning of the record is noted under each trace. C: example of a record demonstrating the lack of effect of Ba<sup>2+</sup> (1.0 mM) on pericyte membrane potential after prolonged exposure to ANG II (10 nM, 20 min). D and E: summaries showing membrane potential measurements from different cells before, during, and after exposure to Ba<sup>2+</sup> at either 0.1 or 1.0 mM. Values shown above the graph are means ± SE of measurements before, during, and after Ba<sup>2+</sup> application. *P < 0.05, Ba<sup>2+</sup> vs. baseline. F: summary of membrane potential measurements from cells previously exposed to ANG II (10 nM, 20 min). Measurements were taken before, during, and after exposure to Ba<sup>2+</sup> (1.0 mM).
During the oscillations, the effects of Ba$^{2+}$ and TEA to augment ANG II-induced depolarization remained unaltered over time (Fig. 4), suggesting that ANG II might be less effective in inhibiting K$^{+}$ conductance when pericyte membrane potential is oscillating. Serendipitous occurrences of oscillations were too few to permit further exploration of that issue.

**Effect of ANG II on whole cell currents.** In a series of pericytes, whole cell current was measured during voltage clamp before and after 20-min exposure to ANG II (10 nM). The protocol employed is illustrated in Fig. 5A. Cells were held at −80 mV and pulsed from −150 to 40 mV (corrected for junction potential) in 20-mV increments for 500 ms (10 s between pulses). Figure 5B shows the currents (I$_m$) elicited by that protocol. For all $V_m > −90 \text{ mV}$, where electrochemical forces favor K$^+$ efflux from the cell, ANG II caused a reduction of I$_m$. Note that in the range of $V_m$ that spans physiological membrane potential, the reduction of I$_m$ after ANG II was a large fraction of the total current that was present before ANG II. That implies that ANG II has an important effect on ensemble ion conductance in the pericytes. If ANG II inhibits K$^+$ channels, then residual currents after should largely be due to transport of Cl$^−$ ion. To test this, we measured the effect of the Cl$^−$ channel blocker niflumic acid (100 μM) on post-ANG II currents. Niflumic acid nearly eliminated post-ANG II currents and shifted the reversal potential from −48 to −5 mV (Fig. 5, C and D).

**Effect of increasing [K$^+$]o on whole cell currents.** To test whether ANG II inhibition of K$^+$ conductance is responsible for the reduction of whole cell current in Fig. 5B, we augmented the electrochemical driving force favoring K$^+$ entry by increasing [K$^+$]o from 5 to 50 mM. Cells were held at −80 mV and pulsed to a range of −150 to +40 mV (10-mV increments). As shown in Fig. 6C, 50 mM K$^+$ increased the inward

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Fig. 3. Effect of tetraethylammonium (TEA$^+$) on DVR pericyte membrane potential. A: sample tracing showing the effect of extracellular TEA$^+$ (30 mM) on resting potential of a DVR pericyte. The membrane potential at the beginning of the record (in mV) is shown under each trace. B: example of a record demonstrating the lack of effect of TEA$^+$ (30 mM) on pericyte membrane potential after prolonged exposure to ANG II (10 nM, 20 min). C: summary of membrane potential measurements from different cells before, during, and after exposure to TEA$^+$ (30 mM). *P < 0.05, TEA$^+$ vs. baseline. D: summary of membrane potential measurements from cells previously exposed to ANG II (10 nM, 20 min). Measurements were taken before, during, and after exposure to TEA$^+$.

Fig. 4. Effect of TEA$^+$ and Ba$^{2+}$ during ANG II-induced membrane potential oscillations. Tracings show 2 records in which pericyte membrane potential undergoes sustained oscillations after ANG II exposure. In each case, exposure to either TEA (30 mM) or Ba$^{2+}$ (1.0 mM) augments depolarization. These K$^+$ channel blockers do not appear to lose effectiveness even after a prolonged exposure to ANG II (contrast with Figs. 2 and 3). The resting membrane potential (in mV) before ANG II exposure is shown at the beginning of each trace.
current at negative pulse potentials. ANG II exposure (10 nM, 20 min) eliminated the augmentation of inward currents by 50 mM KCl (Fig. 6D), favoring the interpretation that conductance(s) specific to the K⁺ ion are suppressed by ANG II.

To further confirm that ANG II inhibits K⁺ conductance, parallel protocols were performed in which inward current was continuously monitored while pericytes were held at –80 mV and [K⁺]ₒ was raised from 5 to 50 mM by isosmotic substitution for extracellular Na⁺ concentration. In one group, the [K⁺]ₒ change was performed 20 min after patch formation. As expected, raising [K⁺]ₒ to 50 mM induced a reversible inward current (Fig. 7A). In another group, pericytes were treated with ANG II for 20 min before patch formation and then exposed to 50 mM [K⁺]ₒ. The inward currents were abolished whether cells were exposed to ANG II before or after establishment of electrical access to the cytoplasm via nystatin (Fig. 7, B and C; summarized in Fig. 7D). The results in Fig. 7 confirm inhibition of pericyte K⁺ conductance by ANG II and show that the duration of nystatin pore formation does not influence the results; i.e., K⁺ channel rundown cannot explain the insensitivity of inward currents to [K⁺]ₒ in the experiments.

Inhibition of outward currents by K⁺ channel blockers. Pericytes held at –80 mV have membrane potential clamped near the predicted Keq (–89 mV) for our buffers. Depolarizing Fig. 5. Effect of ANG II on whole cell current. A: protocol used for depolarizations. Pericytes were depolarized from a holding potential of –80 mV to pulse potentials ranging from –150 to –40 mV (1,000 ms, 20-mV increments). B: ordinate shows means ± SE of cell current (Im; n = 6) as a function of pulse potential (Vm) at baseline and after exposure to ANG II (10 nM, 20 min). The abscissa is corrected for junction potential. The arrow indicates the theoretical equilibrium potential for the Cl⁻ ion in our buffers (–43 mV). *P < 0.05 vs. baseline. C: example of cell current traces in ANG II (10 nM, 20 min) before and after inhibition by niflumic acid (100 µM). D: ordinate shows means ± SE of cell current (n = 7) vs. pulse potential in cells that have been exposed to ANG II (10 nM, 20 min). Data show end pulse current before and after addition of niflumic acid (Nifl A; 100 µM) to the bath. The abscissa is corrected for junction potential. *P < 0.05 vs. ANG II.

Fig. 6. Effect of ANG II on K⁺ inward currents. A and B: tracings showing whole cell current in 5 (A) and 50 mM KCl (B). Pericytes were depolarized from a holding potential of –80 mV to pulse potentials ranging from –150 to +40 mV (500 ms, 10-mV increments). C: ordinate shows means ± SE of cell current (n = 6) as a function of pulse potential in PSS (KCl, 5 mM) and after extracellular KCl concentration was increased to 50 mM by isosmotic substitution for NaCl. The abscissa is corrected for junction potential. At negative pulse potentials, inward current increased when [K⁺]ₒ was raised to 50 mM. *P < 0.05 vs. 5 mM KCl. D: ordinate shows means ± SE of cell current (n = 6) as a function of pulse potential in PSS (KCl, 5 mM) and after extracellular KCl concentration was increased to 50 mM by isosmotic substitution for NaCl. Cells had been preexposed to ANG II (10 nM, 20 min). The abscissa is corrected for junction potential. After ANG II treatment, inward currents at negative pulse potentials are not enhanced by increasing [K⁺]ₒ from 5 to 50 mM (compare with C).
Fig. 7. Effect of ANG II on K⁺ inward currents. A: pericytes were held at −80 mV (after junction potential correction) for 20 min while cell current was measured continuously. After 20 min, [K⁺]o was transiently increased from 5 to 50 mM by isosmotic substitution for NaCl. The example shows that increasing [K⁺]o induced an inward current, reinforcing the findings obtained with pulse protocols in Fig. 6. B: pericytes were held at −80 mV for 20 min in the presence of ANG II (10 nM). After exposure to ANG II, increasing [K⁺]o failed to induce a transient inward current. C: pericytes were exposed to ANG II (10 nM) for 20 min before electrical stimulation to −40 mV generated an outward current (Fig. 5). It is likely that a portion of that outward current is carried by the K⁺ ion because −40 mM is near Cl_eq. We tested whether ANG II could reduce the outward current of pericytes held at −40 mV and whether any residual post-ANG II currents remain sensitive to K⁺ channel blockade. The means ± SE of holding currents at −80 and −40 mV are summarized for control pericytes (n = 34) and pericytes treated with ANG II (10 nM, 20 min, n = 21) in Fig. 8. Net currents at −40 mV were much lower in ANG II-treated pericytes, a finding that is consistent with inhibition of an outward current carried by K⁺. The effects of K⁺ channel blockade in those cells are provided in Figs. 9 and 10. The examples in Fig. 9, A–C, show continuous measurement of whole cell current in pericytes shifted from a holding potential of −80 to −40 mV generated an outward current (Fig. 5). It is likely that a portion of that outward current is carried by the K⁺ ion because −40 mV is near Cl_eq. We tested whether ANG II could reduce the outward current of pericytes held at −40 mV and whether any residual post-ANG II currents remain sensitive to K⁺ channel blockade. The means ± SE of holding currents at −80 and −40 mV are summarized for control pericytes (n = 34) and pericytes treated with ANG II (10 nM, 20 min, n = 21) in Fig. 8. Net currents at −40 mV were much lower in ANG II-treated pericytes, a finding that is consistent with inhibition of an outward current carried by K⁺. The effects of K⁺ channel blockade in those cells are provided in Figs. 9 and 10. The

FIG. 8. ANG II reduces whole cell currents in pericytes held (Vhold) at −40 mV. Whole cell current measurements are shown from DVR pericytes held at −80 and −40 mV in the control state (n = 34) and after prolonged ANG II exposure (n = 21). Currents at −40 mV were much lower in ANG II-treated cells (*P < 0.01 vs. controls at −40 mV).

**DISCUSSION**

Membrane potential of smooth muscle controls Ca²⁺ entry into the cytoplasm through voltage-gated channels and is an important determinant of contractility (18, 25). Depolarization, coupled with intracellular events leading to Ca²⁺ sensitization, is often the means by which vasoconstrictors such as ANG II activate myosin light chain kinase to favor myosin cross-bridge formation and contraction (29, 31). Depolarization of smooth muscle generally occurs by increasing Cl⁻ conductance. At membrane potentials below the Cl⁻ equilibrium potential, activation of Cl⁻ channels favors Cl⁻ efflux from the cell, shifting membrane potential away from K_eq (about −90 mV) toward Cl_eq (−20 to −40 mV). It has been frequently shown that smooth muscle of renal cortical afferent arterioles behaves in that way (4, 5, 12, 14, 21, 22, 36); however, studies of the contraction of the efferent arteriole have produced varied results. Several investigations found that efferent smooth muscle cells do not always depolarize and that depolarization...
that ANG II can depolarize pericytes by activating a Ca\textsuperscript{2+} channel entry is modulated by membrane potential change (26, 38, 39). Strong evidence has recently been obtained that the channel architecture of efferent smooth muscle varies with the depth of the parent glomerulus in the renal cortex. Voltage-gated Ca\textsuperscript{2+} channels are important to control [Ca\textsuperscript{2+}]\textsubscript{CYT} in juxtamedullary but not superficial efferent arteriolar smooth muscle (13). DVR are branches of juxtamedullary efferent arterioles, and we have repeatedly observed that DVR pericytes are, in fact, depolarized by ANG II and that Ca\textsuperscript{2+} entry is modulated by membrane potential change (26, 38, 39).

Resting membrane potential of the DVR pericyte, measured with artificial buffers and nystatin-perforated patches, is in the range of −50 to −70 mV, similar to that of other smooth muscle (25, 26, 39). Measurement of membrane potential with patch-clamp techniques necessitates dialysis of the cell interior with the buffer in the electrode. As such, we cannot ensure that current or prior membrane potential measurements replicate exact levels that exist in vivo. Despite this, we are confident that ANG II can depolarize pericytes by activating a Ca\textsuperscript{2+}-sensitive, niflumic acid-inhibitable Cl\textsuperscript{−} conductance (26). The goal of this study was to determine whether inhibition of pericyte K\textsuperscript{+} conductance is also a mechanism by which ANG II acts to induce depolarization. Our first approach to that question was to study the influence of changing K\textsubscript{eq} and Cl\textsubscript{eq} on membrane potential. When [K\textsuperscript{+}]\textsubscript{o} was raised to increase K\textsubscript{eq}, the membrane potential of pericytes shifted from its preexisting baseline to a close approximation of K\textsubscript{eq} (Fig. 1A). That finding simply verifies that K\textsuperscript{+} conductance dominates over other ions in the determination of resting membrane potential. After treatment with ANG II, membrane potential depolarized, as previously described (26, 38, 39), but became increasingly insensitive to raising K\textsubscript{eq} (Fig. 1A). As responsiveness to raising K\textsubscript{eq} was lost, responses to raising Cl\textsubscript{eq} from −43 to −3 mV increased (Fig. 1B). That finding implies that, after ANG II, Cl\textsuperscript{−} conductance rather than K\textsuperscript{+} conductance is dominant in the determination of membrane potential. We also tested whether K\textsuperscript{+} channel blockers (Ba\textsuperscript{2+} and TEA\textsuperscript{−}) affect membrane potential before and after ANG II exposure. Those agents depolarized resting pericytes but had no effect after prolonged ANG II treatment (Figs. 2 and 3). Taken together, the data in Figs. 1–3 fully support concomitant activation of Cl\textsuperscript{−} channels and inhibition of K\textsuperscript{+} channels by ANG II, but they do not rigorously prove the latter because an overwhelming increase in Cl\textsuperscript{−} conductance relative to a stable K\textsuperscript{+} conductance could hypothetically yield similar results.

The relative magnitude of K\textsuperscript{+} vs. Cl\textsuperscript{−} conductance after ANG II treatment was investigated by measuring whole cell current in pericytes subjected to depolarizing pulses (Fig. 5).
In a final series, we examined the ability of ANG II to reduce outward current in pericytes voltage-clamped near Cl$_{eq}$ at −40 mV (Figs. 8–10). In pericytes that had not been exposed to ANG II, depolarization from −80 to −40 mV generated a net outward current, raising $I_m$ from a mean of −103 to +102 pA (Fig. 8). In voltage-clamped cells exposed to ANG II for 20 min, the same depolarization only increased current from −117 to −40 pA, a finding that is consistent with elimination of K$^+$ efflux by ANG II. To verify that the outward current generated by depolarization to −40 mV is at least partially carried by K$^+$, we tested whether the classic, nonselective K$^+$ channel blockers Ba$^{2+}$ and TEA inhibit it. Both agents were effective in doing so in resting cells (Figs. 9E and 10C) but not in cells preexposed to ANG II (Figs. 9F and 10D).

The results of this study do not establish which classes of K$^+$ channels control resting potential or are modulated by vasoconstrictors such as ANG II. K$^+$ channel architecture of smooth muscle is generally complex: multiple classes of K$^+$ channels contribute to the ensemble of currents that affect cell function (18, 25). Ca$^{2+}$-activated K$^+$ channels (K$_{Ca}$) and voltage-operated K$^+$ channels (Kv) activate with depolarization, probably serving to limit microvessel contraction by holding the membrane potential low enough to reduce voltage-gated Ca$^{2+}$ entry. Large- and small-conductance K$_{Ca}$ channels are widely expressed in smooth muscle (25). If they are present in DVR pericytes, it is likely that they are inactivated by ANG II because ANG II elevates pericyte [Ca$^{2+}$]$_{cyt}$ (30, 39) to activate K$_{Ca}$ channels if present. ANG II-mediated inhibition of K$_{Ca}$ channels in other renal microvessels does occur. Experiments by Roman and colleagues (2, 23, 33) established the existence of K$_{Ca}$ inhibition through the cytochrome P-450A $\omega$-hydroxylase product 20-HETE. ANG II inhibition of K$_{Ca}$ channels mediated by protein kinase C and c-SRC has also
been verified (1, 3). Kv channels are common in smooth muscle, and inhibition of their activity by ANG II occurs in various cell types, including arterial smooth muscle (15, 28, 35). KATP channels formed by association of inward rectifier (KIR) 6.x with sulfonurea receptors are widely expressed in smooth muscle (18, 25). We have shown that the KATP channel activator pinacidil hyperpolarizes DVR pericytes and dilates preconstricted vessels (39). Precedent for ANG II inhibition of KATP channels is well established (16, 20, 34, 37). It seems probable that ANG II inhibits KATP channels in DVR pericytes, but extensive investigations will be needed to support this hypothesis. Inward rectifier K⁺ channels (KIR) are expressed in smooth muscle. Strongly rectifying classes of KIR would be difficult to investigate through antisense or RNA day makes assignment of measured currents to a specific ion channel activity. The need to harvest vessels throughout the and phosphorylation events responsible for modulation of 9 and 10) favor that interpretation. These studies have been inward rectification below Keq 1070, 1998.


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REFERENCES


