Ca²⁺ signaling and membrane potential in descending vasa recta pericytes and endothelia

KRISTIE RHINEHART, ZHONG ZHANG, AND THOMAS L. PALLONE
Division of Nephrology, University of Maryland School of Medicine, Baltimore, Maryland 21201-1595

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Special Communication

In addition to the key role of pericytes, DVR endothelia are important in modulation of transport functions and vasomotor tone. In vitro studies have shown that they generate nitric oxide at a rate influenced by oxygen free radical formation (21). In addition, DVR endothelia exhibit unusual Ca²⁺-signaling events (16, 18). Angiotensin II (ANG II), a hormone that is expected to elevate intracellular Ca²⁺ concentration ([Ca²⁺]i) by activating the G protein-coupled angiotensin type 1 (AT₁) receptor, has been shown to suppress basal [Ca²⁺]i and [Ca²⁺]i responses to bradykinin (BK) or the sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase (SERCA) blocker thapsigargin (18).

Renal medullary perfusion is a determinant of diuretic state and appears to influence blood pressure, sodium balance, and extracellular fluid volume (3, 12). Despite the role that DVR pericytes and endothelia play in this scheme, information concerning the ion channel activity and signaling processes that govern their function has been lacking. For this purpose, we adapted electrophysiological and microfluorescent methods to investigate that regulation (14, 16, 18, 24). Those efforts have been hampered by two technical barriers. First, when the Ca²⁺-sensitive fluorescent probe fura 2 is loaded into isolated vessels, an exclusively endothelial signal is obtained, so that pericyte [Ca²⁺]i responses have yet to be reported (16). Second, patch clamp of abluminal pericytes on isolated vessels is possible, but the luminal endothelial cells are inaccessible to study. Thus fura 2-based [Ca²⁺]i studies and electrophysiological measurements have been restricted to endothelia and pericytes, respectively. We describe two methods that overcome those technical barriers. After collagenase digestion, a micropipette can be used to strip pericytes from isolated vessels to isolate either cell population. Patch clamp can be performed on DVR endothelia isolated after pericyte

RENAL MEDULLARY BLOOD FLOW is supplied by branches of juxtamedullary glomerular efferent arterioles called descending vasa recta (DVR). DVR traverse the renal outer medulla sequestered with ascending vasa recta into vascular bundles. The radial arrangement of DVR within the bundles suggests that DVR function to regionally distribute blood flow within the outer and inner medulla of the kidney (10, 15, 17). Pericytes surround DVR and impart contractile function and are therefore an important regulatory element in determination of medullary perfusion. Vasomotor tone of isolated DVR responds to a large number of agents (15).

Address for reprint requests and other correspondence: T. L. Pallone, Div. of Nephrology, N3W143, University of Maryland at Baltimore, Baltimore, MD 21201-1595 (E-mail: tpallone@medicine.umaryland.edu).

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stripping or on DVR “everted” by turning the vessels inside out with a micropipette (5, 23). In the presence of the anion transport blocker probenecid, pericytes load well with fura 2, so that their \([\text{Ca}^{2+}]_i\) responses can be examined (4). By using these methods, we have shown that ANG II suppresses endothelial \([\text{Ca}^{2+}]_i\), and increases pericyte \([\text{Ca}^{2+}]_i\). Pericyte and endothelial membrane potentials \((\Psi_m)\) are depolarized by ANG II, and both are hyperpolarized by the vasodilator BK.

METHODS

Isolation of endothelia by pericyte stripping. Kidneys were removed from Sprague-Dawley rats (70–150 g; Harlan), sliced into sections along the corticomedullary axis, and stored at 4°C in a physiological saline solution (PSS) composed of (in mM) 145 NaCl, 5 KCl, 1 MgCl₂, 1 CaCl₂, 10 HEPES, and 10 glucose, pH 7.4, at room temperature. As previously described, small wedges of renal medulla were separated from kidney slices by dissection and transferred to CaCl₂-free PSS containing collagenase 1A (0.45 mg/ml; Sigma), protease XIV (0.4 mg/ml; Sigma), and albumin (1.0 mg/ml) (14). These were incubated at 37°C for 22 min and then returned to 1 mM CaCl₂ containing PSS and held at 4°C in a petri dish. At intervals, vessels were isolated from the digested renal tissue by microdissection and transferred to a perfusion chamber on an inverted microscope (Nikon Diaphot). In the chamber, the vessels were captured by a micropipette and positioned onto the coverslip, and after 15–30 min were allowed for cell attachment and stabilization, bath flow was initiated. We previously showed that this approach to enzymatic digestion allows gigaohm seals to be obtained on pericytes for electrophysiological recording (14, 24).

To separate the pericytes from the enzymatically digested vessels, the maneuver illustrated in Fig. 1 was performed. Isolated DVR were aspirated into a microperfusion-style holding pipette (19) with an opening of 5–10 μm. During the aspiration, pericytes strip from the abluminal surface of the vessel and are retained at the pipette tip (Fig. 1, A and B). Once the vessel has been completely drawn into the pipette, a preparation of pericytes remains as a group of cells suspended in the bath, adherent to the pipette. Finally, the aspirated, pericyte-denuded vessel can be ejected from the pipette to yield a preparation of DVR endothelia that is free of pericytes (Fig. 1C). The ejected endothelia were positioned on the chamber coverslip and given 15–30 min to adhere for stabilization before bath flow was initiated.

Whole cell patch-clamp recording. \(\Psi_m\) was monitored by patch-clamp recording from endothelial cells or pericytes at room temperature. To accomplish this, outer medullary DVR were digested to remove basement membrane as described above and to enable establishment of gigaohm seals with patch pipettes (14, 24). Patch-clamp studies of pericytes were always done on intact vessels. Stripped pericytes were used for \(\text{Ca}^{2+}\) measurements but not for electrophysiological studies. Patch pipettes were made from borosilicate glass capillaries (model PG52151-4, World Precision Instruments, Sarasota, FL; 1.5 mm OD, 1.0 mm ID) using a two-stage vertical pipette puller (model PP-830, Narishige) and heat polished. To obtain electrical access for whole cell perforated patch-clamp recording, nystatin was used as the pore-forming agent (6, 8). The pipette solution contained 120 mM potassium aspartate, 20 mM KCl, 10 mM NaCl, 10 mM HEPES, pH 7.2, and nystatin (100 μg/ml with 0.1% DMSO) in ultrapure water. Nystatin in DMSO was kept frozen at −20°C and renewed weekly. Each day, the nystatin stock was thawed, dispensed into the potassium aspartate pipette solution at 37°C by vigorous vortexing for 1 min, and subsequently protected from light. To clear slight remaining nystatin precipitate from the saturated electrode solution, pipettes were backfilled from a syringe via a 0.2-μm filter.

\(\Psi_m\) was measured using a CV201AU head stage and Axopatch 200A amplifier (Axon Instruments, Foster City, CA) in current-clamp mode (current = 0) at a sampling rate of 10 Hz. \(\Psi_m\) was recorded with pipettes of 8- to 15-MΩ resistance. Lower resistance pipettes proved technically difficult to use and led to premature loss of seals. Pipettes with nystatin-containing electrode solution were inserted into the bath under positive pressure and positioned near the cell, and the offset of the amplifier was adjusted to null the junction and electrode potentials. The final approach to the cell was con-
trolled with a piezoelectric drive (model PCS-5000, Burleigh). Gigaseals were established by pressing the pipette tip against the cell and applying light suction. The progress of seal formation was followed on a digital oscilloscope (model M305, Hameg) by observing the current elicited by test pulses of 5-mV amplitude. Seal formation was facilitated by gradually reducing the holding potential from 0 to −70 mV. After seal formation, the appearance of the cell capacitance transient and the access resistance were monitored using a Digidata analog-to-digital converter and Clampex 7.0 (Axon Instruments, Union City, CA) with 10-mV pulses at a holding potential of −70 mV. Final access resistance was generally 15–40 MΩ. Junction and Donnan potential corrections were applied as previously described (8, 14).

Measurement of $\left[Ca^{2+}\right]_i$. Incubation in 10 μM fura 2-AM (Molecular Probes, Eugene, OR) for 20 min at 37°C yields a strong signal when loaded into a collection of pericytes isolated as shown in Fig. 1B from a single vessel. Figure 1D shows the fluorescent emission of fura 2-loaded pericytes (510 nm) during excitation of fura 2 at the isosbestic point (360 nm). Results are shown for vessels incubated and then maintained in the presence or absence of the anion transport inhibitor probenecid (1 mM). Probenecid has a marked effect of preventing the leak of fura 2 from the pericyte cytoplasm. Endothelial cells were loaded with fura 2 as previously described (16) by exposure to bath containing 2 μM fura 2-AM. In this study, the fura 2 incubation buffer and all solutions involved in $\left[Ca^{2+}\right]_i$, signaling studies of both cell types contained 1 mM probenecid.

A photon-counting photomultiplier assembly was employed to measure fluorescent emission at 510 nm. Light for excitation of fura 2 was provided from a 75-W xenon arc lamp. Fura 2-loaded cells were excited using 350/380-nm wavelength combinations. The excitation wavelengths were isolated with a computer-controlled monochromator (PTI, Lawrenceville, NJ). A Nikon CF fluor ×40 oil-immersion objective with numerical aperture of 1.3 was used for fura 2/ $\left[Ca^{2+}\right]_i$, measurement. Fluorescent emission was isolated with a 510WB40 filter (Omega Optical, Brattleboro, VT). The background-subtracted ratio of fluorescent emission (R510/380) was converted to the equivalent $\left[Ca^{2+}\right]_i$, with the assumption of a dissociation constant of 224 nM for fura 2/ $\left[Ca^{2+}\right]_i$.

Reagents. ANG II, BK, probenecid, ionomycin, bovine serum albumin (A2153, Cohn fraction V), nystatin, collagenase IA, and protease XIV were obtained from Sigma (St. Louis, MO). ANG II or BK in water was stored in 200-μl aliquots at −20°C and diluted on the day of an experiment. The enzyme digestion solution was prepared in 50-ml batches, frozen in 2-ml aliquots, and thawed daily as needed. Cyclopiazonic acid (CPA; Calbiochem, San Diego, CA) was stored in DMSO at 10 mM. Fura 2 (Molecular Probes) was stored at 1 mM in anhydrous DMSO. Reagents were thawed once, and the excess was discarded at the end of the day.

Fig. 2. $\left[Ca^{2+}\right]_i$, responses of DVR pericytes and endothelia. A: intracellular $Ca^{2+}$ concentration ($\left[Ca^{2+}\right]_i$) response of fura 2-loaded pericytes isolated by the process described in Fig. 1B. After baseline recording for 1 min, 10 nM ANG II was introduced into the bath for 10 min. $\left[Ca^{2+}\right]_i$, increased from a baseline of $\sim$65 nM to a peak of 555 ± 100 nM and then fell to a plateau near 200 nM (n = 10). B: $\left[Ca^{2+}\right]_i$, response of fura 2-loaded endothelia isolated as described in Fig. 1C. After 2 min, 10 nM ANG II or vehicle (n = 7, each group) was introduced into the bath. $\left[Ca^{2+}\right]_i$, fell from resting value to $\sim$20 nM in the ANG II-treated group (P < 0.05, ANG II vs. vehicle, continuously after 5 min). C: $\left[Ca^{2+}\right]_i$, response of fura 2-loaded endothelia to 100 nM bradykinin (BK). Endothelia were studied by stripping pericytes and then loading fura 2 (n = 5) or by loading fura 2, stripping, and immediately proceeding with $\left[Ca^{2+}\right]_i$, measurements without equilibration (n = 5). BK induced a response in both cases, but the peak was blunted in the latter group.
Statistics. Values are means ± SE. The significance of differences between means was calculated using Student’s t-test (paired or unpaired, as appropriate) and analysis of variance. Where sampling rates were high, the majority of error bars were suppressed to clarify display of data.

RESULTS

Ca\(^{2+}\) signaling in DVR pericytes and endothelia. We previously showed that ANG II reduces basal [Ca\(^{2+}\)]\(_i\) and suppresses BK- or thapsigargin-induced [Ca\(^{2+}\)]\(_i\) elevations in DVR endothelia. We had been unable to examine ANG II-induced [Ca\(^{2+}\)]\(_i\) changes in pericytes because of poor loading of fura 2 (16). Experiments verifying the endothelial [Ca\(^{2+}\)]\(_i\) responses and showing previously undocumented pericyte responses are shown in Figs. 2 and 3. These data were obtained in enzymatically isolated pericytes and endothelia in the presence of probenecid. Figure 2A shows the effect of abluminal application of 10 nM ANG II on [Ca\(^{2+}\)]\(_i\) in isolated pericytes (mean ± SE, n = 10). A classic peak-and-plateau response was observed. The contrasting effect of 10 nM ANG II on endothelial [Ca\(^{2+}\)]\(_i\), is shown in Fig. 2B. In pericyte-stripped vessels, basal endothelial [Ca\(^{2+}\)]\(_i\), which is between 50 and 100 nM, fell to a mean of 20 nM after exposure to ANG II. This effect of ANG II reproduces prior results obtained in isolated DVR that had not been subjected to enzymatic digestion or pericyte stripping (18).

Endothelial [Ca\(^{2+}\)]\(_i\) responses to BK were also reexamined in isolated endothelia (Fig. 2C). BK stimulated the expected peak-and-plateau [Ca\(^{2+}\)]\(_i\) elevation. In vessels that had been stripped and then equilibrated at 37°C during fura 2 loading, the response was similar to that previously observed (16). When vessels were loaded with fura 2 and then stripped of pericytes so that [Ca\(^{2+}\)]\(_i\) was immediately examined after the stripping process, the peak response to BK was blunted (Fig. 2C). This suggests that the stripping process might cause intracellular Ca\(^{2+}\) store depletion and

Fig. 2. (A) DVR pretreated with vehicle or 10 nM ANG II for 5 min were exposed to 10 μM CPA. Inhibition of internal store sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\)-ATPase by this agent resulted in elevation of [Ca\(^{2+}\)]. Final [Ca\(^{2+}\)], was much lower in ANG II-pretreated group. These experiments are a continuation of those in Fig. 2B, with abscissa relabeled to time 0. *P < 0.05, ANG II vs. vehicle, continuously after 4 min. B: endothelial [Ca\(^{2+}\)], was measured for 1 min, and then 10 μM CPA was introduced into the bath. After 5 min of CPA exposure, the bath was exchanged to also introduce 10 nM ANG II. DVR endothelial [Ca\(^{2+}\)], was markedly suppressed after ANG II exposure.

Fig. 3. [Ca\(^{2+}\)], inhibition in DVR endothelia treated with cyclopiazonic acid (CPA). A: DVR pretreated with vehicle or 10 nM ANG II for 5 min were exposed to 10 μM CPA. Inhibition of internal store sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\)-ATPase by this agent resulted in elevation of [Ca\(^{2+}\)]. Final [Ca\(^{2+}\)], was much lower in ANG II-pretreated group. These experiments are a continuation of those in Fig. 2B, with abscissa relabeled to time 0. *P < 0.05, ANG II vs. vehicle, continuously after 4 min. B: endothelial [Ca\(^{2+}\)], was measured for 1 min, and then 10 μM CPA was introduced into the bath. After 5 min of CPA exposure, the bath was exchanged to also introduce 10 nM ANG II. DVR endothelial [Ca\(^{2+}\)], was markedly suppressed after ANG II exposure.

Fig. 4. Eversion of DVR for endothelial patch-clamp recording. A: a single descending vas rectum in which an elongated perfusion-style pipette (eversion pipette) has been advanced through a holding pipette to turn the vessel inside out and expose endothelial cells at its distal end. A patch-clamp pipette deformed under heat to bend its tip ~90° has been advanced to form a seal with luminal endothelial surface. B: endothelial membrane potential (\(V_m\)) recordings often showed incremental variations indicative of channel openings.

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points to the need for an equilibration period after the mechanical trauma of stripping.

The ability of ANG II to inhibit endothelial \([\text{Ca}^{2+}]_i\) responses is difficult to demonstrate in resting vessels, because basal \([\text{Ca}^{2+}]_i\) is low and ANG II-induced changes are small (Fig. 2B). We also reexamined the ability of ANG II to block \([\text{Ca}^{2+}]_i\) elevations in isolated endothelia treated with the SERCA inhibitor CPA (10 \(\mu\text{M}\); Fig. 3). CPA markedly increased endothelial \([\text{Ca}^{2+}]_i\). Compared with vehicle-treated controls, ANG II reduced the endothelial \([\text{Ca}^{2+}]_i\) elevation achieved by CPA (Fig. 3A). Similarly, addition of 10 nM ANG II to the bath after treatment with 10 \(\mu\text{M}\) CPA markedly reduced endothelial \([\text{Ca}^{2+}]_i\) (Fig. 3B). These results reproduce our prior observations that were obtained in intact vessels when thapsigargin was used as the SERCA inhibitor (18).

Electrophysiological measurements of \(\Psi_m\) in DVR endothelia and pericytes. We obtained recordings of \(\Psi_m\) from DVR endothelia by two methods. First, pericyte-denuded vessels (Fig. 1C) were ejected onto a coverslip and accessed for whole cell recording by patches formed on their abluminal surface. Second, intact, nondigested microdissected DVR were everted by turning them inside out (Fig. 4A). This was done by deliberately crushing one end during microdissection. The crushed end was then cannulated, as typically done for microperfusions (5, 23). By continuously advancing an elongated “perfusion pipette,” portions of the endothelial surface were exposed and made accessible for patch-clamp recording on their luminal surface. For better visualization of the process, most patch pipettes were bent \(\sim 90^\circ\) by

Fig. 5. Effect of ANG II on \(\Psi_m\) in DVR endothelia. A–D: individual recordings of \(\Psi_m\) from endothelial cells as 10 nM ANG II was introduced into and then removed from the bath (10-min exposure). A and B are from everted DVR; C and D are from pericyte-stripped DVR. Depolarization was observed. Voltage shown at the beginning of each trace is resting potential at time 0. Trace in C shows evidence of incremental changes due to channel activity. E and F: summary of endothelial \(\Psi_m\) changes induced by ANG II in pericyte-stripped \((n = 12)\) and everted \((n = 8)\) vessels. NS, not significant.

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heating on a microforge. This permitted orientation of the tip so that the approach of the patch pipette tip to the endothelial cell could be visualized. Alternatively, some gigaseals were formed with unaltered patch pipettes by lowering the tip onto the preparation until variations in the current visualized as pulses on the oscilloscope showed that the cell membrane had been touched. With the latter method, the perfusion pipette obscures visualization on an inverted microscope, making choice of the cell impossible. During recording, incremental variations in $\Psi_m$ were sometimes observed that indicated single-channel or groups of channel openings. A particularly dramatic example is shown in Fig. 4B.

The effect of 10 nM ANG II on endothelial $\Psi_m$ is illustrated in Fig. 5. Most often, spiking, rapid oscillations of $\Psi_m$ occurred that were often superimposed on some elevation of the baseline (Fig. 5, A–D). Reversibility after ANG II washout was variable but often present. Summaries of the effects of ANG II on $\Psi_m$ are provided in Fig. 5, E and F, for stripped and everted vessels, respectively. The baseline, ANG II, and recovery values are shown as 10-s averages at the end of each period. In pericyte-stripped vessels, $\Psi_m$ depolarized from $-53.1 \pm 3.2$ to $-43.4 \pm 4.4$ mV ($P < 0.05$) and recovered to $-48.3 \pm 3.7$ mV ($P = 0.07$, ANG II vs. recovery). ANG II tended to depolarize the endothelia more in everted than in stripped vessels [$-56.3 \pm 4.2$ (baseline) to $-35.8 \pm 3.8$ mV (ANG II)], but the difference in the ANG II-depolarized values ($-43.4 \pm 4.4$ vs. $-35.8 \pm 3.8$ mV) did not achieve significance ($P = 0.24$) for the two configurations.

The effect of 100 nM BK on endothelial $\Psi_m$ is illustrated in Fig. 6. The response was characterized by transient hyperpolarization, which tended to return to baseline before BK washout (Fig. 6, A–D). Summaries of the effects of BK on $\Psi_m$ are provided in Fig. 6, E and F, for stripped ($n = 16$) and everted ($n = 9$) vessels,
respectively. Values show 10-s averages for the baseline and recovery periods. Because the BK effect was transient, the minimum \( \Psi_m \) achieved during the hyperpolarization, rather than a time average, is displayed. In stripped vessels, \( \Psi_m \) changed from \(-39.9 \pm 3.4\) mV to a minimum of \(-62.7 \pm 2.8\) mV \((P < 0.01)\) and recovered to \(-45.2 \pm 4.5\) mV \((P < 0.01, \text{BK vs. recovery})\). Everted vessels showed similar hyperpolarization \((P < 0.01, \text{BK vs. recovery})\).

To test whether variation of \( \Psi_m \) in DVR pericytes might be part of the mechanism by which BK dilates these vessels, we obtained eight records from pericytes. As shown in Fig. 7, baseline \( \Psi_m \) was low: \(-65.0 \pm 1.8\) mV. Despite the low resting potential of this group of pericytes, 100 nM BK hyperpolarized them to \(-72.0 \pm 1.0\) mV \((P < 0.05)\), an effect that reversed to \(-62.7 \pm 3.6\) mV after washout \((P = 0.05, \text{BK vs. recovery})\).

DISCUSSION

We previously demonstrated that complex Ca\(^{2+}\)-signaling events occur in DVR endothelia. The endothelium-dependent vasodilator BK produces classic peak-and-plateau elevations of \([\text{Ca}^{2+}]_i\), as expected for stimulation of a G protein-coupled receptor that signals through inositol trisphosphate-mediated Ca\(^{2+}\) release-and-entry mechanisms \((2)\). Also as expected, BK enhances production of nitric oxide (NO), probably through stimulation of NO synthase type III \((21)\). In most cell types, ANG II acts through the AT\(_1\) receptor to increase \([\text{Ca}^{2+}]_i\) \((22)\). Furthermore, infusion of ANG II leads to NO production within the kidney, particularly in the renal medulla \((25)\). On the basis of those expectations, our prior finding, reproduced in this study \((2)\), that ANG II suppresses \([\text{Ca}^{2+}]_i\) signaling in DVR endothelia is particularly surprising. On the basis of this finding, we have speculated that the source of ANG II-stimulated NO production in the renal medulla is epithelial, rather than endothelial, in origin and that this enables NO to provide a feedback mechanism through which oxygen-consuming outer medullary interbundle nephrons can modulate their own perfusion \((18)\). The receptor subtype and signaling mechanisms that mediate this process are unknown.

Given the importance of Ca\(^{2+}\) signaling and NO production in the preservation of medullary blood flow, we were motivated to develop methods that would enable determination of the mechanisms by which DVR cellular processes are regulated. We were previously unable to examine pericyte \([\text{Ca}^{2+}]_i\) signaling, because fura 2 failed to load into pericytes on isolated DVR \((16)\). In contrast, electrophysiological examination of pericytes has been possible because of their abluminal location \((14, 24)\). Conversely, because fura 2 loads avidly into DVR endothelia, we have been readily able to measure endothelial \([\text{Ca}^{2+}]_i\) changes but unable to access those cells for electrophysiological recording.
In this effort, we developed two methods for bridging those gaps. First, enzymatic digestion of the basement membrane makes it possible to form gigaseals on the pericytes for patch clamp (14) and to strip the pericytes from the vessels simply by aspirating a vas rectum into a holding pipette (Fig. 1). The result is that either cell type can be isolated for Ca\(^{2+}\) studies or electrophysiological recording. As a second, somewhat arduous, approach to endothelial electrophysiology, isolated DVR that have not been enzymatically digested can be everted to expose the luminal endothelial surface (5, 23) (Fig. 4). Gigaseals form on the luminal endothelial surface without prior enzymatic treatment.

Studies of endothelial [Ca\(^{2+}\)]\(\text{i}\) signaling in pericyte-denuded vessels differ from our prior examinations in isolated vessels in important ways. First, the endothelial cells have been subjected to enzymatic digestion and the trauma required to denude the abluminal surface of pericytes. Second, endothelial cell responses are studied in the absence of pericytes, so that pericyte-endothelial communications via diffusible factors or myoendothelial junctions would have been disrupted. Motivated by this, we first determined whether we could reproduce our prior finding that BK elevates DVR endothelial [Ca\(^{2+}\)]\(\text{i}\), and that ANG II suppresses it (16, 18). Despite the trauma of isolation, enzymatic digestion, and the absence of pericytes, both responses were intact (Figs. 2 and 3), but an equilibration period after stripping is probably needed to achieve full responsiveness (Fig. 2C). This supports the notion that cellular signaling and receptors for those ligands are preserved. We previously showed that perfusion of DVR Ca\(^{2+}\) entry by increasing the electrochemical driving force for influx via nonselective cation channels while reducing endothelial Ca\(^{2+}\) entry via nonselectivecation channels. The recent demonstration of expression of L- and T-type voltage-operated Ca\(^{2+}\) channels in the juxtamedullary effenter arteriole and DVR is consistent with that possibility (7). We demonstrated that ANG II decreases the rate of refilling of Ca\(^{2+}\) into store-depleted DVR endothelia and decreases the rate of Mn\(^{2+}\) entry into Ca\(^{2+}\)-replete cells. Those findings do not differentiate between ANG II-induced blockade of the Ca\(^{2+}\) entry pathway and \(\Psi_m\) change, because either could inhibit divalent cation influx (16). Further studies are required to study the effects of ANG II on endothelial currents in voltage-clamp experiments.

In summary, we devised a relatively simple method for separating and isolating DVR pericytes and endothelia for examination by electrophysiological and microfluorescent methods. We have, for the first time, verified that ANG II increases [Ca\(^{2+}\)]\(\text{i}\) in the DVR pericycle cytoplasm and reduces [Ca\(^{2+}\)]\(\text{i}\) in DVR endothelia. When pericytes are removed from DVR, the endothelial response to ANG II is preserved, supporting the existence of an endothelial ANG II receptor. ANG II and BK have opposite effects to depolarize and hyperpolarize pericytes and endothelia. A relationship between \(\Psi_m\) changes and [Ca\(^{2+}\)]\(\text{i}\) responses is hypothesized on the basis of Ca\(^{2+}\) entry pathways in the two cell types.

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