Molecular and Functional Identification of Cyclic AMP–Sensitive BK$_{Ca}$ Potassium Channels (ZERO Variant) and L-Type Voltage-Dependent Calcium Channels in Single Rat Juxtaglomerular Cells

Ulla G. Friis, Finn Jørgensen, Ditte Andreasen, Boye L. Jensen, Ole Skøtt

Abstract—This study aimed at identifying the type and functional significance of potassium channels and voltage-dependent calcium channels (Ca v ) in single rat JG cells using whole-cell patch clamp. Single JG cells displayed outward rectification at positive membrane potentials and limited net currents between $-60$ and $-10$ mV. Blockade of K$_+^+$ channels with TEA inhibited 83% of the current at $+105$ mV. Inhibition of K$_V^+$ channels with 4-AP inhibited 21% of the current. Blockade of calcium-sensitive voltage-gated K$_+^+$ channels (BK$_{Ca}^+$) with charybdotoxin or iberiotoxin inhibited 89% and 82% of the current, respectively. Double immunofluorescence confirmed the presence of BK$_{Ca}^+$ and renin in the same cell. cAMP increased the outward current by 1.6-fold, and this was inhibited by 74% with iberiotoxin. Expression of the cAMP-sensitive splice variant (ZERO) of BK$_{Ca}^+$ was confirmed in single-sampled JG cells by RT-PCR. The resting membrane potential of JG cells was $-32$ mV and activation of BK$_{Ca}^+$ with cAMP hyperpolarized cells on average 16 mV, and inhibition with TEA depolarized cells by 17 mV. The cells displayed typical high-voltage activated calcium currents sensitive to the L-type Ca v blocker calciseptine. RT-PCR analysis and double-immunofluorescence labeling showed coexpression of renin and L-type Ca v 1.2. The cAMP-mediated increase in exocytosis (measured as membrane capacitance) was inhibited by depolarization to $+10$ mV, and this inhibitory effect was blocked with calciseptine, whereas K$_+^+$-blockers had no effect. We conclude that JG cells express functional cAMP-sensitive BK$_{Ca}^+$ channels (the ZERO splice variant) and voltage-dependent L-type Ca$^{2+}$ channels. (Circ Res. 2003;93:213-220.)

Key Words: BK$_{Ca}^+$, Ca$^{2+}$, juxtaglomerular cells

Renin release from juxtaglomerular (JG) cells in the renal afferent arterioles is controlled by several intracellular messenger systems. Vasodilators generally stimulate renin release by increasing the intracellular concentration of cAMP and vasoconstrictors generally inhibit renin release by increasing the intracellular calcium concentration.

The membrane potential often acts as an integrator in cellular signaling and it has been suggested to be involved in the control of renin release. Based on electrophysiological measurements, Fishman$^1$ suggested that hyperpolarization was associated with stimulation and depolarization with inhibition of renin secretion. Cell membrane depolarization, followed by activation of Ca$,^+$ and calcium influx, has been suggested to inhibit renin release.$^2,^3$ However, the inability of depolarization to change calcium concentration in JG cells and the lack of effect of calcium channel blockers on renin release have questioned the relevance of Ca$,^+$ in the control of renin secretion.$^4,^5$ The ion channels responsible for setting the membrane potential of JG cells are not known with certainty. When measured in the whole-cell patch-clamp mode, there are limited net currents in JG cells at membrane potentials between $-60$ and $-10$ mV, whereas at more positive potentials, an outward current is activated.$^4,^7$ This outward current is activated by cAMP.$^8$

We have used the patch-clamp technique in combination with molecular techniques to define the ion channels involved in the outward current and to study the presence of Ca$,^+$ in JG cells and their possible interaction with the secretory process.

Materials and Methods

Isolation of Rat JG Cells
Male Sprague-Dawley rat (60 to 80 g; Animal Facility, University of Southern Denmark, Denmark) JG cells were isolated from renal cortex.$^8$ All animal procedures conformed to the Danish law on experiments on animals and with the guidelines for the care and handling of animals established by the US Department of Health and Public Services and published by the National Institutes of Health. For patch clamp, the cells were transferred to cover slips, and for the renin secretion studies the cells were separated on a Percoll density gradient (25% Percoll at 27 000 g for 30 minutes), resuspended in RPMI-1640 medium, and seeded in 96-multiwell plates.
Renin Secretion From JG Cell Cultures
Cultured cells were incubated for 20 hours, washed, and experimental agents added. After 20 hours, the medium was removed, and the cells were harvested. Renin concentration was determined by RIA for ANG 1. Renin secretion rates were calculated as fractional release of total renin content.

Immunofluorescence
JG cells attached to glass slides were rinsed in TBS, fixed in 99.9% EtOH, and air-dried. Kidney cryosections were blocked with TTBS containing 5% goat serum +1% BSA and JG cells with 5% skim milk. The specimens incubated with primary antibodies (mouse monoclonal anti-renin [SWANT, Bellinzona, Switzerland], polyclonal rabbit anti-rat αs2 [Alomone Labs, Jerusalem, Israel], polyclonal rabbit anti-rat BKCa [Chemicon, Temecula, Calif]) for 2 hours and were washed in TTBS. Next, the specimens incubated with secondary antibody for 30 minutes. Secondary antibodies were as follows: goat-anti rabbit IgG conjugated to Alexa Fluor 488 combined with goat anti-mouse IgG conjugated to Alexa Fluor 488 for JG cells (Molecular Probes). The specimens were mounted with fluorescence medium (DAKO). Negative controls with omission of primary antibody were always run in parallel, and where peptides were available, we included preabsorption controls (αs2 peptide).

Reverse Transcription–Polymerase Chain Reaction
RT-PCRs were performed as previously described. Templates for RT-PCRs were total RNA isolated from rat organs, from microdissected rat renal peliglomerular vessels, and from single JG cells sampled with patch pipettes. RNA was isolated by acid guanidinium-thiocyanate phenol-chloroform method using yeast tRNA as carrier. To amplify rsLO (BKCa) ZERO and STREX splice site junctions, we used primers that span the splice site 2 junction: 5′-TTTTTCATCGCAAGTGA-3′; antisense, 5′-TATTACCTTACGTCAAGTGA-3′; antisense, 5′-GTGAAA-CATTCCGACGAGCTA-3′. The primers anneal to bases 2335 to 2635/H11032; high-chloride solution, K-glutamate 135, NaCl 10, KCl 10, MgCl2 1, HEPES-NaOH 10, Mg-ATP 0.5, Na2GTP 0.3, EGTA 11, pH 7.07; high-chloride solution, K-glutamate 135, NaCl 10, KCl 10, MgCl2 1, HEPES-NaOH 10, Mg-ATP 0.5, Na2GTP 0.3, EGTA 11, pH 7.0; solution, HEPES-NaOH 10, NaCl 140, KCl 2.8, MgCl2 1, CaCl2 2, glucose 11, sucrone 10, TEA 5 (osmolality was 317 mOsm/kg; pH 7.17); 4-AP-containing solution, HEPES-NaOH 10, NaCl 140, KCl 2.8, MgCl2 1, CaCl2 2, glucose 11, sucrone 10, 4-AP 1 (osmolality was 309 mOsm/kg; pH 7.25); and solution for Ca2+ currents, TEA-acetate 148, HEPES-CsOH 10, KCl 2.8, MgCl2 1, BaCl2 10.8 (osmolality was 325 mOsm/kg; pH 7.2).

Reagents
4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES), Tris-HCl, glucose, sucrose, insulin, penicillin, streptomycin, K-glutamate, Mg-ATP, Na2-ATP, forskolin, TEA, dithiothreitol, and trypsin were from Sigma Chemical Co. RPMI 1640 and FCS were from Gibco Life Technologies. Collagenase A, Na2-GTP, and cAMP were from Roche. Percoll was from Pharmacia (Sweden). 4-AP was from Merck (Germany), and BaCl2 was from ICN Biomedicals Inc. Charybdotoxin, iberiotoxin, and calciseptine were from Alomone Labs (Israel). All other chemicals were of analytical grade.

Statistics
All values are given as mean±SEM. Paired Student’s t test was used to calculate statistical difference. The change in Cm was calculated as the difference (in percent) in Cm (at t=0 minutes and t=10 minutes). A value of P<0.05 was considered statistically significant.

Results
Electrophysiological Identification of K+ Channels in JG Cells
The whole-cell recording configuration was obtained in 149 single cells isolated from 80 preparations. The average Cm was 2.42±0.10 pF, and the cells displayed outward current rectification at positive membrane potentials (Figure 1A). Inward current rectification was never observed, not even when the internal solution was changed to a high-chloride solution, and the pulse protocol was changed to cover a voltage range from −200 mV to +70 mV (Figure 1B).

Addition of the potassium channel blocker tetraethylammonium (TEA, 5 mmol/L) resulted in a large inhibition of the outward current (Figures 1C and 1D; P<0.05, n=4), indicating that most of the outward current in JG cells is carried by K+.

The voltage-gated Kc channel blocker 4-aminopyridine (4-AP) inhibited 20.6±9.5% of the outward current at +105 mV (Figure 1D; P<0.05, n=12), indicating that only a minor part of the outward current is carried by K+ through 4-AP sensitive Kc channels. The effects of TEA and 4-AP were additive: at +105 mV, TEA (5 mmol/L) together with 4-AP (1 mmol/L) inhibited 91.5±2.9% of the current (Figure 1D; P<0.05, n=4).

The identity of the Kc channels was investigated by superfusion with charybdotoxin or iberiotoxin, which inhibit calcium-sensitive voltage–gated BKc channels (Figures 2A and 2B). At +105 mV, charybdotoxin (1×10−7 mol/L) inhibited 88.7±1.2% of the outward current (P<0.05, n=4). Similarly, iberiotoxin (3×10−7 mol/L) inhibited 81.6±2.8% of the outward current at +105 mV (n=4, P<0.05). The effects of iberiotoxin (Figure 2C) and charybdotoxin (not
shown) were reversible. Thus, most of the outward current is carried through BK$_{Ca}$ channels.

The gating of BK$_{Ca}$ channels is sensitive to calcium. The Ca$^{2+}$ dependence of the outward current was, therefore, tested with EGTA (2 mmol/L) in the pipette solution, which almost completely abolished the outward current (Figure 2D; n = 7).

Outward current in JG cells is enhanced by cAMP, and the ZERO splice variant of the SLO-BK$_{Ca}$ channel gene is sensitive to cAMP. We therefore examined whether BK$_{Ca}$ channels carry cAMP-activated currents. Figure 2E shows the I-V relationships recorded immediately after the whole-cell configuration was obtained (circles), after the cells have been dialyzed with cAMP (1 μmol/L) for around 3 minutes (squares), and after the cells have been superfused with iberiotoxin (10$^{-7}$ mol/L) (triangles). Dialysis with cAMP for 3 minutes resulted in an 1.6-fold increase in the outward current at +105 mV (P < 0.05, n = 5), and iberiotoxin inhibited 74.1% ± 5.7% of this current (P < 0.05, n = 5), indicating that the cAMP-mediated increase in outward current is carried through BK$_{Ca}$ channels.

**Molecular Identification of BK$_{Ca}$ Channels in JG Cells**

The sensitivity of BK$_{Ca}$ channels to cAMP is determined by alternative splicing of the SLO gene at splice junction 2, where the presence of a 59 amino acid insert (STREX-variant) abolishes cAMP sensitivity. RT-PCR analysis using primers adjacent to splice junction 2 of the SLO gene was applied to discriminate between STREX and ZERO BK$_{Ca}$ channel splice variants. First, rat organs with reported differ-
rSLO. Messenger RNA was isolated from single JG cells, reverse transcribed with and without reverse transcriptase, and amplified for cDNA. Double-amplification of ZERO only. In kidney preglomerular vessels (Figure 3B) and single-sampled JG cells (Figure 3C), there was a measure of exocytosis. An original trace from a cell dialyzed with 1 mM vessel/reaction) for BKCa-rSLO and renin. Predominant variant was ZERO. No amplification was seen in the absence of reverse transcriptase. Negative controls without reverse transcriptase (−RT) and without cDNA (“0”) were double-amplified in parallel. Size marker is X174DNA/HaeIII fragments. B, PCR amplification (32 cycles) of cDNA from microdissected rat renal preglomerular (PG) vessels (1 mm vessel/reaction) for BKCa-rSLO and renin. Predominant variant was ZERO. No amplification was seen in the absence of reverse transcriptase. Negative controls without reverse transcriptase (−RT) and without cDNA (“0”) were double-amplified in parallel. Size marker is X174DNA/HaeIII fragments. C, PCR amplification (32 cycles) of cDNA from microdissected rat renal preglomerular (PG) vessels (1 mm vessel/reaction) for BKCa-rSLO and renin. Predominant variant was ZERO. No amplification was seen in the absence of reverse transcriptase. Negative controls without reverse transcriptase (−RT) and without cDNA (“0”) were double-amplified in parallel. Size marker is X174DNA/HaeIII fragments. D through G, Immunolabelings of freshly isolated JG cells. D and E, JG cells were double-labeled with mouse anti-renin (D) and rabbit anti-BKCa (E), F, Overlay of D and E. G, Bright-field image of the labeled cell shown in D through F. Bar=10 μm.

Significance of cAMP-Mediated BKCa Activation for Cell Capacitance Changes and Renin Secretion

To address the functional significance of BKCa activation, the patch-clamp technique was used to record cell capacitance \( (C_m) \) changes in response to cAMP. An increase in \( C_m \) indicates addition of membrane to the cell surface area and is a measure of exocytosis. An original trace from a cell dialyzed with 1 mM cAMP is shown in Figure 4A. Iberiotoxin (1×10⁻⁷ mol/L) was added via a pipette from t=200 seconds (arrow). \( C_m \) increased by 13.5±4.9% (P<0.05; n=5; Figure 4B), which is similar to the increase obtained in the absence of iberiotoxin. This indicates that in spite of the effect on whole-cell currents, iberiotoxin does not influence the cAMP-mediated increase in membrane capacitance.

Renin release was studied over prolonged time in primary cultures of JG cells. Under control conditions, these cells released 20.4±3.1% of total content (n=10) (Figure 4C). The adenyl cyclase activator, forskolin (10 μmol/L), increased renin release to 31.2±1.8% of total renin content. 4-AP (10⁻⁴ to 10⁻² mol/L; n=10, with four wells assigned per condition in one experiment) did not affect forskolin-mediated renin release (Figure 4C). Similar (negative) results were obtained with TEA (10⁻⁴ to 10⁻² mol/L) (n=12) (Figure 4D), indicating that K⁺ current activation is not necessary for cAMP-mediated renin release.

Role of BKCa Channels for Regulation of JG Cell Membrane Potential

Next, we addressed the contribution of BKCa to the resting membrane potential under current clamp. Under control (resting) conditions, the membrane potential in JG cells was -32±2.7 mV (n=5). With cAMP (1 μmol/L) in the patch pipette, the membrane potential gradually hyperpolarized (Figure 5A, original trace). On average, the membrane potentials were hyperpolarized by 16.0±3.8 mV (Figure 5C; P<0.05; n=9). Addition of TEA to the bath (5 mmol/L) depolarized the JG cells from -35.7±3.4 to -18.7±4.7 mV (Figure 5B, original trace (first arrow), and Figure 5C; P<0.05; n=3). The effect was reversible (Figure 5B, second arrow), and on average, the membrane repolarized from -18.7±4.7 to -37.8±1.8 mV (n=3) after washout of TEA. Similar results were obtained, when the zero current potential was read from the I-V curves obtained in the voltage-clamp mode and acquired in the beginning and after a 10-minute recording period (cAMP data) or before and after change of extracellular solution (TEA, charybdotoxin, iberiotoxin, and...
Thus, cAMP (1 μmol/L) lead to membrane hyperpolarization (ΔmV, 14.1 mV ± 2.8 mV; P<0.05, n=14), whereas TEA (5 mmol/L) lead to membrane depolarization (ΔmV, 21 mV ± 4 mV; P<0.05, n=3). A similar depolarizing effect was observed after addition of charybdotoxin (1×10⁻⁷ mol/L) (ΔmV, 19.3 mV ± 3.3 mV; P<0.05; n=4) and iberiotoxin (3×10⁻⁷ mol/L) (ΔmV, 18.6 mV ± 8 mV; P<0.05, n=5), whereas 4-aminopyridine (1 mmol/L) was without any affect on the zero current potential (ΔmV, 0.7±5.2 mV, n=6). These data suggest that BK<sub>Ca</sub> channels are predominant in setting the resting membrane potential in JG cells and are responsible for cAMP-mediated hyperpolarization.

Voltage-Gated Calcium Channels in JG Cells

Next, we investigated whether voltage-dependent Ca<sup>2+</sup> channels are expressed in JG cells. Na<sup>+</sup> currents and K<sup>+</sup> currents were eliminated by using Cs<sup>+</sup> and tetraethylammonium in the pipette and external solutions, and BaCl<sub>2</sub> was used as charge carrier. Under these conditions, a step protocol yielded the characteristic I-V relationship for Ca<sup>2+</sup> currents, with slight activation from -20 mV and maximal activation at +10 to +20 mV (Figure 6A). Membrane seals >10 GΩ were considered necessary in order to detect these very small currents, and under such experimental conditions 10 out of 13 JG cells displayed detectable inward (Ba<sup>2+</sup>) current. Under these conditions, a step protocol yielded the characteristic I-V relationship for Ca<sup>2+</sup> currents.
Figure 6. L-type VDCCs. A, Immediately after the whole-cell configuration was obtained, the cell was superfused with a solution that facilitates Ba\(^{2+}\) currents, and the I-V curve was monitored by the response to 12 voltage steps of 10 mV (range, −70 to +40 mV) for 400 ms from a holding potential of −30 mV. B, Repetitive pulse was applied every 10 seconds (from −30 to +10 mV for 40 ms). When the inwardly directed current was maximal and stable (bottom trace), the superfusion system was switched to a solution that also contained calciseptine (2\(×\)10\(^{-4}\) mol/L). Top trace shows the effect of calciseptine in the same cell. C, Histogram showing the inhibitory effect of calciseptine on the inwardly directed current. D, Relative change in JG cell membrane capacitance (\(C_m\)) at different holding potentials. E, Representative capacitance traces from cells dialyzed with cAMP (1 \(\mu\)mol/L) measured at +10 mV in the absence or presence of calciseptine (2\(×\)10\(^{-4}\) mol/L).

Evokes an inward Ba\(^{2+}\) current of around 4 pA (Figure 6B, control), which is blocked by the specific L-type voltage-dependent calcium channel (VDCC) blocker calciseptine (2\(×\)10\(^{-4}\) mol/L) (Figure 6B), indicating that this inward current passes through L-type Ca\(^{2+}\) channels. On average, calciseptine inhibited the current by 91.5\(\pm\)3.6\% (\(P<0.05\); \(n=4\); Figure 6C).

Double-immunofluorescence labeling of rat kidney cryosections and of isolated JG cells with antibodies directed against renin and the smooth muscle/cardiac \(\alpha_{1C}\) subunit of an L-type voltage-gated calcium channel showed that immunoreactive proteins were colocalized in terminal afferent glo-

merular arterioles (Figures 7A through 7C) and in single JG cells (Figures 7D through 7G). Preabsorption of the \(\alpha_{1C}\) antibody with the peptide used to raise the antibody prevented labeling. Omission of primary antibodies prevented fluorescence signals from the cells. RT-PCR analysis of pipette-sampled single JG cells showed expression of mRNA encoding the \(\alpha_{1C}\) subunit (Figure 7H).

**Effect of Calcium Channel Activation on JG Cell Membrane Capacitance Changes**

We tested the effect of depolarization-evoked calcium channel activation on cAMP-induced cell membrane capacitance changes. With control solutions, average \(C_m\) did not change significantly during recording times up to 10 minutes (at holding potentials of −30 mV or +10 mV) (Figure 6D). At negative holding potentials of −55 and −30 mV, with minimal Ca\(^{2+}\)-channel activation, cAMP elicited a large increase in membrane capacitance (Figure 6D). With more positive holding potentials, the \(C_m\) increase was inhibited and at a holding potential of +10 mV, the cAMP-induced increase in \(C_m\) was abolished (Figures 6D and 6E, lower trace). To establish a causal link between diminished CAMP sensitivity of \(C_m\) and calcium channel activation, the experiments were repeated in the presence of calciseptine. At a holding potential of +10 mV, calciseptine re-established the effect of cAMP on \(C_m\) (+10.8\% \(\pm\) 1.8\%) (\(P<0.05\) versus control, \(n=5\)) (Figures 6D and 6E, upper trace). These data indicate that the lack of effect of cAMP at +10 mV is due to inhibition of exocytosis caused by calcium entering the JG cell through VDCCs.

**Discussion**

JG cells are characterized by voltage-dependent outward currents, which are enhanced by cAMP. Over 50% of mouse JG cells display inward currents at negative membrane potentials.\(^4\,7\) Such currents were not observed in 326 rat JG cells,\(^8\) suggesting a species difference.

Inhibition of calcium-sensitive voltage-gated (BK\(_{Ca}\)) channels with charybdotoxin or iberiotoxin blocked most of the current, as well as the increase in membrane capacitance. Inhibition of voltage-gated potassium channels (Kv) by 4-aminopyridine (4-AP) inhibited a minor part of the current. On average, BK\(_{Ca}\) currents with TEA depolarized the JG cells. Similar splice sites exist in the COOH terminal part of the molecule in mammals. At splice site 2, there can either be an inserted exon (STREX [Stress axis regulated exons]) or no insert (ZERO).\(^10\,14\,15\) BK\(_{Ca}\) channels with the STREX insert are inhibited by protein kinase A (PKA), whereas the ZERO variant is activated by PKA.\(^12\) Our demonstration of cAMP-stimulated ZERO variant expression in JG cells is consistent with our electrophysiological finding of cAMP-stimulated BK\(_{Ca}\) in these cells.

In the current clamp configuration, the membrane potential was −32 mV, which is close to the potential of −38 mV in afferent arterioles in the perfused hydrenephrotic kidney.\(^16\) cAMP hyperpolarized the membrane potential, and blockade of K\(^+\) channels with TEA depolarized the JG cells. Similar results were observed by analysis of zero current potentials in the voltage clamp configuration where cAMP led to hyper-
polarization, whereas closure of the BKCa channels with inhibitors depolarized the JG cells. Based on these findings, we conclude that the BKCa channels participate in the setting of the resting membrane potential in JG cells.

The membrane potential of the JG cells will be important for the ability of cAMP to activate the BKCa channels. The JG cells are electrically coupled to the vascular smooth muscle cells of the afferent arteriole.4 In nonperfused vessels, the membrane potential is relatively hyperpolarized. Thus, in nonperfused hydronephrotic kidneys, the JG cell membrane potential is in the order of −60 to −75 mV.17−19 and at this negative potential, cAMP-mobilizing agents never caused hyperpolarization.17−19 When JG cells are uncoupled from the arteriole and the membrane potential is more depolarized, cAMP will open the channels and cause hyperpolarization. This is consistent with the results of Fishman1 who measured a membrane potential of −45 mV in JG cells adhering to enzyme-treated isolated glomeruli, and the cells hyperpolarized on exposure to epinephrine.

It is unlikely that the cAMP-induced hyperpolarization per se stimulates renin secretion, because blockade of BKCa did not inhibit the cAMP-induced increase in cell membrane capacitance and because forskolin-induced renin release from isolated JG cells was unaffected by TEA. In isolated perfused rat kidneys, TEA was also without effect on isoproterenol-stimulated renin release.20

The presence of mRNA and protein for L-type Ca2+ (Ca1.2) and the demonstration of currents through Ca2+ which are blocked by an L-type specific inhibitor, demonstrate that the JG cells possess L-type Ca2+. The finding that strong depolarization of the JG cells inhibits cAMP-stimulated increases in cell membrane capacitance is consistent with the view that activation of the L-type Ca2+ is associated with inhibition of renin secretion. The ability of calciseptine to reverse the inhibition supports this interpretation. The whole-cell currents through the Ca2+ were small, but correction for cell surface area (Ca2+) showed that the channel density was similar to that of the smooth-muscle cell line, A7r5, and rat tail artery myocytes.13 A reason why it has been difficult to measure Ca2+-currents in JG cells may be that the cells are about 10 times smaller than vascular myocytes. The expression of Ca2+ channels in JG cells and their inhibitory effect on cAMP-induced renin secretion is consistent with the finding that the calcium channel opener Bay K 8644 inhibits isoproterenol-induced renin release in anesthetized rats,21 and that depolarization with a high K+ concentration reversed forskolin-induced renin secretion in the isolated rat by a mechanism that was dependent on extracellular calcium.22

Previous attempts to address this question by whole-cell patch clamp have been made on isolated glomeruli with attached afferent arterioles and JG cells.4−6,23 Because of cell-to-cell coupling in this preparation,4,23 capacitative currents cannot be fully compensated, and it is therefore difficult to clamp the voltage reliably at voltages deviating from the zero current potential.23 This may explain why it was not possible to observe an increase in Ca2+, using a depolarizing voltage step protocol.4,5 When depolarizing a similar preparation with K+, Russ and coworkers23 reported a small dose-dependent increase in Ca2+, that was abolished by a dihydropyridine Ca2+ antagonist. In many smooth muscle cells, a global increase in intracellular calcium concentration after calcium influx depends on calcium-induced calcium-release from intracellular stores via ryanodine or IP3 receptors. Ryanodine has been shown not to modify calcium release from intracellular stores in the renin-secreting cell line As4.1.24 A less efficient coupling of calcium influx to calcium release in JG cells could contribute to the difficulties in using Ca2+, as a measure of the function of Ca2+.

Renin release is sensitive to changes in the cell volume, and the volume-sensitive step is located later in the secretory pathway than the calcium-sensitive process.25 The change in effective osmolality, which is associated with K+-depolarization (exchange of less permeable Na+ with more permeable K+), may therefore lead to cell swelling and
obscure the effect of activation of Ca, in various preparations. Anisosmotic addition of K’ leads to transient shrinkage and inhibition of renin release.26

During stimulation of renin release by maneuvers that increase the cellular cAMP concentration (sympathetic nervous activity, prostaglandin E2, dopamine, etc), the concomitant activation of the BKCa channels may protect the cells against depolarization and activation of VDCCs. When acute depolarization occurs, the calcium influx may inhibit renin release, but activation of the BKCa channels may repolarize the cells and terminate the inhibitory signal. A 40 mV depolarization (to +10 mV) inhibited cAMP-induced renin secretion, whereas the 20-mV depolarization caused by inhibition of K’ channels was not sufficient. In vivo, the ability of the BKCa channels to stabilize membrane potential may prevent sufficiently strong depolarization and thereby limit the functional significance of Ca, in the control of renin secretion.

In conclusion, we show expression and function of cAMP-sensitive, BKCa channels of the ZERO splice variant and of L-type voltage-dependent calcium channels (Ca, 1.2) in juxtaglomerular renin-secreting cells. The BKCa channels are involved in the control of membrane potential, whereas they do not play a direct role in the control of renin secretion. Activation of the L-type Ca, inhibits cAMP-induced renin release, thereby providing the first evidence at the cellular level for a functional role of these channels.

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References
11. Hansen PB, Jensen BL, Andreassen D, Friis UG, Skott O. Vascular smooth muscle cells express the α1a subunit of a PQ-type voltage-dependent calcium channels and it is functionally important in renal afferent arterioles. Circ Res. 2000;87:896–902.
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