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Angiotensin II induces oscillations of intracellular calcium and blocks anomalous inward rectifying potassium current in mouse renal juxtaglomerular cells

(renin/cyclic AMP/chloride current/guanine nucleotide-binding regulatory protein/guanosine 5′-[γ-thio]triphosphate)

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ABSTRACT **Simultaneous patch-clamp and fura-2 measurements were used to investigate the electrical properties and receptor-mediated changes of intracellular calcium in renal juxtaglomerular cells. Here we report the presence of voltage-activated inward and outward rectifying potassium currents and the inhibition of the anomalous inward rectifying potassium current by angiotensin II (ANG-II). This action of ANG-II was mimicked by guanosine 5′-[γ-thio]triphosphate but not by cAMP, cGMP, inositol 1,4,5-trisphosphate, or phorbol ester, suggesting that ANG-II inhibits the potassium channel directly by means of a guanine nucleotide-binding regulatory protein or by means of an unusual type of second messenger. Blocking of the inward rectifier was paralleled by membrane depolarization, but we obtained no evidence for calcium entry due to voltage-gated calcium channels in juxtaglomerular cells. Instead, under voltage clamp, ANG-II and guanosine 5′-[γ-thio]triphosphate induced release of calcium from intracellular stores followed by a sustained phase of transmembrane calcium influx and oscillations of intracellular Ca^{2+} concentrations. Changes in intracellular Ca^{2+} concentrations were found to depend on the extracellular Ca concentration—i.e., the sustained elevation was abolished in absence of extracellular Ca, and the frequency of repetitive calcium release was directly related to the extracellular concentration of calcium. Moreover, an elevation of extracellular Ca concentration by itself induced release of intracellular calcium in the absence of other stimuli. Changes in intracellular Ca^{2+} concentrations were accompanied by prominent calcium-activated chloride currents, and this mechanism is inferred to be responsible for the inhibitory role of calcium in renin secretion. Intracellular application of cAMP but not cGMP inhibited ANG-II and guanosine 5′-[γ-thio]triphosphate-induced calcium mobilization in juxtaglomerular cells, being consistent with the facilitatory effects of elevated cAMP levels on renin release. The frequency of ANG-II induced oscillations was also markedly attenuated at depolarized membrane potentials, suggesting effective negative feedback control of ANG-II-induced depolarization on repetitive Ca^{2+} transients induced by the hormone.**

Angiotensin II (ANG-II), the classic vasoconstrictor hormone, is an important determinant of blood pressure, and circulating levels of the hormone are controlled by the rate of renin release from the kidneys. Within the kidney renin is synthesized, stored, and secreted from the juxtaglomerular (JG) cells (1). The exocytosis of renin is influenced by a variety of factors including blood pressure, sodium chloride load, sympathetic nerves, and a number of hormones—among them, ANG-II (2). The intracellular control of exocytosis of renin from JG cells is not clearly understood. This control appears to be regulated by second messengers, including calcium, cyclic nucleotides, and protein kinase C activity (3, 4). Among these second messengers calcium is thought to play a key role, and a change of the intracellular calcium concentration $[Ca^{2+}]_i$ has been speculated to be the common final pathway through which renin secretion from JG cells is governed (3, 4). However, in contrast to other secretory cells, exocytosis in JG cells is thought to be inversely related to the intracellular concentration of calcium (4).

To obtain more information about the effect of ANG-II on the electrical properties and the regulation of $[Ca^{2+}]_i$ of JG cells we have used the patch clamp in combination with the fluorescent Ca-indicator dye fura-2 to measure currents in single JG cells located in afferent arterioles obtained from mouse kidneys. In particular, we have examined the effects of ANG-II, cyclic nucleotides, the extracellular calcium concentration $[Ca^{2+}]_o$, and the membrane potential.

MATERIALS AND METHODS

For one preparation both kidneys from a female (National Medical Research Institute strain) mouse (6–8 weeks) were used. The animal was killed by cervical dislocation, and the kidneys were removed, decapsulated, and minced with a scalpel blade. The material was incubated with gentle shaking at 37°C in 30 ml of saline solution (see below) supplemented with 30 mg of collagenase. After 25 min the suspension was sifted by use of 150-μm and 50-μm screens. The material retained by the 50-μm screen was washed in 10 ml of saline and settled in a bench-top centrifuge. The pellet was resuspended in 1 ml of saline and subsequently plated in the recording chamber. The tissues that attached to the glass surface consisted mainly of glomeruli, of which ≈10% had afferent arterioles of different lengths. From these structures cells at a distance between 5 and 100 μm were selected for patch-clamp experiments (see Fig. 1*A*).

Experiments were performed at 23–26°C in a saline solution containing 140 mM NaCl, 2.8 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 11 mM glucose, 10 mM Hepes·NaOH (pH 7.2). In experiments with phorbol ester the cells were preincubated with 100 nM phorbol 12-myristate 13-acetate at 37°C for time periods between 5 and 120 min. Patch-clamp measurements were done with Sylgard-coated pipettes (5–10 MΩ) in whole-cell configuration. The standard solution for filling pipettes (intracellular solution) contained 135 mM potassium glutamate, 10 mM NaCl, 1 mM $MgCl_2$, 10 mM Hepes·NaOH, 0.5 mM Mg-ATP, 0.3 mM GTP, 0.1 mM fura-2 pentapotassium salt (Molecular Probes) (pH 7.2). Guanosine 5′-[γ-thio]triphosphate (GTP[γ-S], inositol 1,4,5-trisphosphate (Amersham), cAMP, and cGMP (Boehringer, Mannheim)

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Abbreviations: ANG-II, angiotensin II; JG, juxtaglomerular; GTP-[γ-S], guanosine 5′-[γ-thio]triphosphate; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; $[Ca^{2+}]_o$, extracellular Ca^{2+} concentration.

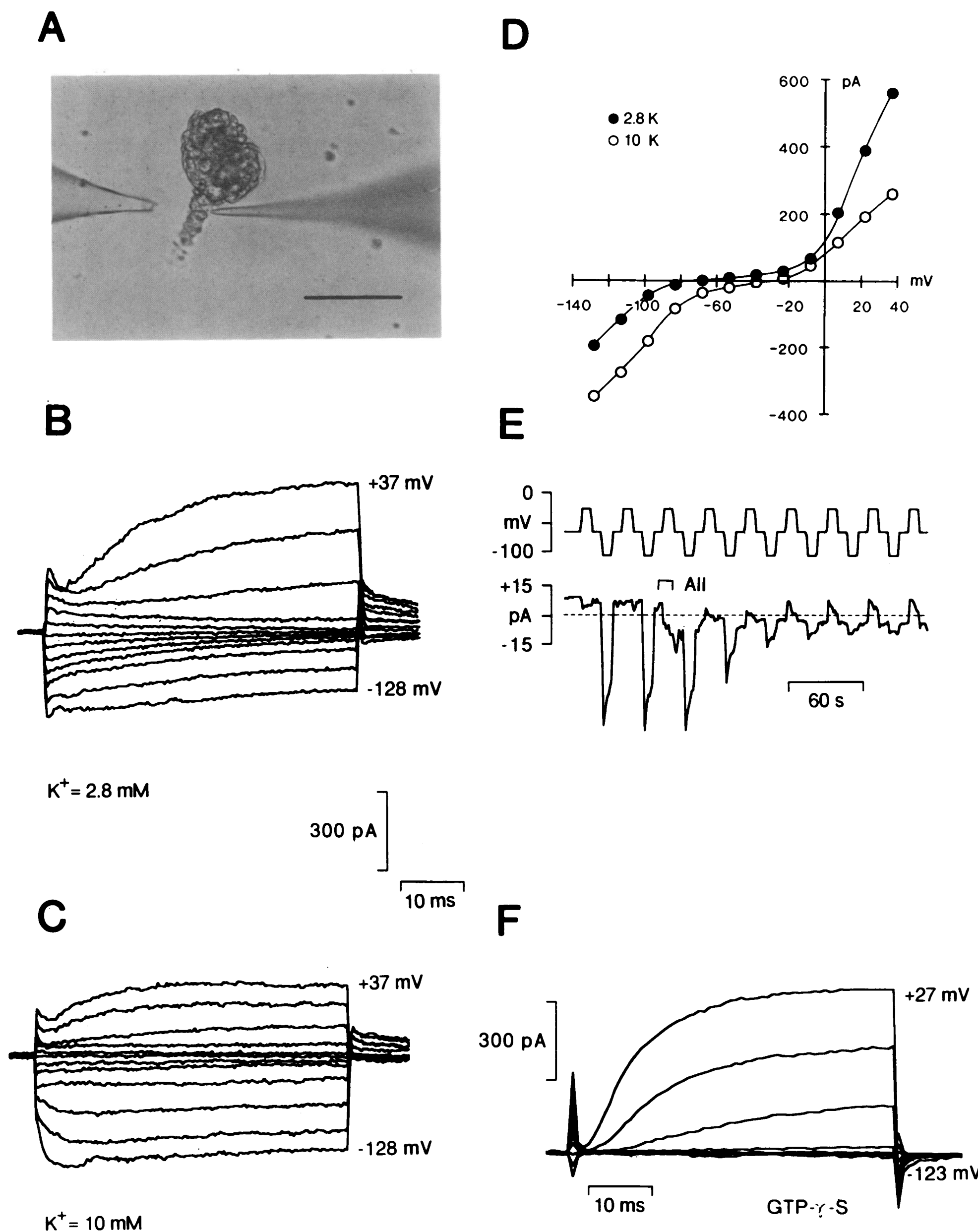

FIG. 1. Ionic currents of JG epithelioid cells. (*A*) Isolated glomerulus from mouse kidney with attached afferent arteriole. (Bar = 100 μm). JG cells are located up to 100 μm from the vascular pole of the glomerulus. (*B*) Steady-state whole-cell currents evoked by stepping the membrane potential from −30 mV to various levels in the presence of standard external solution containing 2.8 mM KCl. (*C*) Same cell as *B* after increasing external K^+ concentration to 10 mM (osmolarity was maintained by reducing NaCl accordingly). (*D*) Steady-state current–voltage relationships derived from *B* and *C*. (*E*) Blocking of the inward rectifier by ANG-II (AII) (1 μM). (*F*) Whole-cell currents after perfusing a cell with GTP[γ-S] (100 μM). Note the absence of inward rectification as compared with *B*.

were used at the concentrations indicated. ANG-II (1 μM, Sigma) or ionomycin (5 μg/ml) were dissolved in extracellular solution and applied onto cells via pressure ejection from a second pipette. Fluorescence measurements on single cells were performed as described (5). Cells were loaded with fura-2 by diffusion from the recording pipette. Fluorescence of fura-2 was excited alternatingly by light at 360 and 390 nm by means of a rotating filter wheel fitted to a slot in the excitation pathway of the microscope. $[Ca^{2+}]_i$ was calculated from the fluorescence ratio (6).

RESULTS

For this study, a total of ≈400 cells at a distance between 5 and 100 μm from the vascular pole of the glomerulus was examined. The membrane potential in whole-cell configuration under our standard experimental conditions was −62 mV ± 2 (mean ± SEM, $n = 16$), which compares to values found in microelectrode studies (7, 8). In most experiments we noticed electrical coupling between cells as judged by relatively high capacitance values (10–20 pF) compared with rather small diameter of cells (5–10 μm).

Typical current records under voltage clamp (Fig. 1*B*) and the resulting current–voltage relationship (Fig. 1*D*) in a JG cell under standard conditions display outward rectification at positive membrane potentials and inward rectification at potentials more negative than −80 mV. Both rectifying currents were absent when intracellular potassium was substituted by cesium and when tetraethylammonium (10 mM) was added to external and internal pipette solutions (data not shown). Tail-current analysis indicated that the reversal potential of the outward current was closely related to the potassium equilibrium potential, and the kinetics of the conductance are similar to those of delayed rectifiers in other cells. The inward rectifying current was also influenced by the extracellular concentration of potassium (Fig. 1*C*). Both the amplitude and the set point of activation were dependent on extracellular K^+ concentration (Fig. 1*D*). This behavior indicates that this conductance is analogous to the anomalous inward rectifying potassium current known from egg cells (9), heart cells (10), and rat basophilic leukemia cells (11).

ANG-II has been found to cause membrane depolarization, indicating that the hormone also alters the electrical behavior

of its target cells (7, 12), but the mechanism for this action has not yet been identified. As seen in Fig. 1*E*, hyperpolarizing voltage pulses activated the inward rectifier. Short-term application of ANG-II (1 μM) for 10 sec to JG cells strongly inhibited this current (n = 12). When recording membrane potential under current clamp, ANG-II caused depolarizations from −69 mV ± 6 (mean ± SEM, n = 9) to −35 mV ± 6 (data not shown). The ANG-II-induced reduction of the inward rectifier and the resulting depolarization were reversible, usually lasting several minutes.

Because there is evidence that the cellular effects of ANG-II are mediated by G proteins (13–15), we have examined the effect of the nonhydrolyzable GTP analogue GTP[γ-S] (100 μM, internal concentration). Like ANG-II, GTP[γ-S] almost completely blocked the inward rectifying current, while leaving the outward K^+ currents unaffected (Fig. 1*F*). An additional effect of GTP[γ-S] was to reduce the total cell membrane capacitance from an average of 11.7 pF ± 2.7 to 7.2 pF ± 0.7 (mean ± SEM, n = 12). We infer from this finding that GTP[γ-S] (directly or indirectly) acts by closing gap junction channels, thus uncoupling JG cells. It should be noted that the inhibitory effects of ANG-II and GTP[γ-S] on the conductance were not related to changes in $[Ca^{2+}]_i$, because the effects manifested also when intracellular calcium was buffered to near-basal values of 150 nM (with a mixture of internal Ca-EGTA/K_2-EGTA at a ratio of 5/5 mM). Second messengers, such as inositol 1,4,5-trisphosphate (1–10 μM), cAMP (50 μM), or cGMP (50 μM), when added to the internal pipette solution did not inhibit the current or prevent the inhibitory actions of ANG-II or GTP[γ-S] (n = 5–7 for each substance, data not illustrated). Similarly, preincubation of JG cells with phorbol 12-myristate 13-acetate (100 nM) for 5 to 120 min at 37°C did not inhibit the current (n = 8).

We obtained no evidence for inward currents typical of voltage-activated sodium or calcium currents. It appears rather that release of calcium from intracellular stores and receptor-mediated Ca^{2+} entry are the primary sources for changes in $[Ca^{2+}]_i$ values. Addition of ANG-II (1 μm) to JG cells typically caused an initial Ca^{2+} transient and a phase of sustained elevation, which was followed by a series of repetitive transients (Fig. 2*A*). Removal of extracellular calcium impaired the sustained elevation of $[Ca^{2+}]_i$ induced by ANG-II but did not prevent the initial Ca^{2+} transient nor the calcium oscillations (Figs. 2*C* and 3*D*). The calcium oscillations, on the other hand, could be blocked by 1 mM internal neomycin (data not shown). Furthermore, Ca^{2+} transients could not be induced by either ANG-II or GTP-[γ-S] after pretreatment of cells with phorbol ester (100 nM) for 30 min at 37°C. The presence of internal GTP was a prerequisite for the calcium mobilization by ANG-II, and the effect of ANG-II was prevented by 100 μM guanosine 5′-*o*-[β-thio]diphosphate (data not shown). Intracellular application of the nonhydrolyzable GTP analogue GTP[γ-S], on the other hand, mimicked all effects of ANG-II on $[Ca^{2+}]_i$—namely, initial calcium transient, sustained elevation, and calcium oscillations (Fig. 2*B*).

Neither cAMP nor cGMP (50 μM) had influence on the resting concentration of calcium when added to the internal (pipette) solution. In the presence of cAMP, however, both ANG-II and GTP[γ-S] were unable to raise $[Ca^{2+}]_i$ in JG cells (Fig. 2*D*), whereas cGMP had no apparent effect on the changes of $[Ca^{2+}]_i$ induced by ANG-II or GTP[γ-S] (Fig. 2*E*).

The secretion of renin from JG cells depends on $[Ca^{2+}]_o$ in an inverse fashion (4). The effect of raising $[Ca^{2+}]_o$ from 2 to 10 mM caused an intracellular Ca^{2+} transient followed by oscillations of $[Ca^{2+}]_i$ (Fig. 3*A*). The Ca^{2+} transients induced by rises in $[Ca^{2+}]_o$ were blunted when neomycin (1 mM) was included in the pipette solution (Fig. 3*B*). Extracellular calcium, moreover, influenced the frequency of the calcium oscillations induced by ANG-II or GTP[γ-S]. An increase of $[Ca^{2+}]_o$ led to an acceleration, whereas a decrease of $[Ca^{2+}]_o$ led to slow-down of internal calcium oscillations (Figs. 3 *C* and *D*).

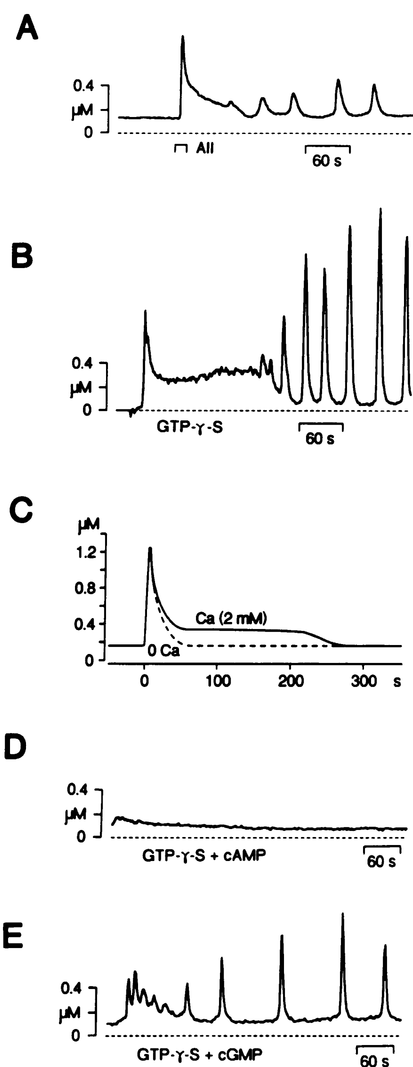

FIG. 2. Changes in $[Ca^{2+}]_i$ after stimulation with ANG-II and GTP[γ-S]. (*A*) ANG-II (1 μM; AII) was applied during the indicated time. The cell was voltage-clamped at −70 mV. Note sustained phase of elevated $[Ca^{2+}]_i$ after the initial Ca^{2+} transient. (*B*) Same as *A*, except that the cell was stimulated by internal GTP[γ-S] (100 μM). (*C*) Schematic drawing of the time course of responses to ANG-II in external Ca^{2+} (2 mM) and in nominally Ca-free external solution (---). The graphs are based on the following mean values ± SEM (n = 15 for 2 mM Ca^{2+} and n = 6 for no added Ca^{2+}; values in parenthesis are for Ca-free saline): basal $[Ca^{2+}]_i$ = 161 ± 52 nM, peak transient = 1234 ± 247 nM (1236 ± 378), and plateau phase = 350 ± 69 nM [no plateau with Ca-free solution; time for return to basal $[Ca^{2+}]_i$ = 280 ± 49 sec (65 ± 21)]. (*D*) Effect of perfusing a cell with internal solution containing cAMP (50 μM) and GTP[γ-S] (100 μM). Note that GTP[γ-S] does not induce changes in $[Ca^{2+}]_i$. (*E*) Same as *D* except that cGMP (50 μM) was included in the internal solution. Stimulation with GTP[γ-S] (100 μM) elicited normal responses of $[Ca^{2+}]_i$. Both traces start immediately after establishment of the whole-cell configuration.

In a next set of experiments we examined whether the $[Ca^{2+}]_i$ affected the electrical behavior of JG cells. As shown in Fig. 4*A* application of ionomycin caused a rise of $[Ca^{2+}]_i$ that was associated with a prominent current. The ionomycin-evoked current was absent when intracellular calcium was buffered to either 50 nM or 150 nM. The current was not affected by substitution of internal potassium by cesium or by

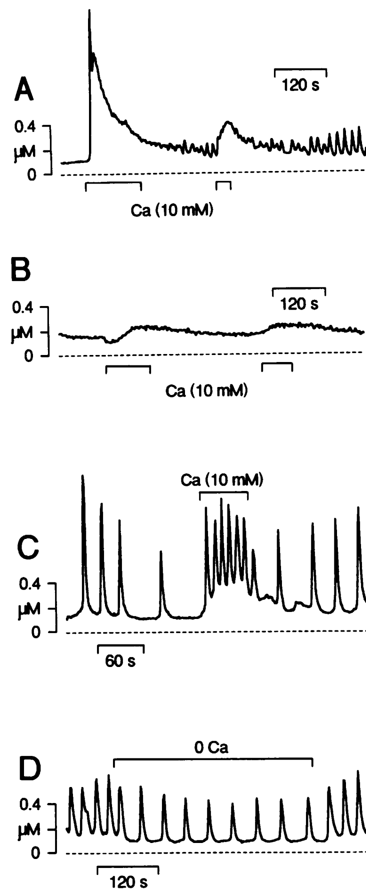

FIG. 3. Effects of extracellular calcium on $[Ca^{2+}]_i$. (A) During the times indicated the cell was flushed with an external solution containing 10 mM calcium instead of the 2 mM solution contained in the standard bath. Holding potential was −70 mV, and the seal resistance did not change during the experiment. The small change in $[Ca^{2+}]_i$ visible after the second application of high $[Ca^{2+}]_o$ may, in part, reflect nonspecific leakage of Ca^{2+} through the pipette–membrane seal. (B) Same as A, except that the cell was perfused with an internal solution that contained neomycin (1 mM). (C) Ca^{2+} oscillations were induced by GTP[γ-S] (100 μM) in standard bath solution (Ca = 2 mM). For the time indicated, the cell was perfused with an external solution containing 10 mM. (D) Same as C, except that the cell was transiently superfused with an external solution containing no added calcium.

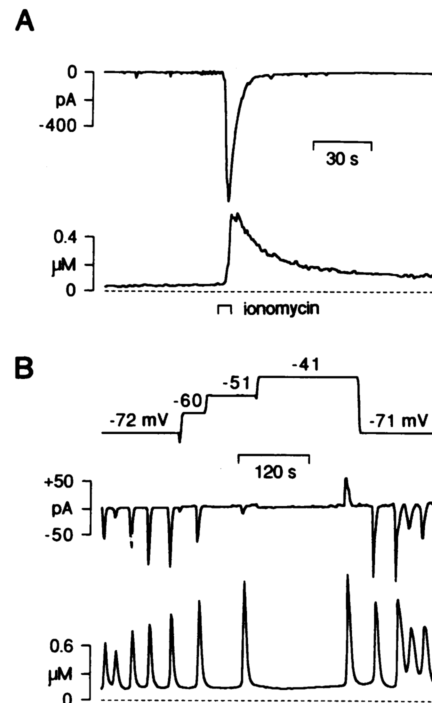

FIG. 4. Interactions of intracellular calcium and membrane voltage. (A) Effect of ionomycin (5 μg/ml) on $[Ca^{2+}]_i$ (lower trace) and the induction of Ca^{2+}-activated chloride current (upper trace). The membrane potential was clamped to −60 mV. In this particular cell, peak conductance was 57 nsec. Mean conductance values (± SEM) for Ca^{2+}-activated currents were 4.9 nsec ± 0.9 (at $[Ca^{2+}]_i$ = 0.2–0.5 μM, n = 9) and 8.3 nsec ± 1.6 (at $[Ca^{2+}]_i$ = 0.5 = 1 μM, n = 7). (B) Repetitive Ca^{2+} transients evoked by ANG-II and the resulting chloride currents at different potentials. Note that the current reverses from inward to outward between potentials of −51 to −41 mV (mean ± SEM = 2 mV, n = 13).

the addition of tetraethylammonium to the external and the internal solutions. However, outward currents (Cl^- influx) were nearly abolished when extracellular chloride was substituted by isethionate (data not shown), suggesting chloride ions as current carriers.

A current with the same characteristics was seen when oscillations of intracellular calcium were induced by ANG-II. Fig. 4B shows the Ca^{2+}-activated currents at different membrane potentials. From a number of similar experiments the reversal potential of these Ca^{2+}-activated currents was determined to be −43 mV ± 2 (mean ± SEM, n = 13). In addition, the frequency of Ca^{2+} transients induced by ANG-II was markedly decreased or even blocked during depolarization (Fig. 4B). Under voltage clamp it is evident that Ca^{2+} oscillations induced by ANG-II at negative membrane potential (close to resting) can be slowed by moderate depolarization and even blocked by stronger depolarization (close to the physiological depolarization induced by ANG-II). Upon hyperpolarization, the oscillatory fluctuations of $[Ca^{2+}]_i$ reappeared (n = 10), although other cells remained refractory (n = 6). Compatible with this finding is the fact that under current-clamp conditions, when ANG-II (1 μM) depolarized the membrane, only one or two Ca^{2+} transients were seen (n = 21).

DISCUSSION

Renin-containing vascular smooth muscle cells have been found to extend up to 100 μm upstream from the vascular pole of mouse glomeruli (7, 16). The probability of patch-clamping a renin-positive cell is inversely correlated to the distance from the vascular pole (7). In agreement with microelectrode studies (7), we obtained no indication for heterogeneities among cells with respect to their electrical properties and the regulation of intracellular calcium. Our findings could, therefore, be relevant for both control of secretion from renin-positive cells and regulation of contraction of renin-negative smooth muscle cells.

Our results provide patch-clamp data on the electrical properties of renin-secreting JG cells. We found that JG cells essentially contain two sets of voltage-activated potassium currents: an outward K^+ conductance that appears as a type of delayed rectifier and an inward rectifying K^+ current that has characteristics similar to anomalous rectifying K^+ currents known from other cells (9–11). The presence of these ionic currents explains the strong dependence of the membrane potential on extracellular K^+ concentration (8).

Clearly, the electrical properties of JG cells were influenced by receptor-mediated hormone actions. ANG-II depolarized the membrane by ≈30 mV, which is probably due to inhibition of the anomalous inward rectifier. Because neither of the "classical" second messengers could mimic block of the inward rectifier, we suggest that ANG-II recep-

tors may either be directly coupled to potassium channels via a G protein or affect the potassium channels by an unidentified type of second messenger. A similar inhibition of inward rectifying K^+ current has been reported in hippocampal horizontal cells by substance P (17).

We could not obtain indications for other voltage-gated currents. In particular, we detected no voltage-activated calcium currents, which are considered as important functional characteristics of JG cells (4). Hence, the ANG-II-induced depolarization is unlikely to trigger Ca^{2+} influx. A functional role for the membrane depolarization may consist of modulating the frequency of intracellular calcium oscillations. Although ANG-II induces oscillations of $[Ca^{2+}]_i$ at hyperpolarized membrane potentials, their frequency is reduced by depolarization. If depolarization of JG cells required higher doses of ANG-II than generation of Ca^{2+} transients, depolarization would appear as a possible regulator of Ca^{2+} oscillations; the underlying mechanism for the effect of membrane voltage on intracellular Ca^{2+} release is not known.

Increases in $[Ca^{2+}]_i$ were paralleled by activation of large chloride currents, and this relationship may offer an intriguing link between changes in $[Ca^{2+}]_i$ and renin secretion. Strong, but still indirect, evidence suggests that exocytosis of renin is inversely correlated to $[Ca^{2+}]_i$ (3, 4). From our findings, a rise in $[Ca^{2+}]_i$ will cause substantial chloride efflux, and this efflux may cause cell shrinkage from osmotic changes. Indeed, JG cells are known to behave as sensitive "osmometers," in which cell shrinkage is paralleled by inhibition of renin secretion (18, 19).

In addition to its effects on membrane currents, ANG-II was found to mobilize internal calcium. The Ca^{2+} transients probably result from inositol 1,4,5-trisphosphate-mediated release of Ca^{2+} from intracellular stores, because ANG-II stimulates inositolphospholipid turnover in JG cells (20). The inhibitory effect of phorbol esters on agonist-induced Ca^{2+} mobilization described here, has been recognized in a variety of cells (21–23) and is consistent with a negative feedback role of protein kinase C on inositolphospholipid hydrolysis. The sustained elevation of $[Ca^{2+}]_i$ after application of ANG-II appears to follow enhanced transmembrane Ca^{2+} influx, because it is not evident in the absence of extracellular calcium. Because the initial Ca^{2+} transient presumably results from the liberation of inositol 1,4,5-trisphosphate, this second messenger is a good candidate for also mediating the sustained phase of calcium entry, as has been documented for mast cells (24).

We found that $[Ca^{2+}]_i$ in JG cells is very sensitive towards changes in $[Ca^{2+}]_o$ values. An increase of $[Ca^{2+}]_o$ causes an intracellular calcium transient followed by calcium oscillations, which can be prevented by internal neomycin. Because neomycin can inhibit inositolphospholipid breakdown (25), inositol 1,4,5-trisphosphate formation is probably involved in the $[Ca^{2+}]_o$-induced Ca^{2+} transients. The observed relation between $[Ca^{2+}]_o$ and $[Ca^{2+}]_i$ could help to explain the dependence of renin secretion on $[Ca^{2+}]_o$. Intracellular calcium mobilization by extracellular calcium has also been seen in parathyroid cells (26) and, as in JG cells, secretion from parathyroid cells is also inversely related to $[Ca^{2+}]_o$ (27).

cAMP is a well-established stimulatory signal for renin secretion (3, 4), and it has been discussed that cAMP could exert its effect on JG cells by interfering with intracellular calcium activity (3, 28). Our results provide direct evidence that cAMP, in fact, blocks changes of $[Ca^{2+}]_i$, induced either by ANG-II or GTP[γ-S]. This agrees with previous results showing that cAMP, but not cGMP, impairs transmembrane ^{45}Ca-uptake in isolated renal JG cells (29, 30). Under the assumption that increased $[Ca^{2+}]_i$ is inhibitory for renin secretion, inhibition of calcium mobilization could explain the apparent stimulatory effect of cAMP on renin secretion.

Finally, we would like to consider some possible implications of our findings for the function of renin-negative vascular smooth muscle cells in the afferent arteriole. These cells are considered to govern glomerular filtration and to mediate the tubuloglomerular feedback by their state of contraction (31). If we assume that a rise of $[Ca^{2+}]_i$ leads to contraction of vascular smooth muscle cells, our findings would imply oscillatory contractions of vascular smooth muscle cells. Under our experimental conditions with internal GTP[γ-S] (100 μM) the frequency of the calcium oscillations was 1.8 ± 0.3 min^{-1} (mean $\pm$ SEM; $n = 12$). This frequency is very close to the physiological oscillations of the proximal tubular pressure (32). Moreover, our findings could help to explain the dependence of the tubuloglomerular feedback response on $[Ca^{2+}]_o$ and the inhibition of tubuloglomerular feedback response by maneuvers that raise intracellular cAMP levels.

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1. Barajas, L. (1979) *Am. J. Physiol.* **237,** F333–F343.
2. Keeton, T. K. & Campbell, W. B. (1981) *Pharmacol. Rev.* **31,** 81–227.
3. Kurtz, A. (1986) *Klin. Wochenschr.* **64,** 838–846.
4. Churchill, P. C. (1985) *Am. J. Physiol.* **249,** F175–F184.
5. Neher, E. (1988) *J. Physiol. (London)* **395,** 193–214.
6. Grynkiewicz, G., Poenie, M. & Tsien, R. Y. (1985) *J. Biol. Chem.* **260,** 3440–3450.
7. Bührle, C. P., Nobiling, R. & Taugner, R. (1985) *Am. J. Physiol.* **249,** F272–F281.
8. Fishman, M. C. (1976) *Nature (London)* **260,** 542–544.
9. Hagiwara, S., Miyazaki, S. & Rosenthal, N. P. (1976) *J. Gen. Physiol.* **67,** 621–638.
10. Sakmann, B. & Trube, G. (1984) *J. Physiol. (London)* **347,** 641–657.
11. Lindau, M. & Fernandez, J. M. (1986) *J. Gen. Physiol.* **88,** 349–368.
12. Bührle, C. P., Scholz, H., Hackenthal, E., Nobiling, R. & Taugner, R. (1986) *Mol. Cell. Endocrinol.* **45,** 37–47.
13. Uhing, R. J., Prpic, V., Jiang, H. & Exton, J. H. (1986) *J. Biol. Chem.* **261,** 2140–2146.
14. Kojima, I., Shibata, H. & Ogata, E. (1986) *FEBS Lett.* **204,** 347–351.
15. Hackenthal, E., Aktories, K. & Jakobs, K. H. (1975) *Mol. Cell. Endocrinol.* **42,** 113–117.
16. Taugner, R., Marin-Grez, M., Keilbach, R., Hackenthal, E. & Nobiling, R. (1982) *Histochemistry* **76,** 61–69.
17. Nakajima, Y., Nakajima, S. & Inoue, M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3643–3647.
18. Frederiksen, O., Leyssac, P. P. & Skinner, S. L. (1975) *J. Physiol. (London)* **252,** 669–679.
19. Skott, O. (1988) *Am. J. Physiol.* **255,** F1–F10.
20. Kurtz, A., Pfeilschifter, J., Hutter, A., Bührle, C. P., Nobiling, R., Taugner, R., Hackenthal, E. & Bauer, C. (1986) *Am. J. Physiol.* **250,** C563–C571.
21. Watson, S. P. & Lapetina, E. L. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2623–2626.
22. Orellana, S. A., Solski, P. A. & Brown, J. H. (1985) *J. Biol. Chem.* **260,** 5236–5239.
23. Penner, R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9856–9860.
24. Penner, R., Matthews, G. & Neher, E. (1988) *Nature (London)* **334,** 499–504.
25. Cockcroft, S., Howell, T. W. & Gomperts, B. D. (1987) *J. Cell. Biol.* **105,** 2745–2750.
26. Nemeth, E. F. & Scarpa, A. (1987) *J. Biol. Chem.* **262,** 5188–5196.
27. Haber, J. R., Rosenblatt, M. & Potts, J. T. (1984) *Physiol. Rev.* **64,** 985–1053.
28. Churchill, P. C. & Churchill, M. C. (1982) *Life Sci.* **30,** 1313–1319.
29. Kurtz, A., Pfeilschifter, J. & Bauer, C. (1984) *Biochem. Biophys. Res. Commun.* **124,** 359–366.
30. Kurtz, A., Della Bruna, R., Pfeilschifter, J., Taugner, R. & Bauer, C. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4769–4773.
31. Wright, F. S. & Briggs, J. P. (1979) *Physiol. Rev.* **59,** 958–1006.
32. Leyssac, P. P. (1986) *Acta Physiol. Scand.* **126,** 271–277.