Direct Demonstration of Exocytosis and Endocytosis in Single Mouse Juxtaglomerular Cells

Ulla G. Friis, Boye L. Jensen, Jeanette K. Aas, Ole Skøtt

Abstract—The rate of renin secretion from renal juxtaglomerular (JG) cells is the major determinant of the activity of the renin-angiotensin system. However, the mechanisms involved in the excretion and turnover of secretory granules in the JG cells remain obscure. Therefore, in the present study, the whole-cell patch-clamp technique was applied to single JG cells from the mouse kidney to measure changes in cell membrane capacitance ($C_m$) as an index of secretory activity. Resting JG cell $C_m$ was stable, on average $3.13 \pm 0.13$ pF (SEM, $n=106$). In isotonic solutions, $C_m$ was unaffected by [Cl$^-$], $C_m$ was consistently increased ($7.0 \pm 1.3\%$ and $7.2 \pm 3.1\%$) by intracellular cAMP (1 to 10 $\mu$mol/L). This effect was mimicked by extracellular application of the $\beta$-agonist isoproterenol to the JG cells ($9.4 \pm 3.1\%$). At 100 $\mu$mol/L, cAMP induced a paradoxical decrease in $C_m$ of $\approx 20\%$, which was mimicked by forskolin. Cell swelling induced by a 7% reduction in osmolality increased $C_m$ with no significant additional effects to [Cl$^-$], and cAMP, cAMP increased whole-cell outward current 2- to 4-fold in all groups, but no correlation between changes in whole-cell currents and $C_m$ existed. We conclude that the whole-cell patch-clamp method allows the study of exocytosis and endocytosis in JG cells. Renin release induced by the cAMP pathway and by cell swelling is exocytotic, and high-intracellular cAMP levels activate membrane retrieval mechanisms. (Circ Res. 1999;84:929-936.)

Key Words: juxtaglomerular apparatus ■ renin ■ electrophysiology ■ exocytosis ■ endocytosis ■ cAMP

Renin is an aspartyl-proteinase hormone that is produced, stored, and released by juxtaglomerular (JG) granular cells in the distal part of the renal afferent arterioles. The rate of renin release from the JG cells is the most important factor that determines the activity of the circulating renin-angiotensin system. Despite this, many of the cellular mechanisms involved in the excretion and turnover of secretory granules in the JG cells remain obscure. Morphological1 and functional data suggest that renin is released by exocytosis of storage granules. However, a clear demonstration of fusion between renin granules and the JG cell membrane after stimulation still has not been provided.

The patch-clamp technique makes it possible to monitor secretory activity in a single cell by measurement of the cell membrane capacitance ($C_m$) as an index of membrane surface area.4 The whole-cell patch-clamp technique has been used to show ion channels in JG cells in isolated afferent arterioles.5,6 but the tight coupling between the JG cells hampers the use of $C_m$ measurements as an estimate of exocytosis. Therefore, an aim of the present study was to develop an experimental model that allowed $C_m$ measurements on single JG cells and to use this model to study renin secretion at the level of the single cell.

Renin secretion is influenced by a number of intracellular messenger systems. cAMP is the common stimulatory second messenger for agonists with receptor coupling to adenyl cyclase (eg, $\beta$-adrenergic agonists, prostaglandin E$_2$, and prostacyclin), but exactly how cAMP affects the secretory pathway is unknown. Cytosolic calcium is an inhibitory second messenger for renin secretion, which increases in response to angiotensin II, endothelin, and $\alpha$-1 adrenoceptor agonists.7 An increase in the cytosolic concentration of calcium activates chloride and potassium channels.5 The ensuing chloride and potassium efflux may cause shrinkage of the cell and/or of secretory granules.3 Shrinkage inhibits renin secretion in vitro and swelling stimulates secretion.5,9 Therefore, intracellular calcium may influence renin secretion through variations in the volume of the JG cells or their secretory granules. The exact cellular mechanism responsible for this osmotic sensitivity is unknown, and a direct demonstration of exocytosis after swelling would contribute significantly to the physiological validity of this hypothesis. In addition to the effects on volume, the intracellular chloride concentration has been hypothesized to directly affect the exocytosis of renin granules.10 For these reasons, we focused on the roles of cAMP, cell volume, and the cytosolic concentration of chloride in the control of JG cell function as studied by the whole-cell patch-clamp technique.

The results show that the model is well suited for the study of granule trafficking in JG cells and that cAMP and cell swelling is associated with increases in $C_m$, which is consistent with the hypothesis that renin release occurs through exocytosis.
Materials and Methods

Isolation of Juxtaglomerular Cells

JG cells were isolated as described by Della Bruna et al. Kidneys from male C57Bl/6J mice (4 to 6 weeks old) were removed, decapsulated, minced, and transferred to 30 mL of isolation buffer supplemented with 0.1% (wt/vol) of collagenase (0.57 U/mg) and 0.25% (wt/vol) of trypsin (1300 BAEE U/mg). The tissue was incubated and stirred gently for 70 minutes at 37°C, then filtered through a 22 μm nylon mesh. The filtrate was washed, centrifuged, and resuspended in 4 mL of isolation buffer. Cells were further separated with a 30% Percoll density gradient, sedimented by centrifugation for 30 minutes at 27 000g (4°C). Four cell layers with different specific renin activities were obtained. The cellular layer (equivalent to a density of 1.049 g/mL) with the highest renin concentration (100-fold increase in specific renin activity) was used for the experiments. These cells were washed twice and resuspended in 3.5 mL of RPMI-1640 medium. Aliquots (100 μL) of this suspension were seeded in 96-multiwell plates for renin secretion studies or transferred (2 to 3 mL) to a 30-mm-diameter Petri dish with coverslips placed at the bottom for patch-clamp experiments. Cells for renin-secretion studies were incubated for 20 hours. Culture medium was then removed, and the cells were washed once with 100 μL of RPMI-1640 medium that contained 2% FCS. Then 100 μL of fresh prewarmed RPMI-1640 medium with agents to be tested was added. Cells for patch-clamp experiments were allowed to settle for at least 45 minutes in an incubation buffer at 37°C in a humidified atmosphere that contained 5% CO₂ in air. Viability of the cells was confirmed by the trypan blue exclusion test performed at different times after the cells were seeded.

Identification of Cells Used for Patch Clamping

Because the isolated cells were not 100% JG cells, the cells used for patch-clamp experiments were confirmed to be renin-containing JG cells by several approaches. First, the cells were selected by their appearance as large granular cells. After the G-seal and the whole-cell configuration were established in these cells, the current-seal and the background current were negligible. After the G-seal was achieved, the cells were superfused with fresh prewarmed RPMI-1640 medium with agents to be tested was added. Cells for patch-clamp experiments were allowed to settle for at least 45 minutes in an incubation buffer at 37°C in a humidified atmosphere that contained 5% CO₂ in air. Viability of the cells was confirmed by the trypan blue exclusion test performed at different times after the cells were seeded.

Patch-Clamp Experiments

One glass coverslip with JG cells was superfused with the experimental buffer, transferred to the recording chamber, and supplemented with buffer to a volume of ~250 μL. Experiments were performed at room temperature in the tight-seal whole-cell configuration of the patch-clamp technique with heat-polished, Sylgard (silicone elastomer)-coated patch pipettes with resistances of 3 to 7 MΩ. Series resistances were in the range of 6 to 15 MΩ, and seal resistances were in the range of 1 to 15 GΩ. High-resolution membrane currents were recorded with an EPC-9 patch-clamp amplifier (HEKA) controlled by E9SCREEN software on an Atari computer (MEGA/STE). High-resolution currents were low-pass filtered at 2.3 kHz and acquired at a sampling rate of 10 kHz, whereas a charting program on another computer synchronously recorded at low resolution (2 Hz) parameters such as Cm and series conductance (low-pass filtered at 500 Hz). The reference electrode was an Ag/AgCl pellet connected to the bath solution through a 150 mmol/L NaCl/agar bridge.

The I-V curve was monitored by the response to 11 voltage steps of 30 mV (range, −200 to +100 mV) for 60 milliseconds from a holding potential of −30 mV (the membrane potential of cells in the wall of pressurized afferent arterioles). The pulses were applied immediately after establishment of the whole-cell configuration. Cm measurements were started maximally 30 seconds after the whole-cell current recording. Cm was measured in all cells by continuous applications of short pulses of −10 mV for 2 milliseconds from a holding potential of −30 mV. Between each of these pulses, an automatic compensation of the slow capacitive current was performed, and the resulting computed values of Cm were recorded on the charting computer for 10 minutes for each cell.

All potentials were corrected for the liquid junction potential between the normal internal and external solution (≈10 mV). To minimize variation due to different cell sizes, whole-cell currents were normalized with the Cm for each individual cell. External solution changes were made by pressure injection from an application pipette (10 to 15 μm in diameter) positioned 10 to 20 μm from the cell.

Statistics

All values are given as mean±SEM. For example, if n = 5, these 5 experiments were performed on 5 JG cells from 5 different mice. ANOVA was used to calculate statistical significance among several groups of experiments. Paired Student’s t test with an appropriate Bonferroni reduction was used to calculate statistical difference from zero. P<0.05 was considered statistically significant.

Solutions

Internal Solutions

Low-chloride buffer was composed of the following (in mmol/L): K-glutamate 135, NaCl 10, KCl 10, MgCl₂ 1, HEPES-NaOH 10, Mg-ATP 0.5, and Na₂GTP 0.3; osmolality was 315 mosmol/kg and pH 7.02 (KOH, 24°C). The osmolality was measured by an osmometer (model 3D3 from Advanced Instruments Inc). Total chloride was 22 mmol/L. cAMP/low-chloride buffer was the same as low-chloride buffer except supplemented with 100 μmol/L cAMP; osmolality was 313 mosmol/kg and pH 6.99 (KOH, 24°C). High-chloride buffer consisted of the following (in mmol/L): K-glutamate 55, NaCl 10, KCl 90, MgCl₂ 1, HEPES-NaOH 10, Mg-ATP 0.5, and...
Na<sub>2</sub>-GTP 0.3; osmolality was 311 mOsm/kg and pH 7.07 (KOH, 24°C). Total chloride was 102 mmol/L. cAMP/high-chloride buffer was the same as high-chloride buffer but was supplemented with 100 μmol/L cAMP; osmolality was 307 mOsm/kg and pH 7.02 (KOH, 24°C). Various concentrations of cAMP (1, 10, and 50 μmol/L) were prepared by diluting the cAMP/high-chloride buffer with the high-chloride buffer.

**External Solutions**

Isolation buffer consisted of the following (in mmol/L): Tris-HCl 10, NaCl 130, KCl 5, CaCl<sub>2</sub> 2, glucose 10, sucrose 20; pH 7.4 (KOH, 37°C). Incubation buffer was composed of RPMI-1640 medium 10.41 g/L, NaHCO<sub>3</sub> 2.2 g/L, FCS (2%) 10 mL, insulin 0.66 U/mL, penicillin (10 000 U/mL), and streptomycin (10 mg/mL) 10 mL and was equilibrated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>; pH 7.2 (KOH, 37°C). Isotonic bath solution was 292 mOsm/kg; pH 7.21 (KOH, 25°C). Hypotonic bath solution was 311 mOsm/kg; pH 7.22 (KOH, 25°C). Hypotonic bath solution was the same as isotonic bath solution but without sucrose; osmolality was 292 mOsm/kg; pH 7.21 (KOH, 25°C). For the solutions for the application pipette, forskolin was dissolved in DMSO and diluted 1000-fold in the isotonic bath solution to a final concentration of 10 μmol/L. Isoproterenol was dissolved in water and diluted 1000-fold in the isotonic bath solution to a final concentration of 10 μmol/L.

Reagents were as follows: RPMI-1640 medium, HEPES, Tris-HCl, glucose, sucrose, FCS, insulin, penicillin, K-glutamate, Mg-ATP, forskolin, isoproterenol, prostaglandin E<sub>2</sub>, 3-isobutyl-1-methylxanthine, and trypsin were obtained from Sigma Chemical Co. Collagenase A, Na<sub>2</sub>-GTP, and cAMP were obtained from Boehringer Mannheim. Percoll was from Pharmacia Biotech. All other chemicals were of analytical grade.

**Results**

**Characterization of Isolated JG Cells**

A typical granular cell used for patch-clamping is shown in Figure 1A. Cell viability was assessed by the trypan blue exclusion test after 1 hour, 24 hours, and 48 hours in primary culture and found to be >99%. Under basal conditions, the JG cells released 0.9±0.08% of their total renin content per hour. The functional state of the cells was tested by incubation with cAMP-dependent agonists of renin secretion. Basal release during 20 hours of incubation amounted to 16.4±1.5% of total content, and renin release was significantly stimulated by forskolin (33.8±2.3%), isoproterenol (39.6±5.1%), prostaglandin E<sub>2</sub> (28.4±1.8%), and 3-isobutyl-1-methylxanthine (41.2±5.6%). Data were mean±SEM of 2 independent cell preparations with 4 wells assigned per condition in 1 experiment.

A single cell contained 0.5 μGU of active renin (the serial dilution was completely linear in the dilution range of 1:5 to 1:125), which is in the same order of magnitude as the renin content of rat JG cells. Conclusive evidence for cell identity was obtained by the demonstration of pre-prorenin mRNA by reverse transcription–PCR as shown in Figure 1B.

**Membrane Capacitance and Current in Single JG Cells**

In this study, the whole-cell recording mode was obtained in 118 isolated cells from a total of 39 preparations. Of these selected cells, 90% (106 cells) displayed a I-V curve that was similar to previously published data from JG cells in afferent arterioles: outward rectification at positive membrane potentials and, depending on the experimental conditions, inward rectification at potentials more negative than −100 mV. Original recordings of the whole-cell currents after applying 11 pulses from −200 mV to +100 mV in 30 mV steps for 60 milliseconds from a holding potential of −30 mV are shown in Figure 1C. The resulting I-V curve from 9 individual experiments is shown in Figure 1D. The outward current has previously been suggested to be the delayed rectifying potassium current, and in accord with this, we noted a 50% reduction of this current by 4-aminopyridine (not shown). The inward rectifier current has been identified as the anomalous inward rectifying potassium current that is also present in small resistance vessels. Figure 1D shows that the net inward current at −200 mV amounts to −117±45 μA/cm<sup>2</sup> (n=9). However, when the cells were dialyzed with a high-chloride buffer ([Cl<sup>−</sup>]<sub>i</sub>=102 mmol/L), the net inward current at negative potentials was almost completely abolished (amounting to −13±4 μA/cm<sup>2</sup> [n=7] at −200 mV; not shown).

The JG cells had an average C<sub>m</sub> value of 3.13±0.13 pF (mean±SEM, n=106), which is equivalent to a cell surface area of 313 μm<sup>2</sup>, a diameter of 10 μm, and a volume of 523 μm<sup>3</sup>, if cells are spherical and the specific capacitance is 1 μF/cm<sup>2</sup>. This estimated size is similar to the directly measured size of isolated mouse JG cells. Figure 1E shows 7 and 10 minute recordings of C<sub>m</sub> in single JG cells, in which the pipette contained control internal solution with a low- or high-chloride concentration. During the recording time (up to 12 minutes), basal C<sub>m</sub> did not change significantly either at [Cl<sup>−</sup>]<sub>i</sub>=22 mmol/L or at [Cl<sup>−</sup>]<sub>i</sub>=102 mmol/L (4.0±3% [n=5] and 1.7±2.8% [n=5], respectively; Figure 1F). Thus, it is possible to obtain prolonged measurements of C<sub>m</sub> in single JG cells, and [Cl<sup>−</sup>]<sub>i</sub>, per se does not alter C<sub>m</sub>.

**Effect of cAMP on Current and C<sub>m</sub> in JG Cells**

In cells dialyzed with cAMP, the outward current increased significantly. At a [Cl<sup>−</sup>]<sub>i</sub>=22 mmol/L, the outward current increased 2.5-fold by 100 μmol/L cAMP at 100 mV (Figure 2A, squares) compared with control (Figure 2A, circles; same as Figure 1D). Also at [Cl<sup>−</sup>]<sub>i</sub>=102 mmol/L, cAMP (100 μmol/L) increased the net outward current. At 100 mV, outward current increased 91% compared with control (not shown).

At cAMP concentrations of 1 and 10 μmol/L, a consistent increase was present in C<sub>m</sub> during the recording time, which was highly significant (7.0±1.3% [n=9] and 7.2±3.1% [n=3], respectively; Figure 2D). An original trace from a cell dialyzed with 1 μmol/L cAMP is shown in Figure 2B (upper trace). To test the specificity of this response, the β-receptor agonist isoproterenol (10 μmol/L) was added to single voltage-clamped JG cells by a second application pipette (Figure 2C, upper trace). Isoproterenol evoked a significant increase in C<sub>m</sub>, which quantitatively mimicked the response induced by internal cAMP (C<sub>m</sub> rose 9.4±3.1%, n=4; Figure 2D). As shown in Figure 2C, we typically observed a lag period of a few minutes after the addition of isoproterenol before C<sub>m</sub> began to increase. This response probably reflects the time required to generate...
Figure 1. Validation of the method. A, Single JG cell attached to the patch pipette. B, Localization of renin expression in cells used for patch clamp by reverse transcription–PCR. PCR determinations used 3 μL of cDNA equivalent to 1.4 JG cells. Lanes 1 and 2, renin (JG cell cDNA; 194 bp); lane 3, negative control without addition of cDNA; lane 4, renin (renal cortex cDNA); and lane 5, standard (λ-Pvu II). Lanes 6 to 9, identical to lanes 1 to 4 but with primers specific for β-actin (190 bp). Agarose gel (2%) was stained with ethidium bromide. C, Original recording of the whole-cell current from a cell dialyzed with a low-chloride buffer (22 mmol/L Cl\(^{−}\)) under isotonic conditions. The currents were measured as the response to 11 pulses from −200 mV to +100 mV in 30 mV steps for 60 milliseconds from a holding potential of −30 mV. D, Steady-state I-V relationship from 9 independent experiments under the same experimental conditions as described in panel C. The maximum outward current at 100 mV amounted to 105±32 μA/cm\(^2\) (n=9), and the maximum inward current at −200 mV amounted to −117±45 μA/cm\(^2\) (n=9). E, Typical time course of C\(_m\) in single mouse JG cells. These 2 cells were dialyzed with low-(22 mmol/L) chloride buffer (lower trace) or with high-(102 mmol/L) chloride buffer (upper trace). F, Relative changes of membrane capacitance, C\(_m\), increased 4±3% (n=5) when the cells were dialyzed with a low-(22 mmol/L) chloride buffer and 1.7±2.8% (n=5) when the cells were dialyzed with a high-(102 mmol/L) chloride buffer. N.S. indicates not significantly different from zero.
**Figure 2.** Effects of cAMP and [Cl−]o on whole-cell currents and Cm of isolated JG cells under isotonic conditions. JG cells were resuspended in an isotonic bath solution. The difference in osmolality between bath and pipette solutions was ±4 mOsm/kg. A, Steady-state I-V relationships. When the cells were dialyzed with cAMP/low-chloride buffer (100 μmol/L cAMP + 22 mmol/L Cl−), the maximal outward current was 265 ± 53 μA/cm² (n=8, squares). When the cells were dialyzed with low-chloride buffer (22 mmol/L Cl−) in the absence of cAMP, the maximal outward current at 100 mV amounted to 105 ± 32 μA/cm² (n=9, circles, same as in Figure 1D). B, Typical time course of Cm in single mouse JG cells. These 2 cells were dialyzed with a low concentration of cAMP (1 μmol/L; upper trace) or with a high concentration of cAMP (100 μmol/L; lower trace). C, Typical time course of Cm in single mouse JG cells. The cells were dialyzed with a high-chloride buffer (102 mmol/L) and stimulated with isoproterenol (10 μmol/L; upper trace) or forskolin (10 μmol/L; lower trace). The arrows indicate the time of extracellular application of secretagogues. The drugs were present throughout the remaining measurement period. D, Relative changes of Cm. CAMP (1 μmol/L) increased Cm by 7.0±1.3% (n=9); CAMP (10 μmol/L) increased Cm by 7.2±3.1% (n=3); cAMP (50 μmol/L) decreased Cm by 3.3±12% (n=4); CAMP (100 μmol/L) decreased Cm by 19.3±6.2% (n=5). Isoproterenol (10 μmol/L) increased Cm by 9.4±3.1% (n=4) and forskolin (10 μmol/L) decreased Cm by 13.1±6% (n=3). Asterisks: value significantly different from zero.

**Effect of Cell Swelling on Current and Cm in JG Cells**

The effect of cell swelling was tested in a separate series of experiments. JG cells were bathed in a buffer that was slightly hypotonic compared with the pipette solution (−19 to −23 mOsm/kg ≈ −6% to −7%). When larger osmotic perturbations were tested, the whole-cell configuration was often lost. Whole-cell currents were reduced in cells that were swollen compared with cells at isotonic conditions (Figure 3A, squares). Original traces of Cm from cells exposed to a slightly hypotonic buffer are shown in Figure 3B (left; lower trace). The arrows indicate the time of extracellular application of secretagogues. The drugs were present throughout the remaining measurement period. D, Relative changes of Cm. CAMP (1 μmol/L) increased Cm by 7.0±1.3% (n=9); CAMP (10 μmol/L) increased Cm by 7.2±3.1% (n=3); cAMP (50 μmol/L) decreased Cm by 3.3±12% (n=4); CAMP (100 μmol/L) decreased Cm by 19.3±6.2% (n=5). Isoproterenol (10 μmol/L) increased Cm by 9.4±3.1% (n=4) and forskolin (10 μmol/L) decreased Cm by 13.1±6% (n=3). Asterisks: value significantly different from zero.

**Effect of cAMP and Cell Swelling on Current and Cm in JG Cells**

To examine the additivity of cAMP and cell swelling on Cm and whole-cell currents, JG cells were swollen (−15 to −21 mOsm/kg ≈ −5 to −7%) during dialysis with a high cAMP concentration. CAMP (100 μmol/L) enhanced outward cur-
rent in swollen cells similarly to the effect under isotonic conditions (Figure 2C, right). Thus, cAMP or an
increased by 11.0 ± 2.6% (n = 5; 7.5% increase), after which the Cm remained constant for another couple of minutes before the seal was finally lost.

**Significance of the Osmolality**
The importance of the absolute level of osmolality versus the difference in osmolality for the change in Cm was tested by the addition of sucrose to both sides of the membrane (to 330 mOsm/kg in the cytosol and to 311 mOsm/kg on the outside) or by removal of sucrose from both sides of the membrane (307 mOsm/kg in the cytosol and 292 mOsm/kg on the outside). The increase in Cm in the 2 situations was identical (10.1 ± 2.6%, n = 5; 11.0 ± 2.7%, n = 7). These experiments were performed in the presence of cAMP and at [Cl−]i = 102 mmol/L. Hence, the JG cell responds to a relative change in osmolality rather than to the absolute level.

**Discussion**
In the present study, we have used the whole-cell patch-clamp technique to study exocytosis and endocytosis at the level of single JG cells. The identity of individual JG cells was confirmed by their I-V characteristics and by their content of pre-prorenin mRNA and active renin. With these cells, we obtained prolonged and reproducible Cm recordings with a stable baseline.

cAMP is a stimulatory second messenger for renin secretion in vivo. In accordance with this, we found stimulation of renin release from cultures of JG cells by agonists with receptor-dependent and -independent coupling to adenyl cyclase. When a single mouse JG cell was dialyzed with cAMP in the range 1 to 10 μmol/L, there was a significant and consistent increase in Cm. Stimulation of endogenous cAMP formation in single JG cells by the β-agonist isoproterenol mimicked the increase in Cm observed after cell dialysis with cAMP, which suggests that functional β-adrenergic membrane receptors are retained in this preparation. Altogether, the data indicate that cAMP initiates
fusion of secretory granules with the plasma membrane in JG granular cells and that cAMP-mediated renin release is exocytotic. At high concentrations of intracellular cAMP (100 μmol/L), we observed a paradoxical decrease of $C_m$. This response could be reproduced by external application of forskolin, a potent direct activator of adenyl cyclase. It can be calculated that 10 μmol/L of forskolin leads to an intracellular concentration of cAMP of $\approx 100$ μmol/L after 5 minutes, assuming 50% cell water (6 pmol cAMP/10^6 JG cells per minute; B.L.J., et al, unpublished data). These results indicate that membrane retrieval mechanisms are activated at high cytosolic concentrations of cAMP. The $C_m$ recording is not able to dissociate ongoing exocytosis from membrane retrieval but yields the integrated net $C_m$ response. Therefore, it is possible that membrane retrieval accompanies exocytosis even at low cAMP concentrations in which net increases in $C_m$ dominate. Conversely, at high cAMP levels the recorded membrane internalization probably obscures a simultaneous exocytosis. Regulated internalization or endocytosis of the JG cell membrane has not previously been reported, but observations at the morphological level are consistent with this idea. The absolute magnitude of membrane internalization after exposure to high intracellular concentrations of cAMP was very large (750 fF in a cell with a whole-cell capacitance of 3.09 pF $\approx 20\%$ of area) and resembles “excess” retrieval recently reported from other secretory cells. The cellular role of excess retrieval is unknown, and it has not previously been shown to be stimulated by cAMP. “Compensatory” retrieval, which primarily replenishes previously exocytosed cell membrane, was observed (rarely) but not systematically investigated in this study. We suggest that membrane recycling is necessary to allow a continuous release of renin during massive stimulation of the secretory process.

The volume status of the cell was found to be important in deciding the directionality of the $C_m$ changes. By itself, slight cell swelling (6% to 7% hypotonic) increased resting cell capacitance by about 10% to 11%, and this response was not altered significantly by intracellular chloride or by 100 μmol/L cAMP in the pipette solution. This increase in $C_m$ corresponds to release of 10 to 11 renin storage granules, because the fusion of a single renin granule is expected to increase $C_m$ by 35 to 40 fF (assuming a volume of mouse renin granules of 0.6 to 0.7 μm³). Given a cell radius of 5 μm, it can be calculated that each cell contains $\approx 200$ granules. Thus, $\approx 5\%$ of the stored granules are released by a decrease in osmolality of 6% to 7%. Because comparable in vitro preparations release about 5% of their total renin content in response to a similar hypotonic stimulus, the present results indicate that exocytosis can quantitatively account for the renin that is released after a moderate decrease in osmolality. Therefore, coupling between intracellular messenger systems and cell volume may constitute a relevant physiological mechanism in the stimulus-secretion coupling of renal JG granular cells.

A high cAMP concentration initiated net membrane retrieval under isotonic conditions, whereas no net membrane retrieval was observed with high cAMP in slightly swollen cells. At present, the mechanism of this inhibition of membrane retrieval by cell swelling in JG cells remains elusive, but it is interesting to note that cell swelling in other cells also has been reported to be associated with specific inhibition of endocytosis.

The cellular mechanism by which a decrease in osmolality stimulates renin release has been hypothesized to involve the swelling of secretory granules. However, in the present experiments, cell swelling was elicited by a pipette solution that was made hypertonic relative to the bath solution by sucrose. Because sucrose does not permeate the membranes of the secretory granules well, the observed exocytosis is unlikely to be induced by swelling of the secretory granules and instead may relate to mechanisms induced by the change in cell volume per se. A pathway by which cell volume regulates exocytosis in chromaffin cells has recently been elucidated. In response to swelling, chromaffin cells depolarize and activate voltage-dependent calcium channels, which allows calcium influx and calcium-mediated stimulation of secretion. For several reasons, this explanation does not pertain to our results: first, voltage-dependent calcium channels are absent in JG cells; second, the intracellular calcium concentration in JG cells does not increase after exposure to hypotonic medium; and third, renin release is inhibited rather than stimulated by intracellular calcium.

On the basis of results from permeabilized JG cells, it has been suggested that the intracellular chloride concentration plays an important role as a stimulator of renin release by promoting swelling of secretory granules. Although we observed a tendency toward enhancement of exocytosis and endocytosis with increasing chloride concentrations, this was not significant, and we must conclude that in voltage-clamped JG cells an increase in intracellular chloride concentration does not elicit net exocytosis.

Whole-cell currents were measured in all experimental groups, but there was no consistent correlation between changes in whole-cell currents and $C_m$. cAMP markedly stimulated the 4-aminopyridine–sensitive outward current in all groups. In keeping with this, the delayed rectifier potassium current, which is present in JG cells, has been reported to be sensitive to cAMP. In addition, the decrease in outward current observed after an increase in [Cl⁻], suggests the presence of a chloride conductance.

In summary, with the patch-clamp technique, we have demonstrated that changes compatible with exocytosis and endocytosis in single JG cells. cAMP and moderate cell swelling increased $C_m$ compatible with exocytotic release of renin. In addition, higher concentrations of cAMP activated a membrane retrieval response, which probably serves to replenish membrane material for granule trafficking.

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References


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