Mechanisms of Regulatory Volume Decrease in Collecting Duct Cells

Wei-Jie Fu, Michio Kuwahara*, and Fumiaki Marumo

Second Department of Internal Medicine, Tokyo Medical and Dental University, Tokyo, 113 Japan

Abstract The mechanisms of cell volume regulation upon osmotic cell swelling were examined in the inner stripe of the outer medullary collecting duct (OMCDi). Segments of OMCDi were dissected from rabbit kidney and perfused in vitro. Using an image processing system, the cross-sectional area of tubule cells was monitored as an index of the relative cell volume. In response to a decrease in extracellular osmolality (290 to 190 mOsm), the tubule cells swelled promptly and restored gradually their original cell volume by a mechanism termed regulatory volume decrease (RVD). The initial response of RVD (the rate of the decrease in cell volume during the first 10 min) was inhibited by 79% at a high concentration of basolateral K⁺ (50 mM). By contrast, the same concentration of luminal K⁺ did not affect the response. When basolateral Cl⁻ was removed 30 min before the experiment, the initial response of RVD was decreased by 77%, whereas the response was not affected 30 min after removal of luminal Cl⁻. Addition of basolateral Ba²⁺ and basolateral anthracene-9-CO₂H inhibited the response by 70 and 65%, respectively. RVD response was accompanied by a transient rise in intracellular Ca²⁺. The Ca²⁺ rise was abolished when intracellular Ca²⁺ was chelated by acetoxymethyl ester of 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA-AM). In this condition, the initial RVD response was decreased by 68%. Our data suggest that the exit of basolateral K⁺ and Cl⁻ via conductive pathways mainly mediates the RVD response in rabbit OMCDi cells, and that intracellular Ca²⁺ is involved in this response.

Key words: K⁺ conductance, Cl⁻ conductance, intracellular Ca²⁺.

The kidney plays an important role in the control of water balance and maintains extracellular fluid volume. Consequently, serum osmolality is kept within a narrow range (285–295 mOsm), whereas urine osmolality varies from 50–1,500

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* To whom correspondence should be addressed.
mOsm between diuretic and antidiuretic states. The osmolality of final urine is determined at the terminal site of the nephron, namely, the collecting duct. Therefore the collecting duct cells tend to be exposed to either a hyperosmotic or a hyposmotic environment. In water-transporting epithelia such as collecting duct cells, cell volume regulation may be especially important for maintenance of intracellular homeostasis.

We previously examined the mechanisms of cell volume recovery from hyperosmotic cell shrinkage in the inner stripe of the outer medullary collecting duct (OMCDi), and suggested that the stimulation of Na\(^+\)-H\(^+\) and Cl\(^-\)-HCO\(_3\)\(^-\) exchanges is predominantly responsible for the recovery [1]. In the present study, we investigated the mechanisms of volume recovery from hyposmotic cell swelling in OMCDi. In general, this recovery, termed regulatory volume decrease (RVD), is initiated by a loss of intracellular osmolytes, followed by a concomitant loss of intracellular water. K\(^+\) and Cl\(^-\) are major intracellular ion species, and the activation of K\(^+\) and Cl\(^-\) transport systems has been observed during the process of RVD in many cell types. K\(^+\) and Cl\(^-\) conductive pathways, K\(^+\)-Cl\(^-\) cotransport, and functionally coupled K\(^+\)-H\(^+\) exchange and Cl\(^-\)-HCO\(_3\)\(^-\) exchange have been described as the mechanisms of RVD (see Hoffman and Simonsen [2] for review). Similar transport processes are identified in renal tubule cells as well [3-12]. Thus, the present study focused on the transport of K\(^+\) and Cl\(^-\) during cell volume change after hyposmotic cell swelling. Furthermore, we investigated the role of intracellular Ca\(^{2+}\) in cell volume change.

**METHODS**

In vitro microperfusion. Renal tubules were perfused in vitro as described previously [1, 13]. Briefly, female Japanese white rabbits weighing 2.0–2.5 kg were anesthetized with pentobarbital. The kidney was removed and cut in coronal slices. Segments of OMCDi were dissected under \(\times 80\) magnification using fine forceps. OMCDi were mounted between holding pipettes and perfused at 37°C in a 150-μl thermoregulated bath. The bath solution was exchanged at \(\sim 10\) ml/min by gravity. At a 10-ml/min exchange rate, new fluid replaced old fluid by \(>90\%\) within 1 s. The bath solution was changed by rotating a four-way valve. The tubule lumen was perfused at \(>10\) nl/min. Luminal perfusate was changed in 20 s. The solutions used are shown in Table 1. HCO\(_3\)\(^-\)-containing and HCO\(_3\)\(^-\)-free solutions were bubbled with 5% CO\(_2\)-95% O\(_2\) and 100% O\(_2\) gases, respectively. The solution pH was adjusted to 7.40±0.05 and the solution osmolality was adjusted to 290±2 mOsm.

Measurement of cell volume. Tubules were viewed with an inverted epifluorescence microscope (Olympus IMT-2) using a \(\times 40\) objective and displayed with a television monitor. Tubule images were recorded at 2-min intervals using an image processor (ARUGUS 200, Hamamatsu Photonics). The focus was adjusted to the lateral-wall cells. Relative tubule cell volume was estimated by the cross-
### Table 1. Composition of solutions.

<table>
<thead>
<tr>
<th></th>
<th>1 Control</th>
<th>2 High K⁺</th>
<th>3 Cl⁻-free</th>
<th>4 Na⁺-free</th>
<th>5 HCO₃⁻-free</th>
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</thead>
<tbody>
<tr>
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<td>143</td>
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<tr>
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<tr>
<td>HCO₃⁻</td>
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<td>25</td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
<tr>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
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<td>SO₄²⁻</td>
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<td>1.2</td>
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<tr>
<td>HEPES-Tris</td>
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<td></td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Gluconate⁻</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>129</td>
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<tr>
<td>Glucose</td>
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<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Units are mM.

Sectional area as described [1, 14]. The outer margin of lateral-wall cells was traced and the number of pixels in this area was counted by a computer.

To observe the response of cell volume to hyposmolality, the solution osmolality was decreased from 290 to 190 mOsm by removing the major salts in the solutions. In all experiments, the luminal and basolateral solutions were replaced simultaneously with the corresponding solution of low osmolality to eliminate the effect of transepithelial water movement on the cell volume. Some experiments were performed in the presence of 50 mM K⁺. Extracellular K⁺ concentration was raised from 5 to 50 mM 5 min before lowering the solution osmolality. Experiments were also carried out in the nominal absence of Cl⁻, Na⁺, or HCO₃⁻ to test the effect of a decrease in intracellular concentration of these ions on the cell volume response. Na⁺ and HCO₃⁻ were removed from the ambient solutions 5 min before lowering the solution osmolality. Previous studies on the proximal tubule indicated that the intracellular Cl⁻ concentration reaches a steady-state in 2–5 min after Cl⁻ removal [15, 16]. However, such an observation has not been reported in the collecting tubule. We preincubated tubules for 30 min with Cl⁻-free solution before lowering the solution osmolality, since we thought that preincubation for 30 min would be enough to deplete intracellular Cl⁻ [11].

In some protocols, 2 mM Ba²⁺, a K⁺ conductance inhibitor, or 100 μM anthracene-9-CO₂H (Aldrich Chemical), a Cl⁻ conductance inhibitor [1, 17] was added to the basolateral solution 5 min before lowering the solution osmolality. SCH28080 (kindly provided by Schering Corp.), a H-K-ATPase inhibitor [12], was applied to the lumen at 10 μM 20 min before lowering the solution osmolality. To examine the effect of vasopressin on RVD response, tubules were exposed to 10⁻⁷ M vasopressin for 20 min before lowering the solution osmolality.
Measurement of cell Ca\textsuperscript{2+}. Intracellular Ca\textsuperscript{2+} of OMCDi cells was measured with Fura-2 using fluorescence ratio imaging as described [18]. Tubules were loaded with 10 \muM of acetoxymethyl derivative of Fura-2 (Fura-2-AM; Molecular Probe) for 20–30 min at 37°C. An emulsifier, pluronic F-127 (Molecular Probe), was added to the loading solution at 0.025% to increase cellular uptake of Fura-2-AM [19]. The dye was excited at wavelengths of 340 and 380 nm alternately, and the emission fluorescence was measured at 500 nm. Intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) was calculated using the equation [Ca\textsuperscript{2+}]=K_d (R-R_{\text{min}})/(R_{\text{max}}-R) \times (Sf_2/Sb_2). K_d is the dissociation constant of fura-2, assumed to be 224 nM [20]; R is the experimental ratio of fluorescence intensity at 340 and 380 nm (340/380); R_{\text{min}} and R_{\text{max}} are the ratios measured at 0 and 1.6 mM Ca\textsuperscript{2+}, respectively, both with ionomycin (5 \muM), Sf_2/Sb_2 is the ratio of fluorescence intensities at 380 nm in 0 and 1.6 mM Ca\textsuperscript{2+} solutions with ionomycin.

In some protocols, the effect of lowering extracellular and/or intracellular Ca\textsuperscript{2+} was tested. Tubules were pretreated for 60 min in Ca\textsuperscript{2+}-free solution containing 1 \muM acetoxymethyl ester of 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA-AM) and/or 0.1 mM ethylene glycol-bis(\beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA).

Statistics. The results are expressed as means±SE. Statistical significance was determined by analysis of variance (ANOVA). Values of p<0.05 were considered significant.

![Graph](image_url)

Fig. 1. Time course of cell volume recovery from osmotic cell swelling in OMCDi cells. Relative cell volume is expressed as the percent of the maximum value. The osmolality of luminal and basolateral solutions was decreased simultaneously from 290 to 190 mOsm 30 s before the zero time point. Each point indicates mean±SE of 14 tubules.

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RESULTS

Figure 1 shows the time course of relative cell volume upon hyposmolality in the control condition. Both luminal and basolateral osmolalities were decreased from 290 mOsm to 190 mOsm simultaneously 30 s before time zero. The tubule cells swelled promptly in response to hyposmolality. After cell swelling, cell volume gradually returned to the basal level, a phenomenon called RVD. The relative cell volume was measured at 2-min intervals and is represented as a percent of maximum cell volume. The cell volume was maximal (100%) at time zero (30 s after the osmolality change). The rate of the decrease in cell volume reduced gradually up to 40 min (Fig. 1, Table 2). However, the rate of decrease was relatively constant during the first 10 min. Since the same holds true in other protocols, we calculated the rate of cell volume decrease for the first 10 min as an index of the initial RVD response.

To examine the mechanisms of RVD, the time course of cell volume recovery

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>290 mOsm</th>
<th>190 mOsm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>71.8±1.9</td>
<td>100</td>
</tr>
<tr>
<td>Total high K⁺</td>
<td>11</td>
<td>72.9±2.0</td>
<td>100</td>
</tr>
<tr>
<td>Basolateral high K⁺</td>
<td>9</td>
<td>70.7±1.6</td>
<td>100</td>
</tr>
<tr>
<td>Luminal high K⁺</td>
<td>8</td>
<td>72.8±1.7</td>
<td>100</td>
</tr>
<tr>
<td>Total Cl⁻-free</td>
<td>12</td>
<td>74.1±1.5</td>
<td>100</td>
</tr>
<tr>
<td>Basolateral Cl⁻-free</td>
<td>10</td>
<td>73.3±1.8</td>
<td>100</td>
</tr>
<tr>
<td>Luminal Cl⁻-free</td>
<td>8</td>
<td>73.1±1.5</td>
<td>100</td>
</tr>
<tr>
<td>Total Na⁺-free</td>
<td>10</td>
<td>73.7±1.8</td>
<td>100</td>
</tr>
<tr>
<td>Total HCO₃⁻-free</td>
<td>10</td>
<td>71.1±2.2</td>
<td>100</td>
</tr>
<tr>
<td>Basolateral Ba²⁺</td>
<td>9</td>
<td>70.5±2.0</td>
<td>100</td>
</tr>
<tr>
<td>Basolateral anthracene-9-CO₂H</td>
<td>9</td>
<td>72.5±1.8</td>
<td>100</td>
</tr>
<tr>
<td>Luminal SCH28080</td>
<td>7</td>
<td>74.1±2.5</td>
<td>100</td>
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<tr>
<td>Basolateral vasopression</td>
<td>7</td>
<td>73.8±2.6</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are mean±SE. n: number of tubules. Relative cell volume was expressed as the percent of maximum volume. High K⁺: extracellular K⁺ was increased to 50 mM. Cl⁻-free, Na⁺-free, HCO₃⁻-free: Cl⁻, Na⁺, and HCO₃⁻ were replaced by gluconate⁻, NMDG⁺, and HEPES-Tris, respectively. Ba²⁺, anthracene-9-CO₂H, SCH28080, vasopression: Ba²⁺, anthracene-9-CO₂H, SCH28080, and vasopressin were added at 2 μM, 100 μM, 10 μM, and 10⁻⁷ M, respectively. See METHODS for details. *p<0.01 compared with controls.

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from hyposmotic swelling was monitored in the presence of 50 mM K⁺. When extracellular osmolality was lowered at a 50-mM extracellular K⁺, the initial response of RVD was inhibited by 93% compared with controls \( (p < 0.01, \text{Fig. 2, Table 2}) \). Next, we determined which side of K⁺ increase was involved in this inhibition. An increase in basolateral K⁺ inhibited the initial RVD response by 79% \( (p < 0.01) \), whereas an increase in luminal K⁺ did not affect the response \( (\text{Fig. 2, Table 2}) \). The time course of cell volume recovery from hyposmotic swelling was also monitored in the absence of Cl⁻. As described in METHODS, tubules were preincubated for 30 min in the absence of ambient Cl⁻ to decrease intracellular Cl⁻. An exposure to Cl⁻-free solution for 30 min in advance resulted in a 70% inhibition of the initial RVD response \( (p < 0.01, \text{Fig. 3, Table 2}) \). The initial RVD response was decreased by 77% after a removal of basolateral Cl⁻ for 30 min \( (p < 0.01) \), but was not inhibited after removal of luminal Cl⁻ for 30 min \( (\text{Fig. 3, Table 2}) \). In addition, the response was not affected in the total absence of ambient Na⁺ or HCO₃⁻ \( (\text{Table 2}) \). These results suggested that the exit of K⁺ and Cl⁻ across the basolateral membrane is mainly responsible for the initial response of RVD.

To characterize the mechanisms of K⁺ and Cl⁻ exit, the effects on RVD response of a K⁺ conductance inhibitor, Ba²⁺, and a Cl⁻ conductance inhibitor, anthracene-9-CO₂H, were examined \( (\text{Fig. 4, Table 2}) \). The addition of 2 mM
basolateral Ba\(^{2+}\) resulted in a 70% inhibition of the initial RVD response (\(p < 0.01\)). Basolateral 100 \(\mu\)M anthracene-9-CO\(_2\)H also significantly decreased the response by 65% (\(p < 0.01\)). After the tubule lumen was perfused for 20 min with a solution containing 10 \(\mu\)M SCH28080, an H-K-ATPase inhibitor, the osmolality was lowered. The initial response of RVD did not change (Table 2). These results suggested that the RVD response was mostly mediated by basolateral K\(^{+}\) and Cl\(^{-}\) exit via conductive pathways.

Since we previously observed a stimulation of the RVI response by vasopressin in OMCDi cells, the effect of vasopressin on RVD was tested. The initial RVD response was not affected by basolateral \(10^{-7}\) M vasopressin (Table 2).

Next, [Ca\(^{2+}\)]\(_i\) was monitored at 30-s intervals during the RVD response in the control condition (Fig. 5). In response to hyposmolality, a transient [Ca\(^{2+}\)]\(_i\) increase was observed at time zero (193±24 to 456±43 nM). [Ca\(^{2+}\)]\(_i\) reached a maximum level at 30 s, and then declined to the basal value in 3 min. We further examined whether the hyposmolality-induced [Ca\(^{2+}\)]\(_i\) spike is due to Ca\(^{2+}\) influx across the cell membrane or to Ca\(^{2+}\) released from intracellular stores. [Ca\(^{2+}\)]\(_i\) monitor was repeated in the nominal absence of extracellular Ca\(^{2+}\). Tubules were preincubated for 60 min in Ca\(^{2+}\)-free medium containing 0.1 mM EGTA. Basal [Ca\(^{2+}\)]\(_i\) was reduced to 76±17 nM (Fig. 5). Even in the presence of EGTA, [Ca\(^{2+}\)]\(_i\) rise was observed in response to hyposmolality. However, peak [Ca\(^{2+}\)]\(_i\) was
Fig. 4. Effects of ion conductance inhibitors on RVD response. Two millimolar Ba$^{2+}$ (□), a K$^{+}$ conductance inhibitor, or 100 μM anthracene-9-CO$_2$H (△), a Cl$^{-}$ conductance inhibitor, was added to basolateral solution 5 min before lowering the solution osmolality. Controls (in the absence of conductance inhibitors, ●) are also presented for comparison. Each point represents mean ± SE of 9 tubules. *$p<0.01$ by ANOVA compared with controls.

observed at 1.5 min, and its level was decreased by 52% compared with the control (1 mM extracellular Ca$^{2+}$). When both extracellular and intracellular Ca$^{2+}$ were chelated by EGTA and BAPTA-AM in the absence of ambient Ca$^{2+}$, [Ca$^{2+}$], rise was completely inhibited (Fig. 5). Chelation of extracellular Ca$^{2+}$ did not affect the initial response of RVD (Fig. 6). By contrast, chelation of both extracellular and intracellular Ca$^{2+}$ significantly decreased the initial RVD response by 68% (Fig. 6). These results suggested that the [Ca$^{2+}$], rise is associated with RVD response, and that intracellular Ca$^{2+}$ stores are, at least in part, the source of Ca$^{2+}$ for the [Ca$^{2+}$], rise.

DISCUSSION

We observed time courses of cell volume change after hypotonic shock in rabbit OMCDi cells. The cell swelling was followed by a gradual volume recovery, indicating the presence of RVD. The initial response of RVD was, 1) mostly inhibited by a 10-fold increase in basolateral K$^+$ concentration (−79%), 2) mostly inhibited by preincubation in basolateral Cl$^-$-free condition (−77%), 3) not inhibited by a 10-fold increase in luminal K$^+$, 4) not inhibited by preincubation in luminal Cl$^-$-free condition, and 5) not inhibited in the total absence of Na$^+$ or
Fig. 5. Time course of \([\text{Ca}^{2+}]_i\) during RVD response. \([\text{Ca}^{2+}]_i\) was observed in the control conditions (1 mM extracellular \(\text{Ca}^{2+}\), ○), after 60 min pretreatment in \(\text{Ca}^{2+}\)-free solution containing 0.1 mM ethylene glycol-bis(\(\beta\)-aminoethyl ether)-\(\text{N},\text{N},\text{N}',\text{N}'\)-tetraacetic acid (EGTA, △), and after 60 min pretreatment in \(\text{Ca}^{2+}\)-free solution containing 0.1 mM EGTA plus 1 \(\mu\text{M}\) acetoxymethyl ester of 1,2-bis-(2-aminophenoxy)ethane-\(\text{N},\text{N},\text{N}',\text{N}'\)-tetraacetic acid (BAPTA-AM, □). Each point represents mean ± SE of 7–8 tubules. All values in the EGTA group and the EGTA plus BAPTA-AM group are significantly (\(p < 0.05\) or \(p < 0.01\)) different from those of controls by ANOVA, except for the values at 1.5 and 2 min in the EGTA group.

\(\text{HCO}_3^-\). These results suggested that the greater part of the initial RVD response is due to \(\text{K}^+\) and \(\text{Cl}^-\) exit across the basolateral membrane.

In the basolateral membrane of renal tubules, \(\text{K}^+\) conductance [3–6], \(\text{Cl}^-\) conductance [3–6], \(\text{K}^+\)-\(\text{Cl}^-\) cotransport [7, 8], and \(\text{Cl}^-\)-\(\text{HCO}_3^-\) exchange [9–11] were identified. In addition, luminal \(\text{H}-\text{K}-\text{ATPase},\) which mediates the exchange of \(\text{K}^+\) for \(\text{H}^+\) at the luminal membrane, was found recently [12]. Thus, we performed additional protocols to clarify the mechanisms of \(\text{K}^+\) and \(\text{Cl}^-\) exit. The initial response of RVD was mostly inhibited by application of basolateral Ba\(^{2+}\) (–70%) or by application of basolateral anthracene-9-CO\(_2\)H (–65%). A previous report showed that functional activity of luminal \(\text{H}-\text{K}-\text{ATPase}\) was mostly inhibited in the presence of 10 \(\mu\text{M}\) luminal SCH28080 in the rabbit collecting duct [12]. However,
the same dose of SCH28080 had no effect on the initial RVD response. Taken together, we conclude that cell osmolyte efflux occurs mainly via basolateral K\(^+\) and Cl\(^-\) conductive pathways during the initial response of RVD. Basolateral K\(^+\) increase or basolateral Cl\(^-\) removal failed to result in a complete inhibition of RVD, suggesting the possibility that processes other than basolateral K\(^+\) and Cl\(^-\) conductances mediate the RVD response. We did not further characterize the nature of these processes in this study because their significance would be minor.

In agreement with our conclusion, electrophysiological studies demonstrated the presence of basolateral Cl\(^-\) conductance in OMCDi cells [5, 6]. Basolateral K\(^+\) conductance of OMCDi was shown to be relatively small but significant under an isosmotic condition [5, 6]. It has been reported that volume-swelling-induced K\(^+\) channel, so-called stretch-activated K\(^+\) channel, is distributed in various cell types, including skeletal muscle cells [21], lens epithelia [22], choroid plexus epithelia [23], proximal tubules [24, 25], and thick ascending limb [26]. In addition, the presence of Ca\(^{2+}\)-activated K\(^+\) channel has been demonstrated in Ehrlich ascites tumor cells [27], choroid plexus epithelia [23], intestine epithelial cells [28, 29], and cortical collecting tubules [30]. Both of these K\(^+\) channels are known to play an important role in RVD response. A stretch-activated K\(^+\) channel and/or Ca\(^{2+}\)-activated K\(^+\) channel may also exist in basolateral OMCDi, contributing to the RVD response, although direct evidence of their presence is lacking. Contribu-
tions of activated K⁺ and Cl⁻ conductances to RVD response have been demonstrated in several cell types [3, 4, 27–29, 31].

RVD response was accompanied by a transient rise in [Ca²⁺], a finding consistent with previous observations [27–29, 32–37]. When intracellular Ca²⁺ was chelated, the [Ca²⁺] spike was abolished and the initial RVD response was inhibited by 68%, suggesting that an increase in [Ca²⁺] is necessary to activate RVD response. After removal of extracellular Ca²⁺ in the presence of EGTA, a peak [Ca²⁺] level was greatly attenuated (−52%), whereas the initial RVD response was not affected. This finding suggests that a small [Ca²⁺] rise (220 nM) is sufficient to activate the initial RVD processes. In regard to Ca²⁺-activated ion channels mediating RVD, the source of Ca²⁺ for the [Ca²⁺] rise is different among cell types. RVD is highly dependent on Ca²⁺ influx from extracellular space in gallbladder epithelial cell [32], red blood cell [33], toad bladder cell [34], intestine epithelial cells [28, 29], and proximal straight tubule [35]. By contrast, RVD is maintained or supplemented by Ca²⁺ release from internal stores in Ehrlich ascites tumor cell [27], medullary thick ascending limb [36], and inner medullary collecting duct [37]. We observed that the RVI response of OMCDi cells was not affected by removal and chelation of extracellular Ca²⁺, and that chelation of intracellular Ca²⁺ significantly inhibited the response. These results suggested that OMCDi cells belong to the latter group.

We previously reported a stimulation of the RVI response by vasopressin in OMCDi cells [1]. In contrast to RVI, we observed that vasopressin was unable to enhance the RVD response. Vasopressin is secreted when renal water reabsorption is necessary, namely, when plasma osmolality is elevated. In such an antidiuretic state, tubule cells shrink and an RVI response takes place. On the other hand, the RVD response is required when tubule cells are exposed to hypotonic fluid under a condition of water excess. In such a diuretic state, vasopressin secretion would cease. Therefore, it may be reasonable from a physiological viewpoint that vasopressin stimulates the RVI response but not that of RVD.

In summary, we have suggested that the response of RVD is mainly mediated by the stimulation of basolateral K⁺ and Cl⁻ efflux via conductive pathways, and that an increase in [Ca²⁺], plays a role in the stimulation of effluaxes of these ions.

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