Alteration of Volume-Regulated Chloride Movement in Rat Cerebrovascular Smooth Muscle Cells During Hypertension

Xiao-Lian Shi, Guan-Lei Wang, Zheng Zhang, Yu-Jie Liu, Jing-Hui Chen, Jia-Guo Zhou, Qin-Ying Qiu, Yong-Yuan Guan

Abstract—The cerebrovascular remodeling is a prominent feature of hypertension and considered a major risk factor for stroke. Cerebrovascular smooth muscle cells meet volume challenge during this pathophysiological process. Our previous studies suggest that volume regulated chloride channels may be critical to the cell cycle of vascular smooth muscle cells. However, it is unknown whether the volume-regulated chloride movement is altered in hypertension. Therefore, we directly measured the concentration of intracellular chloride ([Cl⁻]) in rat basilar arterial smooth muscle cells isolated from control rats and rats that were made hypertensive for 1 to 12 weeks after partial renal artery constriction (2-kidney, 2-clip method) using a 6-methoxy-N-ethylquinolinium iodide fluorescence probe. The [Cl⁻], in isotonic solution showed no difference in all of the groups. After hypotonic perfusion, the reduction in [Cl⁻] was more prominent in hypertensive cerebrovascular smooth muscle cells than in sham control cells. Genistein, a protein tyrosine kinase inhibitor, inhibited hypotonic-induced reduction in [Cl⁻], whereas sodium orthovanadate, a protein–tyrosine phosphatase inhibitor, enhanced hypotonic-induced reduction in [Cl⁻], in both groups. The percentage inhibition of reduction in [Cl⁻] by genistein on volume-regulated chloride movement has a positive correlation with blood pressure levels in the 2-kidney, 2-clip hypertensive group, as is the case for the percentage increase of reduction in [Cl⁻], by sodium orthovanadate. Antihypertensive therapy with the angiotensin-converting enzyme inhibitor captopril completely reversed abnormal volume-regulated chloride movement in hypertensive rats. We conclude that volume-regulated chloride movement is augmented in rat cerebrovascular smooth muscle cells in proportion to the severity of hypertension. (Hypertension. 2007;49:1371-1377.)

Key Words: hypertension (kidney) ▪ hypertrophy/remodeling ▪ chloride channels ▪ cerebral artery ▪ vascular smooth muscle

In cerebral arteries, hypertension induces vascular remodeling, which was defined as an increase in the cross-sectional area of the vessel wall (hypertrophy of the vessel wall) and a reduction in the external diameter of cerebral arteries. These structural changes cause the impairment of cerebral vasodilation and make stroke outcome worse. In response to chronic increase in blood pressure (BP) and vascular hypertrophy, adaptive remodeling of cerebral smooth muscle cells is closely associated with perturbations of ionic movements across the cell membrane. The upregulation of L-type Ca²⁺ channels has been demonstrated in cerebral vessels from several hypertensive animal models. The increase in high-conductance, Ca²⁺-gated K⁺ channels and inward rectifier channels and the reduction in voltage-gated potassium channels and ATP-sensitive K⁺ channels have been reported to contribute to changes in cerebral vasodilation during hypertension. However, it is unclear whether there is a change in anion channels in cerebrovascular smooth muscle (CVSM) cells during hypertension.

The 3D morphometric study has revealed that an increase in cell volume of vascular smooth muscle cells (VSMCs) is the primary change responsible for the hypertrophy of mesenteric arterial media in spontaneously hypertensive rats. However, it is not known whether CVSM cells undergo a similar remodeling process in the 2-kidney, 2-clip (2k2c) renal hypertension model. Recent growing evidence suggests that volume-regulated Cl⁻ channels (I_{Cl,vol}) play an important role in the control of cell volume, proliferation, apoptosis, and membrane potential, especially in VSMCs. Although the molecular nature of I_{Cl,vol} is still under verification, CIC-3, a member of the voltage-gated CIC Cl⁻ channel family, is currently regarded as the most potential molecular component involved in the activation or regulation of I_{Cl,vol} in VSMCs. The evidence supporting the idea includes the following. First, CIC-3 antisense inhibited the functional expression of CIC-3 and endothelin-1–induced proliferation in cultured rat aortic VSMCs in the same time-dependent manner. Second, patch...
clamp experiments combined with gene-targeting studies have found that the ClC-3 Cl\(^{-}\) channel is responsible for the volume regulation of endogenous I\(_{\text{Cl,vol}}\) and the concentration of intracellular chloride ([Cl\(^{-}\)]) in A10 aortic VSMCs.\(^{14}\) We, therefore, hypothesized that volume-regulated Cl\(^{-}\) movement though I\(_{\text{Cl,vol}}\) in CVSM cells might be altered during chronic hypertension.

A major goal of this study was to determine whether the volume-regulated Cl\(^{-}\) movement is altered during hypertension by directly measuring [Cl\(^{-}\)], in rat CVSM cells. In addition, protein tyrosine kinase (PTK) has been reported to mediate volume regulation of I\(_{\text{Cl,vol}}\) in VSMCs.\(^{14}\) It is noteworthy that increased PTK activity was involved in the enhanced contraction of vascular smooth muscle in the development of hypertension.\(^{15}\) Therefore, we further tested whether the volume regulation of [Cl\(^{-}\)], associated with PTK activity would change during hypertension.

**Methods**

For details regarding animal and cell experiments, please see the online supplements available at http://hyper.ahajournals.org.

**Animal Models**

All of the experimental procedures were approved by the Sun Yat-Sen University Committee for Animal Research and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. 2k2c hypertensive rats were prepared as described previously.\(^{16}\)

**Tissue Preparation and Immunofluorescence Analysis**

The brain sections containing 3 mm of basilar artery were removed and fixed in 4% paraformaldehyde. The CVSM cells were visualized with monoclonal \(\alpha\)-smooth muscle actin by an immunostaining method.\(^{17}\) The cross-sectional area of basilar arterial media, the wall diameter, the lumen diameter, and the wall: lumen ratio (the medial thickness to the ID) were assessed after immunostaining.\(^{1}\)

**Cell Isolation**

Rat basilar arterial smooth muscle cells were isolated by enzymatic digestion as described previously.\(^{18}\)

**Measurement of Cell Membrane Capacitance**

The voltage-clamp experiment was performed as described previously.\(^{14}\) The cell membrane capacitance was calculated by integrating the area under an uncompensated capacitive transient elicited by a 5-mV hyperpolarizing pulse from a holding potential of 0 mV.\(^{19}\)

**Measurement of [Cl\(^{-}\)]\text{\textsubscript{i}}**

[Cl\(^{-}\)]\text{\textsubscript{i}} was measured using 6-methoxy-N-ethylquinolinium iodide as described.\(^{14}\)

**Statistical Analysis**

All of the values are expressed as mean±SEM. Comparisons between 2 groups were analyzed using Student’s t test and among 3 groups by ANOVA followed by a posthoc comparison using the least significant difference test (SPSS 11.0). Values of P<0.05 were considered statistically significant.

**Results**

**Development of Hypertension and Morphological Change in Basilar Arterial Smooth Muscle Media in 2k2c Models**

BP in the 2k2c group rose progressively after operation as described previously.\(^{16}\) At 4, 8, and 12 weeks postoperatively, the mean values of BP in the 2k2c group were significantly higher than those in sham-operated groups. Development of hypertension in the 2k2c group could be prevented by chronic captopril treatment (see details in data supplement).

The cell size of CVSM cells, as assessed by cell membrane capacitance, was significantly increased in hypertensive groups at all of the time points, whereas there was no significant difference between sham controls. Moreover, the cell membrane capacitance of hypertensive CVSM cells tended to be larger as time after surgery increased (\(P<0.01\); Table S1). Immunostaining demonstrated a time-dependent increase in \(\alpha\)-smooth muscle actin staining in basilar arteries from the 2k2c group as BP increased. At 4 weeks postoperatively, there was no significant difference in the structure parameters among all of the groups. However, at the end of weeks 8 and 12, the mean values of cross-sectional area, wall diameter, lumen diameter, and wall:lumen ratio in the hypertensive groups were significantly higher than those in the sham-operated group, which could be reversed by captopril treatment (Table S2).

**Comparison of [Cl\(^{-}\)]\text{\textsubscript{i}}, in Rat CVSM Cells**

From the Stern–Volmer equation, the \(K_{o}\) was 37.1 mmol/L and the resting [Cl\(^{-}\)]\text{\textsubscript{i}}, of rat CVSM cells in isotonic solution was 29.0 ± 1.0 mmol/L (\(n=60\) cells from 12 rats). The result shown in Figure 1 summarizes [Cl\(^{-}\)]\text{\textsubscript{i}}, measured from different groups at different time points after operation; there were no significant differences in [Cl\(^{-}\)]\text{\textsubscript{i}}, in isotonic solution among all of the groups (\(P>0.05\)). Change of the medium from isotonic solution to hypotonic solution decreased [Cl\(^{-}\)]\text{\textsubscript{i}}, in CVSM cells, which is consistent with our previous report in A10 VSMCs.\(^{14}\) The mean values of [Cl\(^{-}\)]\text{\textsubscript{i}}, in isotonic solution in CVSM cells from 1, 4, 8, and 12 weeks sham-operated rats were 28.7±1.0, 29.1±1.2, 29.2±1.1, and 29.2±0.9 mmol/L, respectively, which were decreased to 24.4±1.0, 24.6±1.0, 24.5±1.1, and 24.7±1.0 mmol/L; when cells were perfused with hypotonic solution (\(n=30\) cells from 8 rats). Hypotonic perfusion results in 15.1±2.4%, 15.5±3.0%, 16.1±2.3%, and 15.4±2.0% decrease in [Cl\(^{-}\)]\text{\textsubscript{i}}; as compared with their isotonic controls; there is no significant difference between sham-operated groups at different time points.

In the hypertension group, hypotonic solution induced more of a decrease in [Cl\(^{-}\)]\text{\textsubscript{i}}, than those in age-matched sham-operated groups. The mean values of [Cl\(^{-}\)]\text{\textsubscript{i}}, from the 1-, 4-, 8-, and 12-week hypertensive group were 28.5±0.9, 28.6±1.0, 28.3±0.9 and 28.6±1.0 mmol/L, under isotonic solution, respectively, which were decreased to 22.7±1.7, 20.7±1.0, 19.6±0.9, and 18.9±0.8 mmol/L, after exposure to hypotonic solution, respectively, resulting in 20.4±2.8%, 27.6±4.1%, 30.9±3.7%, and 33.7±3.8% reduction in [Cl\(^{-}\)]\text{\textsubscript{i}}, respectively. The percentage decrease of [Cl\(^{-}\)]\text{\textsubscript{i}}, induced by hypotonic solution in CVSM cells from different hypertension groups showed a greater degree of reduction (range: 20% to 35%) of [Cl\(^{-}\)]\text{\textsubscript{i}}, as compared with the degree of reduction (≈15%) observed in age-matched sham controls. The hypotonic-induced reduction in [Cl\(^{-}\)]\text{\textsubscript{i}}, in
hypertensive CVSM cells tended to be greater as time after surgery increased.

We also examined whether the administration of captopril would reverse hypertension-induced change in volume-regulated \([\text{Cl}^-]/\text{H}^+\) in CVSM cells. As shown in Figure 1A through 1D, no significant differences were observed in the mean values of \([\text{Cl}^-]/\text{H}^+\) in CVSM cells between captopril-treated hypertensive rats and age-matched sham controls. Under isotonic condition, the mean values of \([\text{Cl}^-]/\text{H}^+\) in CVSM cells from the 1-, 4-, 8-, and 12-week captopril group were 28.8±1.0, 28.9±1.3, 29.7±1.3, and 29.4±1.3 mmol/L, respectively. In the presence of captopril, subsequent exposure of CVSM cells to hypotonic solution caused a decrease in \([\text{Cl}^-]/\text{H}^+\) with the mean values of 24.5±0.8, 24.1±1.2, 24.6±1.1, and 24.7±1.1 mmol/L, respectively. In the presence of captopril, subsequent exposure of CVSM cells to hypotonic solution caused a decrease in \([\text{Cl}^-]/\text{H}^+\), with the mean values of 24.5±0.8, 24.1±1.2, 24.6±1.1, and 24.7±1.1 mmol/L, respectively (n=30 from 8 rats), and the percentage decrease in \([\text{Cl}^-]/\text{H}^+\) induced by hypotonic solution was 15.9%±2.4%, 16.3%±3.0%, 16.9%±2.0%, and 15.7%±2.2%, respectively. Captopril treatment prevented the enhancement of volume-regulated Cl⁻ movement during hypertension. The reduction in \([\text{Cl}^-]/\text{H}^+\) induced by hypotonic solution had a significant positive correlation with BP levels in the hypertensive group (n=30 cells from 8 rats; \(r=0.8501; P<0.001\); Figure 1E).

**Effects of Genistein and Vanadate on \([\text{Cl}^-]/\text{H}^+\), in Rat CVSM Cells**

Table 1 summarizes effect of genistein (30 \(\mu\)mol/L; a PTK inhibitor) on osmotic-regulated \([\text{Cl}^-]/\text{H}^+\), in CVSM cells from sham and hypertensive rats. When CVSM cells were exposed to hypotonic solution containing genistein, inhibition of PTK caused \([\text{Cl}^-]/\text{H}^+\) to rise consistent with our previous reports in aortic VSMCs. The pooled data from all of the groups were summarized in Figure 2A. The percentage inhibition of reduction in \([\text{Cl}^-]/\text{H}^+\), by genistein (for calculation, see online supplement) in CVSM cells from sham-operated rats was \(\approx 50\%\), showing no significant difference between different time points. However, in the hypertensive group, the percentage inhibition of reduction in \([\text{Cl}^-]/\text{H}^+\), by genistein was higher at every sampling point compared with those of sham controls. The mean values of percentage inhibition in CVSM cells from 1-, 4-, 8-, and 12-week hypertensive rats were 55.8%±8.7%, 72.4%±5.2%, 80.1%±4.5%, and 83.9%±4.5% (n=30 from 8 rats), respectively, which in-
creased gradually as time after operation increased. Captopril treatment completely restored the mean values of percentage inhibition of reduction in \([\text{Cl}^-]\) to the level (~50%) similar to those of sham controls. As shown in Figure 2B, there is a significant positive correlation between the percentage inhibition of reduction in \([\text{Cl}^-]\) by genistein and BP levels in the hypertensive group (n=30 cells from 8 rats; \(r=0.8653; P<0.001\)).

To further test whether PTK is indeed involved in the volume regulation of \([\text{Cl}^-]\), in CVSM cells, we examined the effect of protein–tyrosine phosphatase inhibitor sodium orthovanadate (500 \(\mu\)mol/L) on osmotic regulation of \([\text{Cl}^-]\). As shown in Table 2, exposure of the cells to sodium orthovanadate in hypotonic solution caused a further decrease in \([\text{Cl}^-]\), suggesting that the osmotic regulation of \([\text{Cl}^-]\) in CVSM cells may be linked to a PTK-mediated phosphorylation and dephosphorylation activity. As shown in Figure 3A, hypotonic-induced reduction in \([\text{Cl}^-]\) in CVSM cells from all of the sham-operated rats was further decreased by orthovanadate by ~45%, showing no significant difference when sham control groups at different time points were compared with each other. However, in the 1-, 4-, 8-, and 12-week hypertension group, the reduction in \([\text{Cl}^-]\) in CVSM cells by hypotonic solution was further decreased much more than those of sham controls, and the mean percentage increase in hypotonic-induced reduction in \([\text{Cl}^-]\) by orthovanadate (for calculation, see online supplement) was 44.0%\(\pm\)4.2%, 30.6%\(\pm\)3.3%, 25.1%\(\pm\)3.9%, and 18.8%\(\pm\)2.6%, respectively. The abnormal response of hypotonic-induced reduction in \([\text{Cl}^-]\) to sodium orthovanadate was also normalized by captopril treatment. Moreover, correlation analysis showed that the percentage increase in hypotonic-induced reduction in \([\text{Cl}^-]\) by sodium orthovanadate had a negative correlation with BP levels in the hypertensive group (n=30 cells from 8 rats; \(r=-0.8021; P<0.001;\) Figure 3B).

**Discussion**

Several new findings are presented in this study. First, in 2k2c hypertensive models, our study is the first to show basilar arterial media undergoing remodeling, with an increase in CVSM cell size and cross-sectional area of the media. Second, although the \([\text{Cl}^-]\) showed no difference between hypertensive rats and sham controls, hypotonic perfusion induced much more decrease in \([\text{Cl}^-]\) in hypertensive rats than those of sham controls, suggesting that the volume-regulated \(\text{Cl}^-\) movement through \(I_{\text{vol}}\) could be

### Table 1. Effect of Genistein on Hypotonicity-Induced \([\text{Cl}^-]\)

<table>
<thead>
<tr>
<th>([\text{Cl}^-]), mmol/L</th>
<th>1 Week</th>
<th>4 Weeks</th>
<th>8 Weeks</th>
<th>12 Weeks</th>
</tr>
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<tbody>
<tr>
<td>Sham-operated group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>([\text{Cl}^-]) (_{\text{iso}})</td>
<td>29.1±1.0</td>
<td>29.1±1.0</td>
<td>29.1±1.0</td>
<td>29.2±0.9</td>
</tr>
<tr>
<td>([\text{Cl}^-]) (_{\text{hypo}})</td>
<td>24.6±0.9</td>
<td>24.4±1.0</td>
<td>24.6±0.9</td>
<td>24.4±0.9</td>
</tr>
<tr>
<td>([\text{Cl}^-]) (_{\text{gen+hypo}})</td>
<td>26.9±1.0</td>
<td>26.9±0.9</td>
<td>26.9±1.0</td>
<td>26.8±0.9</td>
</tr>
<tr>
<td>2k2c group</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>([\text{Cl}^-]) (_{\text{iso}})</td>
<td>28.7±1.4</td>
<td>28.5±1.1</td>
<td>28.4±1.2</td>
<td>28.4±1.3</td>
</tr>
<tr>
<td>([\text{Cl}^-]) (_{\text{hypo}})</td>
<td>23.6±1.1</td>
<td>20.9±1.1*</td>
<td>19.8±1.1*</td>
<td>18.8±1.0*</td>
</tr>
<tr>
<td>([\text{Cl}^-]) (_{\text{gen+hypo}})</td>
<td>26.4±1.3</td>
<td>26.5±1.4</td>
<td>26.7±1.0</td>
<td>26.8±0.8</td>
</tr>
<tr>
<td>2k2c + captopril group</td>
<td></td>
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<tr>
<td>([\text{Cl}^-]) (_{\text{iso}})</td>
<td>28.9±0.7</td>
<td>28.9±1.0</td>
<td>29.1±0.9</td>
<td>29.0±0.9</td>
</tr>
<tr>
<td>([\text{Cl}^-]) (_{\text{hypo}})</td>
<td>24.2±0.7</td>
<td>24.5±1.0†</td>
<td>24.3±0.9†</td>
<td>24.4±0.9†</td>
</tr>
<tr>
<td>([\text{Cl}^-]) (_{\text{gen+hypo}})</td>
<td>26.4±0.8</td>
<td>26.8±0.9</td>
<td>26.8±1.0</td>
<td>26.7±1.1</td>
</tr>
</tbody>
</table>

gen indicates genistein; hypo, hypotonic; iso, isotonic. *\(P<0.01\) vs corresponding sham group; †\(P<0.01\) vs corresponding 2k2c group (n=30 from 8 rats).
enhanced during the development of hypertension. We also showed a correlation between hypertonic-induced decreases in [Cl$^{-}$], and an increase in BP in hypertensive animals. These findings suggest the enhanced I$_{\text{Cl,vol}}$ activity in hypertension may be associated with cerebrovascular structural remodeling. In addition, cell volume regulation of Cl$^{-}$ movement in CVSM cells is closely coupled to phosphorylation/dephosphorylation mediated through PTK. This regulatory mechanism could also change as hypertension proceeds.

The present structural remodeling in the basilar artery in 2k2c hypertensive models is in agreement with previous findings from spontaneously hypertensive rats, suggesting that both genetic and nongenetic hypertension models share similar morphological alterations in the cerebral basilar arterial media wall during hypertension. However, it is still not known whether the increase in the cerebrovascular media wall in hypertension is because of either hypertrophy and/or hyperplasia of the VSMCs.

Hypotonic-induced cell swelling has been used to mimic the increase in cell volume in several cell types in response to physical stresses. CVSM cells may not meet osmotic challenge during hypertension; however, our present study demonstrated that the cell volume of CVSM cells became larger as BP increased in the 2k2c renal hypertension model. Therefore, in response to increased cell volume, it is likely that I$_{\text{Cl,vol}}$ would be activated by cell shape change when CVSM is exposed to increased BP.

The pharmacological and electrophysiological properties of the volume-regulated Cl$^{-}$ current in CVSM cells were similar to those of I$_{\text{Cl,vol}}$ reported in other VSMCs and numerous other cell types. In addition, nonspecific I$_{\text{Cl,vol}}$ blockers, indanyloxyacetic acid, and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid have been demonstrated to hyperpolarize and dilate rat cerebral artery smooth muscle cells. Because the Nernst equilibrium potential for Cl$^{-}$ (−20 to −30 mV) is positive to the resting membrane potential in VSMCs (−70 mV), activation of I$_{\text{Cl,vol}}$ would theoretically cause depolarization of CVSM cells. Thus, there is a possible explanation for enhanced activity of I$_{\text{Cl,vol}}$ in hypertension: the increasing BP or vascular hypertrophy may activate I$_{\text{Cl,vol}}$, cause Cl$^{-}$ efflux, and provide a depolarizing influence on CVSM cells. Alteration of I$_{\text{Cl,vol}}$ activity thereby may contribute to altered excitation-contraction coupling in hypertensive

### Table 2: Effect of Sodium Orthovanadate on Hypotonicity-Induced [Cl$^{-}$]

<table>
<thead>
<tr>
<th>[Cl$^{-}$]$_{\text{i}}$, mmol/L</th>
<th>1 Week</th>
<th>4 Weeks</th>
<th>8 Weeks</th>
<th>12 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated group</td>
<td></td>
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</tr>
<tr>
<td>[Cl$^{-}$]$_{\text{i,iso}}$</td>
<td>29.2±0.9</td>
<td>28.8±1.1</td>
<td>29.3±0.9</td>
<td>29.4±0.8</td>
</tr>
<tr>
<td>[Cl$^{-}$]$_{\text{i,hypo}}$</td>
<td>24.7±0.7</td>
<td>24.5±0.9</td>
<td>24.7±0.9</td>
<td>24.8±0.8</td>
</tr>
<tr>
<td>[Cl$^{-}$]$_{\text{i,2k2c+hypo}}$</td>
<td>20.4±0.8</td>
<td>20.7±1.1</td>
<td>20.7±0.9</td>
<td>20.7±1.0</td>
</tr>
<tr>
<td>2k2c group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Cl$^{-}$]$_{\text{i,iso}}$</td>
<td>28.7±1.4</td>
<td>28.7±1.0</td>
<td>28.5±1.1</td>
<td>28.4±1.2</td>
</tr>
<tr>
<td>[Cl$^{-}$]$_{\text{i,hypo}}$</td>
<td>23.3±0.9</td>
<td>21.0±0.8†</td>
<td>19.7±1.0†</td>
<td>18.7±1.0†</td>
</tr>
<tr>
<td>[Cl$^{-}$]$_{\text{i,2k2c+hypo}}$</td>
<td>19.0±0.5*</td>
<td>17.6±1.0†</td>
<td>16.8±0.7†</td>
<td>16.4±0.7†</td>
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<tr>
<td>2k2c+captopril group</td>
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<tr>
<td>[Cl$^{-}$]$_{\text{i,iso}}$</td>
<td>29.0±0.9</td>
<td>29.1±0.9</td>
<td>29.2±0.9</td>
<td>28.7±0.8</td>
</tr>
<tr>
<td>[Cl$^{-}$]$_{\text{i,hypo}}$</td>
<td>24.4±0.6</td>
<td>24.4±0.8§</td>
<td>24.3±0.9§</td>
<td>24.2±0.7§</td>
</tr>
<tr>
<td>[Cl$^{-}$]$_{\text{i,2k2c+captopril+hypo}}$</td>
<td>19.8±0.9‡</td>
<td>20.5±0.9§</td>
<td>20.5±0.9§</td>
<td>20.6±0.8§</td>
</tr>
</tbody>
</table>

*P<0.05 vs corresponding sham controls; †P<0.01 vs corresponding hypertensive group (n=30 from 8 rats). §P<0.01 vs corresponding 2k2c group (n=30 from 8 rats).
CVSM cells. On the other hand, recent studies have found that $I_{\text{Cl,vol}}$ plays dual roles in regulating cell proliferation and apoptosis, although it is not clear whether alteration of cell proliferation or apoptosis is involved in hypertrophy of CVSM; the possible contribution of enhanced activity of $I_{\text{Cl,vol}}$ to cerebral arteriolar remodeling may be related to cell cycle regulation.

Our present study could not determine whether the enhanced $I_{\text{Cl,vol}}$ is the cause or effect of the proposed remodeling. However, our results have found that the enhancement of volume-regulation of $I_{\text{Cl,vol}}$ and alteration of its regulatory mechanism occurred before any large difference in BP existed between hypertensive animals and sham-operated controls. Because hypertrophic remodeling is well known as the primary change in the development of hypertension, we proposed that the activity of $I_{\text{Cl,vol}}$ may be enhanced as the volume of CVSM cells increased.

Numerous studies have demonstrated that PTK phosphorylation and dephosphorylation are involved in the volume regulation of $I_{\text{Cl,vol}}$ in a variety of cell systems, including cultured VSMCs, atrial myocytes, and lymphocytes. Our results in CVSM cells and smooth muscle cells indicated that tyrosine phosphorylation favors stimulation of $I_{\text{Cl,vol}}$ by exposure to hypotonic solution, and tyrosine dephosphorylation blocks hypotonic-induced $I_{\text{Cl,vol}}$ activation. These conclusions were consistent with those reported in human T lymphocytes and rat astrocytes but different from those in human atrial myocytes. The possible explanation for the discrepancy between these studies may be because of different cell types and species and/or nonspecific effects of PTK or protein–tyrosine phosphatase inhibitors. We further found that the hypotonic-induced Cl$^+$ movement was more sensitive to these PTK/protein–tyrosine phosphatase inhibitors as BP levels increased in hypertensive rats. These data suggested that an increase in the tyrosine kinase activity in hypertension could lead to an enhanced activity of osmotic-sensitive Cl$^+$ movement. The present data are in line with the previous report demonstrating that an increase in the activity of PTK in hypertensive aorta could lead to an enhanced Ca$^{2+}$-linked aorta contraction. This finding also suggests that PTK is a shared potential candidate contributing to aortic remodeling in different pathological models.

It is important to know the molecular mechanism responsible for the osmotic-regulated Cl$^+$ movement in CVSM cells. It is, therefore, reasonable to perform additional gene targeting experiments and patch-clamp studies to answer the above questions. In aortic smooth muscle cells, our previous studies have provided strong evidence showing that CIC-3, a member of CIC channel family, may be the molecular component involved in the activation or regulation of $I_{\text{Cl,vol}}$. However, the molecular identity of $I_{\text{Cl,vol}}$ remains controversial and needs specific Cl$^+$ channel blockers. Therefore, the gene targeting experiments were not performed in this study.

**Perspectives**

The present study has shown for the first time that there is an enhancement of volume-regulated Cl$^+$ movement across the CVSM cell membrane mediating through PTK/protein–tyrosine phosphatase phosphorylation and dephosphorylation in the development of hypertension. It is becoming apparent that $I_{\text{Cl,vol}}$ may play a key role in more general cell functions, including cell volume regulation, cell proliferation, and cell apoptosis. Our data provide a new insight into our understanding of the role of $I_{\text{Cl,vol}}$ in some pathological processes, such as cerebrovascular remodeling, leading to stroke in hypertension.

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**Disclosures**

None.

**References**


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