Na\textsuperscript{+}, K\textsuperscript{+}-Adenosine Triphosphatase Regulation in Hypertrophied Vascular Smooth Muscle Cells

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Vascular smooth muscle cell hypertrophy is a normal compensatory state that may play a pathogenic role in hypertension. Angiotensin II stimulates a hypertrophic response in cultured vascular smooth muscle cells. As part of the growth response, angiotensin II rapidly activates the Na\textsuperscript{+}-H\textsuperscript{+} exchanger, increasing Na\textsuperscript{+} influx. Because Na\textsuperscript{+}, K\textsuperscript{+}-ATPase is the major cellular mechanism for regulating intracellular Na\textsuperscript{+}, we studied the effects of angiotensin II-induced hypertrophy on Na\textsuperscript{+}, K\textsuperscript{+}-ATPase expression and activity. Angiotensin II caused rapid increases in both steady-state Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity (ouabain-sensitive 86Rb uptake) and intracellular [Na\textsuperscript{+}]. Angiotensin II also caused a sustained increase in Na\textsuperscript{+}, K\textsuperscript{+}-ATPase at 24 hours with a 73\% increase in maximal 86Rb uptake per milligram protein and a fourfold increase in Na\textsuperscript{+}, K\textsuperscript{+}-ATPase \( \alpha \)-1 messenger RNA levels. Thus, angiotensin II hypertrophy was associated with rapid increases in Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity due to increased Na\textsuperscript{+} entry and sustained increases due to a specific increase in Na\textsuperscript{+}, K\textsuperscript{+}-ATPase expression. These data demonstrate dynamic regulation of Na\textsuperscript{+}, K\textsuperscript{+}-ATPase at the functional and molecular level and suggest that similar compensatory mechanisms should be present in vivo. Alterations in such compensatory pathways may be fundamental to the pathogenesis of hypertension. (Hypertension 1992;20:144–150)

KEY WORDS • sodium • angiotensin II • adenosine triphosphatase, sodium-potassium • muscle, smooth, vascular • hypertrophy • essential hypertension

Hypertrophy is a compensatory, physiological cell process in which the cell does not divide, but increases its size and protein content. In the cardiovascular system, myocytes hypertrophy in response to increased work load and pressure.\textsuperscript{1} The pathophysiological role of this adaptive response is suggested by the genetic model of hypertension seen in the spontaneously hypertensive rat (SHR). In these animals, hypertension is associated with a sustained hypertrophic increase in vascular smooth muscle cell (VSMC) mass.\textsuperscript{2} Recent data have shown that hypertrophied VSMC from hypertensive rats maintain a higher intracellular calcium concentration\textsuperscript{3} than do nonhypertensive cells. Because abnormalities in intracellular Na\textsuperscript{+} concentration ([Na\textsuperscript{+}]) are also thought to be an important feature of hypertensive VSMC, we have chosen to study the effects of VSMC hypertrophy in vitro on [Na\textsuperscript{+}], and Na\textsuperscript{+} regulatory pathways.

In cultured rat aortic smooth muscle cells, the vasoconstrictor angiotensin II (Ang II) has been shown to induce a "hypertrophic" growth response,\textsuperscript{4,5} as defined by increases in cell size and protein synthesis without change in cell number or DNA synthesis. The hypertrophic response induced by Ang II shares many of the same early signaling mechanisms that are induced by mitogens during proliferation.\textsuperscript{6} Ang II rapidly and transiently stimulates phospholipase C, generating inositol trisphosphate and diacylglycerol. Inositol trisphosphate releases calcium stores, and the resulting increase in intracellular Ca\textsuperscript{2+} initiates events leading to cell contraction. Increased diacylglycerol formation and activation of protein kinase C ultimately stimulate Na\textsuperscript{+}-H\textsuperscript{+} exchange, resulting in intracellular alkalinaization. The increased Na\textsuperscript{+} entry mediated by Na\textsuperscript{+}-H\textsuperscript{+} exchange has been shown to cause a rapid increase in ouabain-sensitive Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity in cultured rat aortic VSMC.\textsuperscript{7} Other Na\textsuperscript{+} transporters activated by Ang II include Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange\textsuperscript{8} and Na\textsuperscript{+}-K\textsuperscript{+}-2Cl cotransport.\textsuperscript{9} Thus, the Ang II-mediated hypertrophic growth response is associated with stimulation of many Na\textsuperscript{+} transport pathways.

Ang II hypertrophy is associated with a 45\% increase in cell volume.\textsuperscript{4} This suggests that, as in renal hypertrophy, there should be long-term alterations in Na\textsuperscript{+} regulatory mechanisms such as Na\textsuperscript{+}, K\textsuperscript{+}-ATPase, Na\textsuperscript{+}-H\textsuperscript{+} exchange, and Na\textsuperscript{+}-K\textsuperscript{+}-2Cl cotransport. In the present study we proposed that the increased Na\textsuperscript{+} influx induced in Ang II–hypertrophied VSMC\textsuperscript{10} should necessitate a compensatory increase in Na\textsuperscript{+} efflux mechanisms. Specifically, because alterations in Na\textsuperscript{+}, K\textsuperscript{+}-ATPase abundance have been related to intracellular ion concentration,\textsuperscript{11} we studied the relation in Ang II–treated cells between changes in [Na\textsuperscript{+}], and Na\textsuperscript{+}, K\textsuperscript{+}-ATPase expression and transport function.

Methods

Cell Isolation and Cell Culture

VSMC were isolated from the aortas of 200–300-g male Sprague-Dawley rats (Harlan Sprague Dawley,
Indianapolis, Ind.) by enzymatic dissociation. Cells were grown weekly and used between passages 8 and 20. For experiments, cells were plated in Costar 6-, 12-, or 24-well cluster dishes at 2 × 10^4 to 1 × 10^5 cells/ml in Dulbecco's modified Eagle's medium containing 10% heat-inactivated calf serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were grown to 85% confluency and then grown arrested for 48 hours in 0.4% calf serum (mitotic index less than 10% over 24 hours). To achieve hypertrophy, cells were treated with 100 nM Ang II in 0.4% calf serum for 24 hours.

**Measurement of Protein Content and Protein Synthesis**

Protein content was measured using a modified Lowry procedure that allows the assay to be performed in the culture dish.\(^\text{13,14}\) Protein synthesis was measured by [3H]leucine incorporation.\(^\text{4}\) After quiescence, cells were incubated for 24 hours in medium containing 1 μCi/ml [3H]leucine. Next, the cells were washed with ice-cold saline solution (150 mM NaCl, 10 mM Tris, pH 7.5), and protein was precipitated in 10% trichloroacetic acid. The 10% trichloroacetic acid was aspirated, and the plates were allowed to dry in 95% ethanol. The [3H]leucine was extracted in 0.2 M NaOH and counted by liquid scintillation spectrometry.

**Rubidium-86 Uptake**

Na\(^+\),K\(^+\)-ATPase activity was quantified by measuring ouabain-sensitive \(^8\)Rb flux based on the assumption that \(^8\)Rb transport by Na\(^+\),K\(^+\)-ATPase shows nearly identical kinetics to the normal substrate K\(^+\).\(^\text{7,19}\) VSMC were grown in 24-well dishes and exposed to conditions cultured adult rat VSMC, Ang II has no effect on Na\(^+\) influx and efflux mediated, respectively, by the Na\(^+\)-H\(^+\) exchanger and by Na\(^+\),K\(^+\)-ATPase.\(^\text{7,19}\) To prove that this mechanism was present in these cultured VSMC, Ang II has no effect on Na\(^+\) influx and efflux mediated, respectively, by the Na\(^+\)-H\(^+\) exchanger and by Na\(^+\),K\(^+\)-ATPase.\(^\text{7,19}\) Na\(^+\),K\(^+\)-ATPase activity was quantified by measuring Na\(^+\),K\(^+\)-ATPase

**Statistics**

For individual experiments, comparisons were performing using an unpaired Student's t test with a significant difference being \(p<0.05\). For group comparisons among several experiments, a one-way analysis of variance using Fisher coefficients was performed.

**Results**

**Effect of Angiotensin II on Protein Content and Protein Synthesis**

Ang II induced a hypertrophic response, defined by increases in protein content and protein synthesis without change in cell number. VSMC treated with 100 nM Ang II for 24 hours showed a 41±6.1% (\(p<0.05\)) increase in protein content relative to cells maintained in 0.4% calf serum (\(n=8\)). This compared with a 63±9.5% (\(n=2\)) increase in response to 10% calf serum. Ang II stimulated protein synthesis as demonstrated by a significant increase of 82±17% (\(n=4\)) in [3H]leucine incorporation, compared with an increase of 151±12% (\(n=4\)) by 10% calf serum. The increases in protein content and protein synthesis after treatment with Ang II were comparable to those observed previously.\(^\text{4}\) In these cultured adult rat VSMC, Ang II has no effect on the rates of protein degradation or cell division.\(^\text{4,5}\)

**Effect of Angiotensin II on Steady-State Na\(^+\),K\(^+\)-ATPase Activity**

Ang II has been reported to stimulate rapid increases in VSMC Na\(^+\) influx and efflux mediated, respectively, by the Na\(^+\)-H\(^+\) exchanger and by Na\(^+\),K\(^+\)-ATPase.\(^\text{7,19}\) To prove that this mechanism was present in these cultured VSMC, ouabain-sensitive \(^8\)Rb influx was measured in the presence or absence of amiloride. As shown in Figure 1, \(^8\)Rb uptake was linear for 10 minutes for both control and Ang II–treated cells. Ang II (100 nM)
FIGURE 1. Line plot shows effect of angiotensin II (Ang II) on 86Rb uptake at early time points. Vascular smooth muscle cells were growth arrested in 0.4% serum for 48 hours. Ang II and 86Rb (1 μCi/ml) were then added simultaneously for the indicated times. Na+,K+-ATPase activity was quantified by measuring ouabain-sensitive 86Rb flux. Ouabain-sensitive uptake was calculated by subtracting ouabain-insensitive uptake (1 mM ouabain) from total uptake. Results are mean±SEM for two experiments. Where not indicated, errors are smaller than symbols. Basal values for total and ouabain-sensitive flux were 22±2 and 56±4 cpm/mg in control cells.

Angiotensin II Effects on Na+,K+-ATPase Activity

rapidly increased both total uptake (58%) and ouabain-sensitive 86Rb uptake (64%). More than 90% of this increase was inhibited with 30 μM ethyisopropylamiloride, indicating a predominant role for the Na+-H+ exchanger (data not shown).

The effect of Ang II on Na+,K+-ATPase activity was next examined over the duration of the hypertrophic response. The time course for Ang II effects on ouabain-sensitive 86Rb uptake (Figure 2) showed a significant increase in Na+,K+-ATPase activity after 24 hours of exposure to Ang II. Ang II rapidly increased Na+,K+-ATPase activity when added initially, as shown in Figure 1. Subsequently, Na+,K+-ATPase activity returned to near baseline (at 30 minutes and 1 hour) and then showed a gradual increase up to 24 hours. The first increase in 86Rb uptake was likely due to increased activity of existing Na+,K+-ATPase, while the later, gradual increase in Na+,K+-ATPase activity could be due to increased expression of Na+,K+-ATPase protein. Ouabain-insensitive 86Rb uptake also was increased during the hypertrophic response, from 22±2 cpm/μg protein to 32±3 cpm/μg protein. Most of this increase was bumetanide sensitive and therefore represents Na+-K+-2Cl cotransport.

Angiotensin II Effects on Na+,K+-ATPase Activity Versus Na+,K+-ATPase Expression

To determine whether the Ang II-stimulated increase in Na+,K+-ATPase activity was part of a generalized increase in protein content, total and ouabain-sensitive 86Rb uptake were normalized for cell protein. As shown in Figure 3 (left panels), 24-hour treatment with Ang II increased total 86Rb uptake (nmol 86Rb/min) by 59±19% and increased ouabain-sensitive 86Rb uptake (nmol 86Rb/min) by 41±16%. Because Ang II did not increase cell number,4 these findings indicate that Ang II stimulated VSMC Na+,K+-ATPase activity on a per cell basis. However, as shown in Figure 3 (right panels), there was no effect of Ang II on total ouabain-sensitive 86Rb uptake when normalized to protein (nmol 86Rb/min/mg protein). These data demonstrate that the increase in steady-state Na+,K+-ATPase activity in the Ang II–treated cells was proportional to the increase in protein content.

Steady-state Na+,K+-ATPase activity is not a measurement of total Na+,K+-ATPase capacity, because Na+,K+-ATPase functions at a level necessary to maintain Na+ homeostasis. Therefore, to measure total Na+,K+-ATPase capacity, maximal Na+,K+-ATPase activity was assayed in cells loaded with Na+. To load Na+, cells were incubated for 3 hours in RPMI salts that contained no K+. This increased [Na+], from 6 to 47

FIGURE 2. Line plot shows effect of angiotensin II (Ang II) on 86Rb uptake during hypertrophy. Vascular smooth muscle cells were growth arrested for 48 hours, then fresh 0.4% serum was added for the indicated times. 86Rb uptake was determined after addition of 1 μCi/ml 86Rb for 10 minutes. Results were normalized to protein in each dish. Results are mean±SEM for three experiments.

FIGURE 3. Bar graph shows effect of angiotensin II (Ang II) on steady-state Na+,K+-ATPase activity. Total and ouabain-sensitive Na+,K+-ATPase activity were measured in cells treated for 20 hours with Ang II. Data were converted to nanomoles 86Rb per minute and nanomoles 86Rb per milligram protein and expressed as a percentage increase over the 0.4% calf serum control. Results are mean±SEM for six experiments.
Na⁺,K⁺-ATPase in Hypertrophied Smooth Muscle

This study investigated the effects of angiotensin II (Ang II) on Na⁺,K⁺-ATPase in quiescent vascular smooth muscle cells. The authors measured the concentration-response relation for Ang II induction of Na⁺,K⁺-ATPase α-1 subunit messenger RNA (mRNA) accumulation. Quiescent vascular smooth muscle cells were exposed to Ang II for 24 hours. Total RNA was prepared, and 15 μg per lane was size fractionated. Northern blot hybridizations with rat Na⁺,K⁺-ATPase α-1-specific and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) complementary DNAs (cDNAs) were performed as described in Methods. A typical autoradiogram is shown above the graph. After exposure in the linear film range, densitometry was performed on three autoradiograms. The levels of α-1 and GAPDH at hour 0 were arbitrarily set to 1.0. This resulted in values for GAPDH and α-1 at 24 hours (in response to 10 nM Ang II) of 1.6±0.2 and 3.8±0.3.

Angiotensin II Effects on Na⁺,K⁺-ATPase α-1 Messenger RNA

To gain insight into the mechanisms for Ang II-induced increases in Na⁺,K⁺-ATPase capacity, messenger RNA (mRNA) levels for the α-1 subunit of Na⁺,K⁺-ATPase were determined. Northern blot analysis showed that Na⁺,K⁺-ATPase α-1 mRNA levels increased at 24 hours in a dose-dependent manner. The maximal increase (3.8±0.3-fold) occurred in response to 10 nM Ang II (Figure 4). The increase in response to 10 nM Ang II was significantly larger than the increase in GAPDH (1.6±0.2-fold, p<0.05, n=3) observed in these experiments. The time course for induction of the α-1 subunit by Ang II (100 nM) revealed a peak increase (3.7±0.5-fold) at 6–8 hours, which was sustained (3.5±1.8-fold) at 24 hours (Figure 5). In comparison, GAPDH showed a steady progressive increase over 24 hours. Thus, although both Na⁺,K⁺-ATPase α-1 mRNA and GAPDH mRNA increased in response to Ang II, their time courses differed.

Regulatory Mechanisms for Na⁺,K⁺-ATPase: Effect of Angiotensin II on [Na⁺]

It is possible that changes in [Na⁺] might be the basis for the mechanism leading to increased Na⁺,K⁺-ATPase activity and expression. Therefore, the effects of Ang II on Na⁺ content and cell volume were measured to determine [Na⁺]. Ang II–treated cells showed a significant increase in total Na⁺ content at 4 hours from 18.0±0.2 to 21.6±0.4x10⁻¹⁵ mol/cell (p=0.013, n=9). This increase was sustained and was maximal at 24 hours, increasing to 24.6±0.4x10⁻¹⁵ mol/cell. Ang II initially decreased cell volume after 4 hours from 4.1±0.2 pl/cell to 3.6±0.1 pl/cell, p=0.04, consistent with a change in cell shape. This was followed by a gradual increase until the volume of Ang...
II–treated cells exceeded control at 24 hours (6.0±0.7 pl/cell versus 5.2±0.6 pl/cell, p>0.05). The small increase in control cells likely reflects the fact that, in 0.4% serum, approximately 10–15% of cells continue to grow as shown by [3H]thymidine autoradiography (unpublished observations from our laboratory). The resulting effect of Ang II on [Na+]i is shown in Figure 6C. Treatment with Ang II raised [Na+]i, by 36±13% (6.0±0.7 pl/cell, p<0.05). This increase was due to both a significant decrease in cell volume and a significant increase in Na+ content. Over 24 hours, however, [Na+]i, recovered to the same concentration as the control despite a sustained increase in Na+ content.

Discussion

The purpose of this study was to determine the effect of Ang II–induced hypertrophy on VSMC Na+,K+-ATPase function and expression. The results obtained for ouabain-sensitive 86Rb uptake and Na+,K+-ATPase α-1 subunit mRNA expression lead to three conclusions concerning Na+,K+-ATPase activity in Ang II–hypertrophied VSMC. First, Ang II stimulated Na+,K+-ATPase activity directly at early time points. As shown in Figures 1 and 2, Ang II specifically increased 86Rb uptake by Na+,K+-ATPase in the first 4 hours of treatment. This increase in Na+,K+-ATPase activity appeared to be due to increased Na+ influx, which was primarily mediated by the Na+-H+ exchanger.7,22 Brock et al previously have demonstrated a similar increase in Na+ entry and Na+,K+-ATPase activity during the first 20 minutes of Ang II stimulation. Previous studies suggest that an increase in Na+ influx may be required for Ang II hypertrophy. Inhibition of Na+ influx by amiloride derivatives completely blocked VSMC growth,23 and Ang II-stimulated increases in [3H]leucine incorporation were inhibited when extracellular Na+ was lowered, even to 30 mM.4

Second, Ang II also increased steady-state Na+,K+-ATPase activity on a per cell basis at later time points (24 hours) when hypertrophy was manifest (Figure 3). Because Ang II increased cell size and protein content but not cell number, the increase in 86Rb uptake was likely due to increased Na+,K+-ATPase activity per cell. However, when the increase in 86Rb uptake was corrected for protein content (Figure 3), no change in steady-state Na+,K+-ATPase activity was observed in the Ang II–treated cells. This indicates that Na+,K+-ATPase activity increased in proportion to the increase in cell protein. These data suggest that Na+,K+-ATPase functions at the same basal level in hypertrophied and growth-arrested cells. This is expected, based on the finding that [Na+]i, increased to a maximum of 6.0 mM, considerably below the Km for Na+,K+-ATPase.

Third, Ang II specifically increased Na+,K+-ATPase expression at 24 hours. Maximal ouabain-sensitive 86Rb uptake was increased by 73% in Ang II–treated cells compared with control (14.9±3.3 versus 25.9±4.4 nmol 86Rb per mg protein per min) even when normalized for protein. This suggests that Na+,K+-ATPase protein was specifically increased in each hypertrophied cell even though the steady-state activity was unchanged. These results suggest that hypertrophied cells express an increased number of Na+,K+-ATPase subunits. Also, Na+,K+-ATPase α-1 mRNA expression increased within 6 hours of Ang II treatment and maintained a fourfold increase after 24 hours. The time course for α-1 mRNA accumulation differed from that of the glycolytic enzyme GAPDH (peak for α-1 at 6–8 hours versus peak for GAPDH at 24 hours). This suggests that the increase in Na+,K+-ATPase was not merely a generalized response to hypertrophy.
The major goal of these studies was to relate Ang 2-mediated effects on [Na\(^+\)], to Na\(^+\),K\(^+\)-ATPase expression. Our results indicate an association, since increases in [Na\(^+\)]\(_i\) induced increases in Na\(^+\),K\(^+\)-ATPase \(\alpha\)-1 mRNA induction (compare Figures 5 and 6), and increases in [Na\(^+\)], also preceded increases in maximal Na\(^+\),K\(^+\)-ATPase activity. Other investigators have demonstrated regulation of Na\(^+\),K\(^+\)-ATPase abundance by changes in intracellular and extracellular Na\(^+\). Allen et al\(^{20}\) have shown that exposure of cultured canine VSMC to serum mitogens increased Na\(^+\),K\(^+\)-ATPase activity in association with increased [Na\(^+\)]. Boardman et al\(^{20}\) demonstrated that the increase in Na\(^+\),K\(^+\)-ATPase activity and ouabain binding sites induced in HeLa cells by low K\(^+\) medium was prevented by replacing 40% of extracellular Na\(^+\) with sorbitol. Finally, Kim and Smith\(^{23}\) showed that decreasing extracellular Na\(^+\) without altering K\(^+\) caused a decrease in ouabain binding sites in cultured chick ventricular myocytes. Thus, changes in Na\(^+\),K\(^+\)-ATPase expression occur in response to increased [Na\(^+\)], even when the increase does not appear to saturate transport activity.

There are two important issues raised by our findings that must be explained if our hypothesis that [Na\(^+\)], regulates Na\(^+\),K\(^+\)-ATPase gene expression is correct. First, one must explain how [Na\(^+\)], increases. It appears likely that this is due to an increase in steady-state Na\(^+\) influx. Activation of Na\(^-\)H\(^+\) exchange stimulates entry of \(\approx 30\) mmol Na\(^+\) per mg protein per min, based on the current findings and previous work.\(^{19,22}\) In addition, at early time points there is a significant decrease in cell volume. Second, one must explain how the cell can recognize the small change in [Na\(^+\)]\(_i\) demonstrated here. The most likely explanation is that local [Na\(^+\)], especially nuclear [Na\(^+\)], is considerably higher. This explanation is supported by findings with the Na\(^+\)-sensitive fluorescent dye, SBFI, which show significantly greater [Na\(^+\)] in the nucleus in both hepatocytes\(^{26}\) and VSMC.\(^{27}\)

Alterations in Na\(^+\),K\(^+\)-ATPase gene expression have been demonstrated in several models of hypertrophy. Norman et al\(^{28}\) studied time-dependent changes in steady-state levels of several mRNAs including proto-oncogenes (c-fos and c-myec), structural proteins (actin), and transport proteins (Na\(^+\),K\(^+\)-ATPase) in renal cells induced to hypertrophy (unilateral nephrectomy) and compared them with cells induced to proliferate (folic acid treatment). First, one must explain how [Na\(^+\)], increases. It appears likely that this is due to an increase in steady-state Na\(^+\) influx. Activation of Na\(^-\)H\(^+\) exchange stimulates entry of \(\approx 30\) mmol Na\(^+\) per mg protein per min, based on the current findings and previous work.\(^{19,22}\) In addition, at early time points there is a significant decrease in cell volume.

They found that hyperplasia was associated with an early increase in mRNA expression of all genes studied, whereas the hypertrophied cells showed a gradual accumulation of mRNAs that Norman et al\(^{28}\) termed "sustained message amplification." Specifically, Na\(^+\),K\(^+\)-ATPase \(\alpha\)-1 and \(\beta\) subunit mRNAs gradually increased up to 24 hours after treatment in the hypertrophy model. In the desoxycorticosterone acetate (DOCA)-salt uninephrectomy model of hypertension, that results in VSMC hypertrophy, alterations in Na\(^+\),K\(^+\)-ATPase gene expression have also been observed. Herrera et al\(^{29}\) demonstrated twofold to threefold increases in Na\(^+\),K\(^+\)-ATPase \(\alpha\)-1 mRNA in aortic, cardiac left ventricular, and skeletal muscle tissue 2 weeks after nephrectomy. Thus, increases in \(\alpha\)-1 mRNA in VSMC appear to be a common feature of hypertrophy.

The importance of the present study is twofold. First, it demonstrates that during the early phase of Ang 2-induced hypertrophy, there is an increase in [Na\(^+\)].

This suggests that, during hypertrophy, there is a change in steady-state [Na\(^+\)]. This hypothesis is supported by the recent work of Bobik et al,\(^{30}\) which showed that inhibiting Na\(^+\)-H\(^+\) exchange in VSMC prevented serum-induced cell growth. Second, the present study, like many others, demonstrates dynamic regulation of Na\(^+\),K\(^+\)-ATPase at the functional and molecular level that, over 24 hours, resulted in a return of [Na\(^+\)], to control values.\(^{11,20,29,31}\) If such a compensatory relation is true in vivo, one may hypothesize that changes in VSMC [Na\(^+\)], postulated to occur in hypertension\(^{22}\) should be compensated for by increased Na\(^+\),K\(^+\)-ATPase expression or activity, or both. Based on the present study, the findings of increased VSMC [Na\(^+\)], in hypertension suggest that inhibitors of Na\(^+\),K\(^+\)-ATPase activity are present,\(^{33}\) or other Na\(^+\) homeostatic mechanisms are impaired. Finally, recent studies show that VSMC may express a truncated Na\(^+\),K\(^+\)-ATPase \(\alpha\)-1 subunit isoform generated by alternative RNA processing.\(^{34}\) This finding suggests that complex alterations in different \(\alpha\)-1 subunit abundance and their interaction with the \(\beta\) subunit may be important in determining VSMC Na\(^+\) transport.

**References**


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