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Effect of Na^+ on Na^+,K^+-ATPase α-subunit expression and Na^+-pump activity in aortic smooth muscle cells

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Abstract

In earlier studies we demonstrated that cyclical mechanical strain on vascular smooth muscle cells increases intracellular Na^+ and upregulates the α-1 and α-2 isoform expression of Na^+,K^+-ATPase, and that the increase of intracellular Na^+ and upregulation of the α-2 isoform expression are blocked by Gd^{3+}, which blocks entry of ions (including Na^+) through stretch-activated channels. The present study was designed to investigate the role of intracellular Na^+ in Na^+,K^+-ATPase regulation by increasing intracellular Na^+ with chronic ouabain treatment. In parallel experiments, we measured Na^+,K^+-ATPase α isoform expression, Na^+-pump activity and intracellular Na^+ in cultured aortic smooth muscle cells after treatment with two concentrations of ouabain for various time periods. Treatment with 100 nM ouabain resulted in a significant elevation in intracellular Na^+ after 1 (21%) and 2 h (12%), but the value returned to baseline after 12 h. Both α-1 and α-2 subunits of Na^+,K^+-ATPase were significantly upregulated after 1 through 4 days. Na^+-pump activity was also stimulated, and the time course of this effect closely followed protein expression. At 200 μM of ouabain, the effects on intracellular Na^+, isoform expression and Na^+-pump activity at earlier time points (1 h through 1 day) were similar to those with 100 nM treatment, but prolonged treatment (2 and 4 days) resulted in an accumulation of intracellular Na^+ and inhibition of the isoform expression and Na^+-pump activity, possibly due to general dysfunction of the cells as a result of chronic exposure to high concentrations of ouabain. We conclude that elevated intracellular Na^+ can serve as a signal to mediate the α isoform upregulation and the regulatory process requires less than one day.

Keywords: Smooth muscle cell, aortic; Na^+,K^+-ATPase, α isoform; Na^+-pump activity; Na^+, intracellular; Ouabain

1. Introduction

Na^+,K^+-ATPase is a membrane-associated enzyme that actively establishes transmembrane Na^+ gradients by hydrolyzing ATP as its energy source and is largely responsible for the transport of a range of cellular substrates and electrolytes by using the gradients as the primary driving force (Skou and Esmann, 1992). Digitalis glycosides, including ouabain, are a group of specific inhibitors of this enzyme. The study of Na^+,K^+-ATPase in blood vessels and vascular smooth muscle cells has been difficult because the levels of this enzyme in these tissues are very low (Allen et al., 1986). Nevertheless, the importance of sodium gradient in blood pressure regulation has long been recognized (Friedman et al., 1958), and investigators have attempted to characterize the enzyme and studied its role in modulating vascular contraction (Allen et al., 1986; Hendrickx and Casteels, 1974; Lang and Blaustein, 1980). Since the discovery that both the α and β subunits of Na^+,K^+-ATPase exist in different isoforms, three isoforms of the α-subunit (α-1, α-2 and α-3) with varying affinities for ouabain inhibition and binding, and two of the β subunit (β-1 and β-2) have been reported in mammalian cells (Lingrel and Kuntzweiler, 1994). The existence of multiple isoforms in the vasculature was also speculated and supported by experimental evidence (Weiss et al., 1993). We demonstrated that all three α isoform proteins are expressed in rat arteries and aortic smooth muscle cells (Sahin-Erdemli et al., 1994).

Since Na^+,K^+-ATPase plays an important role in regulating intracellular Ca^{2+} levels through the Na^+–Ca^{2+} exchange mechanism (Blaustein, 1977), numerous studies have investigated the effect of the Na^+-pump inhibition on blood vessels and vascular smooth muscle cells. Ouabain

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has often been chosen as the inhibitor, mostly with acute treatment (Zhu et al., 1994; Woolfson et al., 1990). The present study was based on our previous findings that cyclical mechanical strain on aortic smooth muscle cells increases intracellular Na^+ and upregulates the α-1 and α-2 isoform expression of Na^+,K^+-ATPase (Songu-Mize et al., 1996; Liu et al., 1998). Furthermore, the increase of intracellular Na^+ and upregulation of the α-2 isoform expression were blocked by gadolinium (Gd^{3+}) (Songu-Mize et al., 1996; Liu et al., 1998). Gd^{3+} blocks entry of ions (including Na^+) into the cell through stretch-activated channels. In the present study, by chronic treatment of aortic smooth muscle cells with ouabain, which loads the cells with Na^+, we investigated the role of intracellular Na^+ in regulation of the α subunit expression of Na^+,K^+-ATPase, Na^+-pump activity and the time course of this regulation.

2. Materials and methods

2.1. Preparation of cultured aortic smooth muscle cells

Aortic smooth muscle cells were isolated from male Sprague–Dawley rats weighing 150–200 g as described earlier (Sahin-Erdemli et al., 1994). The aortas were isolated and incubated for 30 min in minimal essential medium supplemented with 2.4 mM Ca^{2+}, 0.33 mg/ml soybean trypsin inhibitor, and 200 U/ml collagenase (type I). The tissues were then cleaned of adventitia, minced with scissors, and incubated further for 90–120 min in a fresh aliquot of the same medium plus 15 U/ml elastase (type III). The cells were then centrifuged, washed and resuspended in culture medium (Medium 199 + 10% fetal bovine serum) and seeded in 100-mm Petri dishes. Individual experiments were performed in 6-well culture dishes on confluent aortic smooth muscle cells between the 3rd and 7th passages.

2.2. Chronic treatment of aortic smooth muscle cells with ouabain

To investigate the effects of ouabain on aortic smooth muscle cells we used two concentrations of ouabain; 100 nM and 200 μM. At nanomolar concentrations ouabain is expected to occupy and inhibit high affinity isoforms of the enzyme. Whereas, at micromolar concentrations, it occupies both the high and low affinity isoforms, thereby inhibiting a much larger number of Na^+ pump sites. To monitor the course of ouabain-induced effects in aortic smooth muscle cells, we performed the experiments after treating the cells for six time periods, i.e., 1 h, 2 h, 12 h, 1 day, 2 days and 4 days. Time controls were run parallel to each time point, and were pooled because of no significant differences among them. Ouabain was added fresh with every change of media every other day. Isoform expression, Na^+-pump activity, and intracellular Na^+ measurements were made in parallel experiments.

2.3. Preparation of samples for Western blot analysis

Following the ouabain treatments, aortic smooth muscle cells from individual 6-well culture dishes were washed twice with cold phosphate buffered saline. The plates were then scraped in a homogenization buffer (in mM: sucrose = 250, Tris = 50, EDTA = 1, pH = 7.4). After an initial centrifugation at $20\,000 \times g$ for 1 min at 4°C, the pellet was resuspended in a lysis buffer (in mM: NaCl = 140, Tris = 10, $MgCl_2$ = 1.5 containing 0.5% Triton-X-100, pH = 8.6) and centrifuged again at $20\,000 \times g$ for 3 min at 4°C. The supernatant was used for electrophoresis/Western blot analysis. Protein concentrations were determined by the bicinchoninic acid protein assay (Smith et al., 1985) using bovine serum albumin as a standard. Final concentrations of the protein in the individual samples were in the range of 2–3 mg/ml.

2.4. Gel electrophoresis and immunoblotting

First, standard curves for the α-1 isoform (cell extract protein ranging from 0–30 μg) and the α-2 isoform (cell extract protein ranging from 0–40 μg) were constructed to determine the linear range and the appropriate protein amount for quantitation of each isoform. We loaded 10 and 20 μg of the cell extract protein onto the gel for α-1 and α-2 isoforms, respectively, for electrophoresis. The cell extracts and prestained molecular weight standards (Bio-Rad) were subjected to polyacrylamide gel electrophoresis on 10% polyacrylamide gels in the presence of 0.1% sodium dodecyl sulfate (Laemmli, 1970) and then transferred to polyvinylidene fluoride membrane by electroblotting (Towbin et al., 1979). After blocking in Tris-buffered saline (in mM: Tris · HCl = 20, NaCl = 137, pH = 7.5) containing 5% (w/v) non-fat dried milk (Carnation) and 1.0% (v/v) Tween 20 for 1 h at room temperature, the blots were probed with monoclonal antibodies, McK1 and McB2, directed against the α-1 and -2 subunits of Na^+,K^+-ATPase, respectively, as described before (Sahin-Erdemli et al., 1995). The α-3 subunit was undetectable because of the amount of cell extract protein available from each culture well. The blots were treated with the secondary antibody, horseradish peroxidase-labeled sheep anti-mouse Ig (Amersham). Blots were then treated with Enhanced Chemiluminescence reagent (ECL, Amersham) and exposed to X-ray film for visualization of the bands. Quantitation of the bands were performed as described previously (Liu et al., 1998). The fluorograms were scanned and the intensity of the bands for α-1 and α-2 was quantified as optical density units using a computerized image analyzer (M-2 Model, Imaging Research, Canada). The band density values were all within the linear range of the standard curve. To normalize band

density values from different blots, an internal control sample of a known amount of aortic smooth muscle cell extract was loaded onto each gel and the density was designated as 1.

2.5. Measurement of Na^+-pump activity in aortic smooth muscle cells

The Na^+-pump activity was determined in cultured aortic smooth muscle cells using a modified ouabain-sensitive $^{86}Rb^+$ uptake technique as previously described (Songu-Mize et al., 1990). The medium was removed and the cells were washed with Krebs–Henseleit buffer (pH 7.4, bubbled with CO_2 5%–O_2 95%; composition in mM: $NaHCO_3 = 27.2$, $NaCl = 117$, $NaH_2PO_4 = 1$, $MgSO_4 = 1.2$, $CaCl_2 = 1.8$, dextrose = 11, $KCl = 5$) and incubated in Krebs–Henseleit buffer without KCl for 30 min at 37°C. After 30 min, the cells were washed with Krebs–Henseleit and supplied with 0.5 ml of fresh Krebs. Some wells contained 2 mM ouabain for determination of ouabain-resistant $^{86}Rb^+$ uptake. After a 2-min preincubation period, $^{86}RbCl$ ($\sim 10^6$ CPM/well, 10–50 nM) was added to start the uptake reaction, which was terminated after 30 min by removing the incubation medium and washing twice with Krebs buffer. Na-pump activity was determined by subtracting the uptake of $^{86}Rb^+$ ($+K^+$) in the presence of 2 mM ouabain, ouabain-resistant uptake, from the total uptake. The Na^+-pump activity is expressed as nanomoles of ($^{86}Rb^+ + K^+$) per mg cell protein per 30 min. The uptake reaction is linear for at least 30 min and the maximum pump capacity is measured under the conditions described above (Songu-Mize et al., 1990).

2.6. Intracellular Na^+ measurement

Intracellular Na^+ was measured as described in an earlier study (Liu et al., 1998). After the ouabain treatment, the cells were washed six times with ice-cold 0.15 M LiCl (EM Science Suprapur®) and treated with 0.6 ml 50 μM nystatin (Sigma) in each well for two days to release intracellular Na^+. In our pilot experiments, treatment of aortic smooth muscle cells with 50 μM nystatin was very effective in releasing intracellular Na^+, and the intracellular Na^+ concentrations obtained from the same group of samples are consistently close to each other. An Na^+ standard curve ranging from 0–217.0 μM (0–500 ppb) was prepared using a standard solution (Atomic Absorption Standard, EM Science). For the intracellular Na^+ measurement of the samples, a 0.5 ml aliquot was taken from each well and diluted to 5 ml with water for measurement with an ICP-Emission Spectrophotometer (Perkin-Elmer Optima 3000) at 589 nm wavelength. The water used in the intracellular Na^+ measurements was obtained from a Milli-Q$^+$ apparatus (Millipore) and had a resistance of ≥ 18.2 MΩ cm. Intracellular volume of cell monolayers was determined by a methylglucose (MG) uptake method (Kletzien et al., 1975). After the medium was aspirated and washed with 1 ml of glucose-free HEPES-buffered saline solution (HBSS, containing in mM: $NaCl = 130$, $KCl = 5$, $MgCl_2 = 1$, $CaCl_2 = 1$, pyruvic acid = 2, HEPES = 10, pH = 7.4) at room temperature,

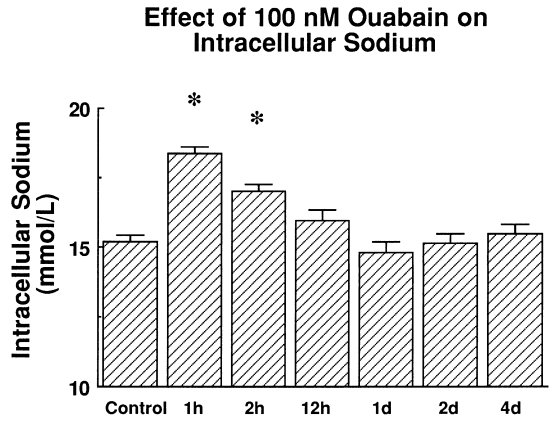

Fig. 1. Effect of treatment with 100 nM ouabain on intracellular Na^+ in cultured aortic smooth muscle cells. The bar graphs represent the mean and standard errors for intracellular Na^+ concentrations in mM. Asterisks indicate a significant difference ($P < 0.05$) compared to the control. ANOVA and Newman–Keuls test were used for multiple and individual comparisons, respectively ($n = 9$).

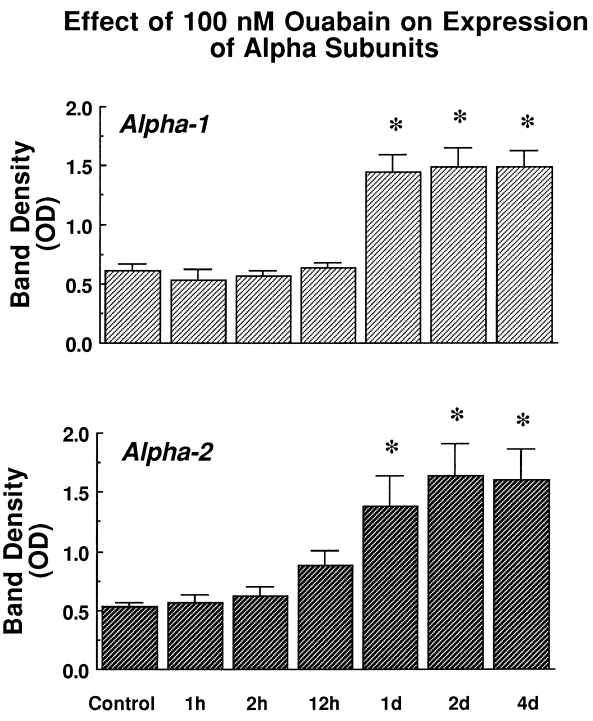

Fig. 2. Effect of treatment with 100 nM ouabain on the expression of α subunits of Na^+,K^+-ATPase in cultured aortic smooth muscle cells using Western blot analysis. The bar graphs represent the mean and standard errors for band densities from immunoblots expressed as optical density units (O.D.). Top panel: α-1 subunit expression, and bottom panel: α-2 subunit expression. Asterisks indicate a significant difference ($P < 0.05$) compared to the control. ANOVA and Newman–Keuls test were used for multiple and individual comparisons, respectively ($n = 6$–9).

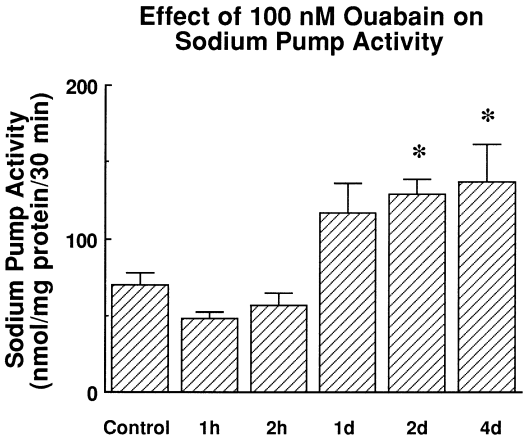

Fig. 3. Effect of treatment with 100 nM ouabain on Na^+-pump activity in cultured aortic smooth muscle cells. The bar graphs represent the mean and standard errors. Asterisks indicate a significant difference ($P < 0.05$) compared to the control. ANOVA and Newman–Keuls test were used for multiple and individual comparisons, respectively ($n = 9$).

0.5 ml of glucose-free HBSS containing 0.2 μCi/ml of [^{14}C]3-*O*-MG and 10 mM cold 3-*O*-MG was added and incubated for 30 min at room temperature. The intracellular volume for each condition was determined in triplicate. Following the incubation period, the wells were rinsed twice with 1 ml ice-cold glucose-free HBSS containing 1 mM phloretin and 1% (v/v) ethanol. The cells in each well were digested with 0.5 ml of HBSS containing 0.1% Triton-X-100 solution and samples were taken for protein determination and scintillation counting. The intracellular volume was determined to be 6.25 μl/10^6 cells. Intracellular Na^+ concentrations of the samples were derived based on the amount of Na^+ extracted from the cells and the total intracellular volume per well as determined by the methods described above.

2.7. Statistical analysis

Analysis of variance (ANOVA) followed by Newman–Keuls test was used. A confidence limit of 95% was considered significant.

3. Results

To establish the time course of Na^+ loading with chronic ouabain treatment, we measured aortic smooth muscle cell intracellular Na^+ concentrations after treatment with 100 nM ouabain for 1 h, 2 h, 12 h, 1 day, 2 days and 4 days. The Na^+ concentration of control samples was 15.20 ± 0.25 mM ($n = 9$). Intracellular Na^+ concentrations significantly increased after 1-h (18.37 ± 0.25, a 21% increase, $n = 9$) and 2-h (17.03 ± 0.23, a 12% increase, $n = 9$) treatment, but returned to control levels when the treatment continued (Fig. 1).

To assess the effect of intracellular Na^+ changes on the expression of the catalytic α-1 and α-2 subunits of Na^+,K^+-ATPase and the functional aspects of the Na^+-pump, we measured these variables in aortic smooth muscle cells after treatment with 100 nM ouabain at corresponding time points. Ouabain at this concentration should only occupy and inhibit a small portion of the binding sites (mostly the high affinity sites) in rat smooth muscle cells. Treatment with 100 nM ouabain stimulated both α-1 and α-2 isoform expression after 1 day (136% and 162% increase), 2 days (144% and 210% increase) and 4 days (144% and 204% increase for α-1 and α-2, respectively compared to control treatments; Fig. 2, top and bottom panels). Treatment with 100 nM ouabain for 1 h, 2 h and 12 h did not affect the expression of either isoform (Fig. 2). The Na^+-pump activity in aortic smooth muscle cells was measured using the $^{86}Rb^+$ uptake technique after treatment with ouabain at similar time points as above. The changes in Na^+-pump activity in aortic smooth muscle cells followed a similar pattern to that of the α-subunit expression. That is, the Na^+-pump activity started to increase after 1-day treatment (a 66% increase) and reached significant levels after 2-day (an 85% increase) and 4-day (a 96% increase compared to control) treatment periods (Fig. 3).

We also used a concentration of ouabain, 200 μM, which is likely to occupy most of the high- and low-affinity Na^+,K^+-ATPase sites in aortic smooth muscle cells. At this concentration ouabain increased the intracellular Na^+

Table 1
Effect of 200 μM ouabain on Na^+,K^+-ATPase α-subunit expression, Na^+-pump activity and intracellular Na^+ concentration

Treatment	(Band density, O.D.)		Na^+-pump activity (nmol [Rb + K]/mg per 30 min)	$[Na^+]_i$ (mM)
	α-1	α-2		
1 h	0.60 ± 0.06	0.52 ± 0.07	87.9 ± 5.6	16.9 ± 0.26^a
2 h	0.61 ± 0.08	0.52 ± 0.06	47.4 ± 3.2	17.2 ± 0.28^a
12 h	0.91 ± 0.05	0.72 ± 0.10	–	17.5 ± 0.25^a
1 day	1.87 ± 0.32^a	1.47 ± 0.12^a	167.4 ± 14.0^a	15.5 ± 0.27
2 days	0.84 ± 0.09	0.56 ± 0.11	102.3 ± 17.6	21.3 ± 0.28^a
4 days	0.91 ± 0.15	0.61 ± 0.10	86.0 ± 7.6	21.0 ± 0.57^a
Control	0.61 ± 0.06	0.53 ± 0.04	70.0 ± 8.0	15.2 ± 0.25

Values are expressed as means $\pm$ standard error.
[a] $P < 0.05$, compared to the control.
ANOVA followed by Newman–Keuls test was used, $n = 6$–9 in each group.

Table 2
Total cell protein and extracted membrane protein of cultured smooth muscle cells: effect of ouabain treatment

Treatment	Total cell protein	Extracted protein
100 nM ouabain		
1 h	337 ± 8.6	64.0 ± 2.26
2 h	335 ± 9.4	65.1 ± 2.11
12 h	336 ± 8.3	63.9 ± 2.34
1 day	335 ± 5.4	65.1 ± 2.62
2 days	336 ± 5.6	64.7 ± 1.93
4 days	335 ± 5.6	65.0 ± 2.50
200 μM ouabain		
1 h	334 ± 6.8	64.8 ± 1.57
2 h	335 ± 6.6	64.4 ± 2.38
12 h	335 ± 9.9	64.3 ± 2.37
1 day	333 ± 10.6	65.2 ± 2.08
2 days	335 ± 8.4	64.6 ± 1.90
4 days	335 ± 7.2	65.2 ± 1.77
Control	338 ± 8.0	64.4 ± 1.92

Values are expressed as means ± standard error, expressed in μg protein per culture well ($n = 9$ in each group).

concentrations significantly after 1-h (an 11% increase), 2-h (a 13% increase) and 12-h (a 15% increase compared to control) treatment periods. Following a transient return to the control level at day 1, intracellular Na^+ concentrations increased again at days 2 and 4 and reached even higher levels (a ~40% increase). Treatment with 200 μM ouabain stimulated the isoform expression only at 1-day time point (a 201% increase for α-1 and 179% for α-2 compared to control). No significant change was observed at other time points. Similar to the protein expression, the Na^+-pump activity was not stimulated until day 1 (a 139% increase), but declined at days 2 and 4. The results from 200 μM ouabain treatment are summarized in Table 1.

Treatment of ouabain for up to 4 days did not affect either the total protein content per well or the yield of membrane protein extraction. (Table 2).

4. Discussion

In two related previous studies we observed upregulation of both the α-1 and α-2 subunits of Na^+,K^+-ATPase (Songu-Mize et al., 1996) in response to chronic cyclical stretch in aortic smooth muscle cells. In addition a transient increase in intracellular Na^+ occurred in these cells during stretch (Liu et al., 1998). The purpose of the present study was to investigate the role of the intracellular Na^+ in the upregulation of the α subunit expression of Na^+,K^+-ATPase in cultured aortic smooth muscle cells (Songu-Mize et al., 1996; Liu et al., 1998). We used a model system in which treatment with a low concentration of ouabain (nanomolar range) mimicked the pattern and time course of changes in intracellular Na^+ as observed during chronic cyclical stretch, and measured the isoform expression as well as Na^+-pump activity. The results demonstrate for the first time that, similar to cyclical stretch, chronic ouabain treatment upregulates the α-1 and α-2 isoforms, which is corroborated by the stimulation of the functional counterpart of the enzyme, the Na^+-pump activity. Since the increase in isoform expression and Na^+-pump activity was preceded by a significant increase in intracellular Na^+ (1 and 2 h of treatment), which subsequently declines to baseline levels, it is likely that intracellular Na^+ serves as a signal, either directly or indirectly, to upregulate the Na^+,K^+-ATPase isoform expression. The time course of the elevation and later normalization of intracellular Na^+ with 100 nM chronic ouabain treatment was very similar to what we observed in the previous study, in which aortic smooth muscle cells were cyclically stretched for similar time periods. In that study, the intracellular Na^+ went up by 30% and 23% following a 1-h and 2-h stretch, respectively, and returned to baseline levels after a 1-day stretch (Liu et al., 1998). In another recent study, we demonstrated that chronic, cyclical stretch for 2 and 4 days upregulates the α-1 and α-2 subunit expression of Na^+,K^+-ATPase in cultured aortic smooth muscle cells (Songu-Mize et al., 1996). Furthermore, Ruiz-Opazo et al. (1997) in a very recent study showed that a 16-h treatment of A10 cells with monensin to increase intracellular Na^+ induced a dose-dependent upregulation of α-1, α-2 and β-1 subunit mRNA levels of Na^+,K^+-ATPase. They also characterized a transcriptional Na^+-response mechanism, defining a positive Na^+-response regulatory region in the α-1 and α-2 genes of Na^+,K^+-ATPase, and detected a Na^+-response nuclear DNA binding protein (Ruiz-Opazo et al., 1997). Taken together, these data suggest a strong causal relationship between intracellular Na^+ increase and the α isoform expression.

Since the function of Na^+,K^+-ATPase is to maintain relatively stable transmembrane Na^+ and K^+ gradients, any approach that results in an increase in intracellular Na^+ will initially stimulate this enzyme; this has been demonstrated by studies on various cell types (Skou and Esmann, 1992; Smith and Rozengurt, 1978). But it still remains to be resolved how increased intracellular Na^+ stimulates Na^+,K^+-ATPase at the expression level and manifests a long-term stimulation of the enzyme. Yamamoto et al. (1994) have found that increased intracellular Na^+ stimulates the transcription of Na^+,K^+-ATPase α and β isoform genes in aortic smooth muscle cells. Whereas, Rayson (1991) demonstrated that, in outer medullary kidney tubular segments, intracellular Ca^{2+} elevations increase the transcription rate of the α-1 and β-1 subunit mRNAs. In our previous studies, Ca^{2+} entry which can be blocked by isradipine appeared to be involved in the basal expression but not stretch-induced regulation of the α subunits (Liu et al., 1998). However, the role of secondary increase in intracellular Ca^{2+} during stretch in the regulation of these isoforms can not be ruled out, since an increase in intracellular Na^+ can also mobilize Ca^{2+} from intracellular stores (Borin et al., 1994). In addition,

even a slight intracellular Na^+ elevation can cause a considerable intracellular Ca^{2+} increase through the Na^+–Ca^{2+} exchanger (Blaustein, 1977). Thus, it is conceivable that even a slight rise in intracellular Na^+ caused by stretch-activated channels could be amplified into a stronger intracellular Ca^{2+} signal via the Na^+–Ca^{2+} exchanger. Indirect contribution of other ions such as H^+, K^+, Cl^-, HCO_3^-, OH^- to the regulation of the Na^+-pump isoforms cannot be ruled out, since modifying the intracellular Na^+ may produce transient changes in the activity of other membrane pumps to maintain cellular homeostasis. To differentiate the roles of other ions, more specific experimental approaches are needed.

Although both the α-1 and α-2 isoforms are upregulated with either ouabain treatment or mechanical stretch, the intracellular events underlying the regulation of these two isoforms may involve additional mechanisms. For example, Gd^{3+}, a potent stretch-activated channel blocker, can fully block the stretch-induced upregulation of α-2, but the α-1 isoform is affected to a lesser degree (Songu-Mize et al., 1996). It is conceivable that intracellular Na^+ serves as a signal, possibly at the transcriptional level (Ruiz-Opazo et al., 1997), that is followed by different intracellular pathways to regulate the differential expression of the α-1 and α-2 isoforms.

The Na^+-dependent upregulation of the Na^+,K^+-ATPase α isoforms may be relevant to the pathophysiology of hypertension. Under in vivo conditions, vascular smooth muscle cells are subject to chronic cyclical stretch exerted by blood pressure. As we have already demonstrated, cyclical stretch increases intracellular Na^+ by opening stretch-activated channels (Liu et al., 1998). In hypertension, vascular smooth muscle cells are exposed to greater strain, which may open more stretch-activated channels. The Na^+ induced upregulation of the Na^+,K^+-ATPase α isoforms can be interpreted as an effort of the cell to increase the membrane Na^+ transport capacity so as to remove excessive Na^+ and restore normal intracellular Na^+ levels.

In our study, two different concentrations of ouabain (100 nM and 200 μM) produced clearly different effects on the α isoform expression, Na^+-pump activity and intracellular Na^+. In a pilot study, we treated aortic smooth muscle cells with 400 μM of ouabain for up to 4 days and the cells were viable at the end of the treatment as assessed by Trypan blue exclusion and were not morphologically different from the control cells. It is also noteworthy that ouabain treatment with either concentration did not affect the total protein content per well or the yield of membrane protein extraction used for Western blotting (Table 1). Therefore, the decline of both Na^+-pump activity and isoform expression with 200 μM of ouabain treatment may not be attributed to lysis and destruction of the cellular structure, but the saturation of the cell's compensatory capacity to pump sodium out due to near complete inhibition of the pump sites.

With 200 μM ouabain the isoform expression declined and the intracellular Na^+ concentration stayed at very high levels after prolonged treatment. If we assume the low affinity K_d in aortic smooth muscle cells is in the μM range (Noel and Godfraind, 1984), 100 nM of ouabain would only occupy and inhibit a portion of the binding sites (mostly the high affinity sites) (Deth and Lynch, 1980; Songu-Mize and Sanford, 1990), and the cells would be still able to compensate by synthesizing more Na^+-pump molecules to restore normal intracellular Na^+ concentrations. But with 200 μM of ouabain, almost all high affinity binding sites and a vast majority of low affinity binding sites should be occupied, and the resulting Na^+-pump blockade might be beyond the compensatory capacity of the cells.

In summary, chronic ouabain treatment of aortic smooth muscle cells results in transiently elevated intracellular Na^+ levels which is followed by the upregulation of the α-1 and α-2 subunit expression of Na^+,K^+-ATPase and an increase in Na^+-pump activity. These findings, together with our similar results from application of mechanical strain, suggest that any stimulus which raises intracellular sodium, even modestly, stimulates α-1 and α-2 subunit expression and thereby increases Na^+ extrusion capacity and eventually restores Na^+ homeostasis.

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