Stimulation of Na,K-ATPase by low potassium requires reactive oxygen species

Xiaoming Zhou,1 Wu Yin,1 Sonia Q. Doi,1 Shawn W. Robinson,2 Kunio Takeyasu,3 and Xuetao Fan1

1Department of Medicine, Uniformed Services University, Bethesda 20814; 2Department of Medicine, University of Maryland, Baltimore, Maryland 21201; and 3Department of Natural Environment Sciences, Kyoto University, Kyoto 606-01, Japan

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Zhou, Xiaoming, Wu Yin, Sonia Q. Doi, Shawn W. Robinson, Kunio Takeyasu, and Xuetao Fan. Stimulation of Na,K-ATPase by low potassium requires reactive oxygen species. Am J Physiol Cell Physiol 285: C319–C326, 2003.—The signaling pathway that transduces the stimulatory effect of low K+ on the biosynthesis of Na,K-ATPase remains largely unknown. The present study was undertaken to examine whether reactive oxygen species (ROS) mediated the effect of low K+ in Madin-Darby canine kidney (MDCK) cells. Low K+ increased ROS activity in a time- and dose-dependent manner, and this effect was abrogated by catalase and N-acetylcysteine (NAC). To determine the role of ROS in low-K+-induced gene expression, the cells were first stably transfected with expression constructs in which the reporter gene chloramphenicol acetyl transferase (CAT) was under the control of the avian Na,K-ATPase α-subunit 1.9 kb and 900-bp 5′-flanking regions that have a negative regulatory element. Low K+ increased the CAT expression in both constructs. Catalase or NAC inhibited the effect of low K+. To determine whether the increased CAT activity was mediated through releasing the repressive effect or a direct stimulation of the promoter, the cells were transfected with a CAT expression construct directed by a 96-bp promoter fragment that has no negative regulatory element. Low K+ also augmented the CAT activity expressed by this construct. More importantly, both catalase and NAC abolished the effect of low K+. Moreover, catalase and NAC also inhibited low-K+-induced increases in the Na,K-ATPase α1- and β1-subunit protein abundance and ouabain binding sites. The antioxidants had no significant effect on the basal levels of CAT activity, protein abundance, or ouabain binding sites. In conclusion, low K+ enhances the Na,K-ATPase gene expression by a direct stimulation of the promoter activity, and ROS mediate this stimulation and also low-K+-induced increases in the Na,K-ATPase protein contents and cell surface molecules.

Madin-Darby canine kidney cells; N-acetylcysteine; catalase

MANY TYPES OF CELLS INCREASE plasma membrane transport capacity of a nutrient or ion when confronted with the decreased concentration of that nutrient or ion in culture medium. For example, depletion of glucose or an amino acid from the culture medium evokes an increase in hexose or amino acid transport capacity (7, 8, 11, 19). Similarly, a decrease in extracellular K+ concentration leads to an enhanced Na,K-ATPase activity (3, 12, 15, 16).

All mammalian cells have inward Na+ and outward K+ gradients. Maintenance of these two ion gradients relies on the Na,K-ATPase that acts through coupled active transport of Na+ and K+ across the plasma membrane. In cell culture media, a decrease in K+ concentration <1 mM inhibits the activity of the Na,K-ATPase. In most mammalian cells, the Na,K-ATPase represents the only pathway to expel intracellular Na+ and uptake extracellular K+. Any malfunctioning or inhibition of the Na,K-ATPase will result in decreased cellular Na+ extrusion and K+ uptake. Under conditions of continuous passive leaks, there will be a net gain of intracellular Na+ and a net loss of intracellular K+, thus creating a decrease in the Na+ and K+ gradients. Cells respond to this stress by upregulation of the Na,K-ATPase. This process is constituted by increases in 1) Na,K-ATPase mRNA abundance within the first few hours, 2) Na,K-ATPase protein synthesis and abundance within 8 to 24 h, and 3) the function of the enzyme within 20 to 24 h (3, 12, 15, 16). A reduction of serum K+ concentration to about 3 mM by restriction of K+ intake also stimulates Na,K-ATPase activity with coordinated increases in the mRNA and protein abundances in the medullary collecting duct of rats (4, 6, 9, 13). However, the K+ concentration used in vitro is much lower than that used in vivo. A similar sequence of reactions is also induced when cells are exposed to a sublethal concentration of ouabain, a specific inhibitor of the enzyme (17, 18). The effect of low K+ on Na,K-ATPase activity is abolished when the Na+ concentration is reduced (2). Subsequent studies demonstrate that veratridine or monensin, used to increase Na+ entry, produces a similar effect on the Na,K-ATPase as does low K+ (15, 23, 26). The critical role of Na+ in the effect of low K+, therefore, has been established.

Although the effect of low K+ on the Na,K-ATPase has been repeatedly demonstrated both in vitro and in vivo, the role of ROS in the effect of low K+ remains largely unknown. The present study was under-
vivo, the signaling pathway that transduces the effect of low K\(^+\) remains largely unknown. Reactive oxygen species (ROS) are generated as byproducts of cellular metabolism. Over the past decade, ROS have emerged as an important integral component of membrane receptor signaling. ROS fulfill important prerequisites for intracellular messenger molecules; they are easily synthesized, highly diffusible, easily degraded, and ubiquitously present within all types of cells. Although ROS can be generated from a variety of sources, the ROS acting as messengers are usually produced from NADPH oxidase, a flavoprotein similar to the phagocytic NADPH oxidase. This enzyme produces ROS with rapid kinetics of activation and inactivation. The rapid kinetics allows a tight up- and downregulation of intracellular ROS levels within the short time required for transduction of signals (5). ROS have been demonstrated to be involved in the signaling pathways originating from growth receptors, cytokine receptors, receptor serine/threonine kinases, G protein-coupled receptors, and ion channel-linked receptors (5). In rat cardiomyocytes, the partial inhibition of the Na,K-ATPase by a nontoxic concentration of ouabain causes a rapid generation of ROS that is prevented by preexposure of the cells to the antioxidants N-acetylcysteine (NAC) or vitamin E. In parallel, these antioxidants also block or attenuate the ouabain-induced expression of genes that are related to cardiac hypertrophy like skeletal α-actin and atrial natriuretic factor (28). In Madin-Darby canine kidney (MDCK) cells, low K\(^-\) induced biosynthesis of the Na,K-ATPase is dependent on the intracellular iron activity that is important to the generation of ROS and is inhibited by catalase (30). In the present study, we examined whether the stimulation of the Na,K-ATPase by low K\(^-\) required ROS, using MDCK cells as a model.

**MATERIALS AND METHODS**

*Chemicals.* All chemicals were purchased from Sigma (St. Louis, MO). NAC was dissolved in control or low-K\(^-\) medium as a 300 mM stock solution, and pH of the stock solution was adjusted with 10 N NaOH.

*Cell culture.* MDCK cells were purchased from the American Type Culture Collection (Manassas, VA). The cells were kept in Dulbecco’s modified essential medium (DMEM) with 10% fetal bovine serum at 37°C in an atmosphere containing 5% CO\(_2\). The cells were collected after exposure to trypsin for 10 min and placed down at a high density. After incubation for 24 h, the cells became confluent and were then treated with either control or low-K\(^-\) medium for appropriate periods of time. The control medium contained K\(^-\)-free DMEM (JRH Biosciences, Lenexa, KS) plus 7.5% horse serum and 2.5% fetal bovine serum that had been dialyzed against K\(^+\)-free Hanks’ solution (150 mM NaCl, 0.5 mM MgCl\(_2\), 0.2 mM Na\(_2\)HPO\(_4\), 0.4 mM NaH\(_2\)PO\(_4\)) and was supplemented with 5.25 mM K\(^+\). All low-K\(^+\) media were identical to the control medium except some K\(^-\) was substituted by Na\(^+\) (3). In most of the experiments, the concentration of K\(^+\) in the low-K\(^-\) group was 0.1 mM, except for in the concentration-response study in which serially reduced K\(^+\) concentrations were used. The antibiotic gentamycin (25 μg/ml; Gibco BRL, Grand Island, NY) was included in all culture media.

*Ouabain binding assay.* The cells were placed down at 6 × 10\(^4\) cells/well in a 96-well plate. After treatments, the confluent cells were washed twice with Ca\(^2+\)-, K\(^-\)-free Hanks’ solution and incubated in the same solution plus 2 mM EGTA at 37°C for 15 min to disrupt tight junctions of the cells. Next, the cells were incubated in ouabain binding media at 37°C for an additional 15 min, washed four times with ice-cold Ca\(^2+\)-, K\(^-\)-free Hanks’ solution, and solubilized in 0.4 N NaOH. Radioactivity remaining in the cells was quantified by scintillation counting. The total binding medium contained 4 × 10\(^{-7}\) M [\(^3\)H]ouabain in Ca\(^2+\)-, K\(^-\)-free Hanks’ solution. This concentration of ouabain was demonstrated to be high enough to saturate binding sites (data not shown). Nonspecific binding was measured in the presence of 10\(^{-4}\) M unlabeled ouabain. Nonspecific binding was <2% of total binding. Specific ouabain binding was defined as the difference between total binding and nonspecific binding. Binding assays done in transwells were essentially the same as those in plastic dishes, except the cells were incubated at room temperature, and 2 mM CaCl\(_2\) was included in both binding media and washing solution to preserve the tight junctions of confluent monolayers of epithelial cells. Western blotting was performed, the medium from the basolateral side was checked for radioactivity to ensure no leakage of [\(^3\)H]ouabain and vice versa.

*Rb uptake assay.* The cells were placed down at 5 × 10\(^5\) cells/well in a 24-well plate. 86Rb was used as a cognate for measuring K\(^-\) uptake. After the tight junctions were disrupted, the cells were washed twice with Ca\(^2+\)-, K\(^-\)-free Hanks’ solution containing a suboptimal K\(^+\) concentration (2 mM KCl) and incubated with the same solution (total uptake) or plus 0.1 mM ouabain (background uptake) at 22°C for 30 min to saturate ouabain binding sites. The uptake was measured by adding 86Rb and incubating for 15 min at 22°C. This period of incubation was within the linear uptake range (data not shown). Rb\(^+\)(K\(^-\)) transport mediated by the Na,K-ATPase is the difference between total and background uptakes.

*Na,K-ATPase activity assay.* The cells were placed down at 5 × 10\(^5\) cells/well in a 24-well plate. After treatments, the confluent cells were washed twice with a hypotonic solution (1 mM MgCl\(_2\), 0.25 mM EDTA, 0.1% bovine serum albumin, and 1 mM imidazole, pH 7.4) and then incubated with the assay buffer (130 mM NaCl, 20 mM KCl, 4 mM MgCl\(_2\), 1 mM EGTA, 3 mM NaAzid, 0.1% saponin, and 30 mM imidazole-HCl, pH 7.4) or plus 10\(^{-4}\) M ouabain in background assays at 37°C for 20 min. Reactions were started by adding 3 mM ATP and incubated at 37°C for an additional 45 min. The release of inorganic phosphate (P\(_i\)) by the enzyme was within the linear range during the incubation period (data not shown). The liberated P\(_i\) was measured as absorption at 850 nm by the method of Baginski et al. (1). The Na,K-ATPase activity was defined as the difference between the total ATPase activity and the background ATPase activity and accounted for about 35% of the total ATPase activity in most cases.

*ROS measurement.* Separation of confluent monolayers of MDCK cells requires prolonged exposure to trypsin, which causes damage to the cells. Thus, for these experiments, cell suspensions trypsinized from plates of subconfluent cultures with 1 × 10\(^5\) cells/assay were used. The cells were preloaded with 20 μM freshly prepared 2′,7′-dichlorofluorescein diacetate (DCF-DA) in phenol red-free DMEM in some cases also with antioxidants at 37°C for 30 min, washed with 0.9% NaCl to remove K\(^+\), and then treated with phenol red-free control or low-K\(^-\) medium. After treatments, the cells were directly analyzed by flow cytometry with excitation at 475 nm and emission at 525 nm, using software System II (Coulter, Miami, FL). The gate was appropriately set to distinguish
oxidant-stressed cells from non-oxidant-stressed cells. The reading in the first control was arbitrarily assigned a value of 100%. The rest of the data were normalized to this value (33).

**Western analysis.** The cells were placed down at $8 \times 10^5$ cells/well in a 12-well plate. After treatments, the confluent cells were rinsed with ice-cold phosphate-buffered saline (PBS) and scraped with a rubber policeman in a loading buffer supplemented with 5% β-mercaptoethanol and 0.1 mg/ml phenylmethysulfonyl fluoride (PMSF) and 0.04 g/ml aprotinin. Samples were loaded into 10% sodium dodecyl sulfate-polyacrylamide gel in 12 g/lane, resolved by electrophoresis, and electrophoretically blotted onto PVDF membranes. The membranes were first hybridized with primary antibodies and then with horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG secondary antibody (Sigma). Antibody binding was visualized by the enhanced chemiluminescence method (Amersham, UK) (32) and semi-quantified by the software GelPro. The antibody against the α1-subunit of the Na$^+$,K$^+$-ATPase was a generous gift from Dr. Thomas A. Pressley (Texas Tech University Health Sciences Center) (14). Antibodies against β1-subunit and GAPDH were purchased from Upstate Biotechnology (Lake Placid.
NY) and RDI Research Diagnostics (Flanders, NJ), respectively.

Transfection and chloramphenicol acetyl transferase assay. The cells were placed down at $6 \times 10^5$ cells/60 mm dish overnight and then transfected with chloramphenicol acetyl transferase (CAT) expression plasmid constructs mixed with a vector pCB6 that confers G418 resistance. LipofectAMINE was used according to the manufacturer’s instructions (GIBCO BRL), and the cells were selected in growth medium supplemented with 600 μg/ml G418. For CAT assays, the cells were placed down at $8 \times 10^5$/well in a 12-well plate. After treatments, the cells were transferred into an Eppendorff tube with the lysis buffer included in the assay kit. A freezing and thawing method was used to rupture the cells. CAT assays were performed according to the manufacturer’s procedures (Promega, Madison, WI).

Statistical analyses. Data are expressed as means ± SE. Statistical analyses were performed using analysis of variance (ANOVA) or Student $t$-test as appropriate. Post hoc comparisons were made by Dunnett’s test. $P < 0.05$ was considered significant.

RESULTS

Low K$^+$ stimulates the function of the Na,K-ATPase. Because the parental MDCK cells are not homogeneous, subcloning was performed. Of nine clones screened, every single clone showed an increase in ouabain binding sites in response to low-K$^+$ stimulation (data not shown). However, clone A4 had the biggest response. Therefore, this clone was chosen for the rest of the study. The concentration-response analysis demonstrated a maximum response at 0.1 mM K$^+$. At this concentration, low K$^+$ increased ouabain binding sites from 18.4 ± 0.6 pmol/mg protein to 32.5 ± 1.9 pmol/mg protein.

Fig. 3. Diphenylene iodonium (DPI) inhibits low-K$^+$-induced ROS activity. A: control. B: 0.1 mM [K$^+$]. C: control plus 5 μM DPI. D: 0.1 mM [K$^+$] plus 5 μM DPI. ROS activity was measured 10 min after low K$^+$ with DCF-DA-based fluorescence imaging. The cells were preincubated with DPI for 30 min before exposure to low K$^+$.

Fig. 4. Catalase or NAC inhibits low-K$^+$-induced Na,K-ATPase promoter activity. The reporter gene, chloramphenicol acetyl transferase (CAT), was under the control of sequentially deleted 5'-flanking region of the avian $\alpha$-subunit designated as pc1.9kbCAT, pc900bpCAT, and pc96bpCAT, respectively. CAT assays were performed 24 h after the addition of low-K$^+$ medium. The reading in each experiment was normalized to the mean of readings of control experiments ($n = 4$). The mean of readings obtained under control conditions was arbitrarily assigned a value of 100%. All data are expressed as relative values to this number. The cells were pre-treated with an antioxidant for 30 min before exposure to low K$^+$ ($^{a}P < 0.05$, Student’s $t$-test).
pmol/mg protein \( (P < 0.01; \text{Fig. 1A}) \). The time-course study revealed that the effect of low K\(^+\) on ouabain binding began after a lag time of more than 12 h and reached a plateau by 20 h. During this interval, the number of ouabain binding sites increased by 60 to 77\% (Fig. 1B). The absolute number of ouabain binding sites did not significantly change in the control medium throughout this time course (data not shown).

Exposure to low-K\(^+\) medium also increased the Na,K-ATPase activity from 5.3 ± 1.1 nmol P\(_i\)/mg min\(^{-1}\) to 11.2 ± 1.1 nmol P\(_i\)/mg min\(^{-1}\) \( (P < 0.005; \text{Fig. 1C}) \), the Na,K-ATPase-mediated Rb\(^+/K\(^+\) transport rate from 11.5 ± 0.6 nmol/mg min\(^{-1}\) to 22.9 ± 1.5 nmol/mg min\(^{-1}\) \( (P < 0.005; \text{Fig. 1D}) \), and \( \alpha_1 \)- and \( \beta_1 \)-subunit protein contents from 98.2 ± 2.5\% to 168.1 ± 5.0\% and 99.0 ± 2.7\% to 267.1 ± 31.5\%, respectively \( (P < 0.005, P < 0.05; \text{Fig. 1E}) \). To examine whether the effect of low K\(^+\) was polarized, ouabain binding assays were conducted on the cells grown in transwells. The effect of low K\(^+\) was mainly restricted to the basolateral domain, although the percentage increase was less than that observed in the apical membrane. Low K\(^+\) increased the number of basolateral ouabain binding sites from 28.3 ± 2.0 pmol/mg to 50.8 ± 5.0 pmol/mg \( (P < 0.005; \text{Fig. 1F}) \).

**Low K\(^+\) stimulates ROS activity.** Low K\(^+\) also increased the intracellular ROS activity in a dose- and time-dependent manner as determined by DCF-DA-based flow cytometry (Fig. 2). Low K\(^+\) rapidly increased ROS activity, and the effect of low K\(^+\) reached the peak within 10 min. Catalase inhibits Cr(VI)-induced generation of ROS and activation of p53 in a human lung epithelial cell line (24). Catalase or NAC, another well-known antioxidant, blocked the effect of low K\(^+\) on ROS production (Fig. 2B). Low-K\(^+\)-induced ROS activity was also suppressed by 5 \( \mu \)M diphenylene iodonium, an inhibitor of the NADPH oxidase (Fig. 3).

**ROS mediate the effect of low K\(^+\) on the Na,K-ATPase.** The increased ROS activity induced by low K\(^+\) that occurs at early stages implies that ROS may be required for the stimulation of the Na,K-ATPase gene expression. To address this issue, the cells were first stably transfected with expression constructs in which the reporter gene CAT was under the control of the avian Na,K-ATPase \( \alpha \)-subunit 1.9-kb and 900-bp 5\'-flanking regions that have a negative regulatory element (31). Because the CAT activity in the mixture of transfectants was not detectable, subcloning was performed, and positive clones were identified by CAT activity. Low K\(^+\) increased the CAT expression in both
constructs. Catalase or NAC inhibited the effect of low K\(^+\) but had no significant effect on basal CAT activity (Fig. 4). To determine whether the increased CAT activity induced by low K\(^+\) was mediated through releasing the repressive effect or a direct stimulation of the promoter, the cells were transfected with a CAT expression construct that is directed by a 96-bp promoter fragment that is located immediately upstream to the coding region and has no negative regulatory element (31). Because the mixture of G418-resistant cells displayed CAT activity, CAT assays were conducted directly in the pool of the G418-resistant cells without subcloning. Low K\(^+\) also augmented the CAT activity expressed by this construct, implying that the effect of low K\(^+\) is due to the direct stimulation of the promoter activity. More importantly, both catalase and NAC abolished the low-K\(^+\)-induced CAT activity, which suggests that ROS mediate the stimulation of low K\(^+\) on the \(\alpha\)-subunit promoter (Fig. 4).

Low K\(^+\) elevates the \(\alpha\)- and \(\beta\)-subunit mRNA levels associated with increased protein abundance of the enzyme (3, 16). To test whether the antioxidants also suppressed the effect of low K\(^+\) at the protein level, Western analyses were employed first to determine the linear relationship between protein loading and the chemiluminescence signal. Figure 5, A and B, shows that the amount of protein loading between 6 and 15 \(\mu\)g was within the linear range of both \(\alpha\)- and \(\beta\)-subunit detections. Next, the same analyses were used to examine the effect of antioxidants. As shown in Fig. 5, C and D, catalase or NAC inhibited low-K\(^+\)-induced increases in \(\alpha\)- and \(\beta\)-subunit protein abundance. The \(\beta\)-subunit showed a bigger increase than the \(\alpha\)-subunit. It is possible that the \(\beta\)-subunit is more responsive than the \(\alpha\)-subunit. The \(\beta\)-subunit also exhibited a greater response to hyperoxia than the \(\alpha\)-subunit in MDCK cells (25). In a low electrical field (20 volts), the \(\beta\)-antibody recognized four closely migrated faint bands that most probably result from different stages of glycosylation (22). In a high electrical field (40 volts), only one band was detected as shown in Fig. 5. Inhibition of low-K\(^+\)-induced increases in protein abundance by the antioxidants would be expected to result in inhibition of low-K\(^+\)-induced increases in cell surface presence of Na,K-ATPase molecules. To test this possibility, the effect of low K\(^+\) on ouabain binding sites was examined in the presence of catalase or NAC. Catalase at 500 U/ml or NAC at 35 mM almost completely inhibited the effect of low K\(^+\), whereas catalase or NAC had no significant effect under the control conditions (Fig. 6, A and B). NAC at 35 mM did not induce cell death. Similarly, diphenylene iodonium also abolished the effects of low K\(^+\) on ouabain binding sites (Fig. 6C).

**DISCUSSION**

The MDCK cell is one of the best-characterized in vitro models for study of the effect of low K\(^+\) on the Na,K-ATPase. With the use of this model, the present study has demonstrated that 1) low K\(^+\) increased ROS activity, and 2) suppression of ROS activity by catalase or NAC blocked low-K\(^+\)-induced promoter activity, protein abundance, and cell surface molecules of the Na,K-ATPase. Because low K\(^+\) stimulates the Na,K-ATPase via increased mRNA abundance and protein synthesis (3, 12, 15, 16), the present study argues for ROS me-
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mediating low K⁺-induced gene expression of the Na,K-ATPase, which leads to the increment in the protein abundance and function of the enzyme.

By CAT activity assays, Yu et al. (31) have identified that the avian α-subunit gene contains a repressive region located upstream to the promoter region. Gel retardation assays reveal the existence of a set of proteins binding to this repressive region, although the binding amount of each individual protein varies in different types of cells (31). Low K⁺ not only increased CAT activity directed by the avian 1.9-kb and 900-bp 5'-flanking regions that have the negative regulatory element, but also the CAT expression controlled by the 96-bp promoter region that does not contain the negative regulatory element. More importantly, both catalase and NAC abrogated the effect of low K⁺ in every construct (Fig. 4). These data suggest that the stimulatory effect of low K⁺ on the α-subunit gene expression results from the direct stimulation of the promoter activity instead of from lifting repressors and that ROS mediate this stimulation. Substantial evidence suggests that the transient production of ROS is an important signaling event that mediates a variety of agonist-induced gene expressions, most of which are related to cell hypertrophy and proliferation. ROS stimulate the promoter activity of a gene directly or through other signaling cascades, like protein tyrosine phosphorylation and MAP kinases (5). The effect of low K⁺ is mitogenic (3). The present finding is within the purview of this paradigm.

However, how ROS stimulate the α-subunit promoter activity remains unknown. ROS regulate activities of several transcription factors, most notably nuclear factor-κB (NF-κB) and activator protein-1 (AP-1). Although the 5'-flanking region of the α-subunit gene has six AP-1 and AP-2 binding sites, none of these is located in this 96-bp region. Rather, this region is nucleotide GC-rich with five putative Sp-1 targeted sequences (31). The Sp-1 binding site is present in both α- and β-subunit promoters across isoforms and species (10, 20, 21). In rat cardiac myocytes, the increased β1-subunit gene transcription by low K⁺ is mediated by increasing Sp-1/Sp-3 binding to the promoter region (34). Sp-1 is known to be modulated by the redox state. Moreover, upregulation of the β1-subunit of the Na,K-ATPase by hyperoxia in MDCK cells is mediated by stimulation of Sp-1/Sp-3 activity (25). It is plausible to speculate that low K⁺ increases the activity of ROS that enhance the binding of Sp-1/Sp-3 to the promoter, thereby increasing transcription of the Na,K-ATPase gene.

The discovery of the inhibitory effect of catalase and NAC on low-K⁺-induced increases in Na,K-ATPase protein abundance and ouabain binding sites is somewhat surprising. In contrast to ARL15 cells, a rat hepatoma cell line, in which low K⁺ induces a constant elevation of the Na,K-ATPase mRNA level (16), in MDCK cells, a previous report (3), in addition to our own studies (data not shown), reveals that low K⁺ only transiently increases the Na,K-ATPase mRNA abundance. The return of the mRNA abundance to the basal level within 3 h of exposure to low-K⁺ medium implies that transcriptional regulation may not be significant in the effect of low K⁺ on the Na,K-ATPase protein abundance. However, the inhibition of the Na,K-ATPase protein abundance by the antioxidants argues against this possibility. In outer medullary kidney tubules or chick skeletal muscle, ouabain- or veratridine-induced increases in Na,K-ATPase activity is constituted by two steps: 1) increased protein synthesis at an early stage and 2) decreased protein degradation at a late stage (18, 26). It is not clear whether ROS are also involved in stabilizing the Na,K-ATPase protein.

Low K⁺ not only stimulates the biosynthesis of the Na,K-ATPase but also induces general protein synthesis (3, 16, 29). The Na,K-ATPase mediates a ouabain-induced mitogenic effect in cardiac myocytes by interacting with neighboring membrane proteins like Src and epidermal growth factor receptors, which send messages to nuclei through organized cytosolic cascades of signaling events. Among those signaling events, ROS and intracellular Ca²⁺ are essential second messengers (27). Whether the Na,K-ATPase also acts as a signal transducer that mediates the pleiotropic effect of low K⁺ remains to be determined.

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DISCLOSURES

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