Modulation of $\text{Na}^+\text{-K}^+$-ATPase cell surface abundance through structural determinants on the $\alpha_1$-subunit

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Address for reprint requests and other correspondence: S. V. Pierre, Dept. of Physiology and Pharmacology, Univ. of Toledo College of Medicine, Health Science Campus, 3000 Arlington Av., Toledo, OH 43614-2598 (e-mail: Sandrine.Pierre@utoledo.edu).

Pierre SV, Belliard A, Sottejeau Y. Modulation of $\text{Na}^+\text{-K}^+$-ATPase cell surface abundance through structural determinants on the $\alpha_1$-subunit. Am J Physiol Cell Physiol 300: C42–C48, 2011. First published November 3, 2010; doi:10.1152/ajpcell.00386.2010.—Through their ion-pumping and non-ion-pumping functions, $\text{Na}^+\text{-K}^+$-ATPase protein complexes at the plasma membrane are critical to intracellular homeostasis and to the physiological and pharmacological actions of cardioioderetic steroids. Alteration of the abundance of $\text{Na}^+\text{-K}^+$-ATPase units at the cell surface is one of the mechanisms for $\text{Na}^+\text{-K}^+$-ATPase regulation in health and diseases that has been closely examined over the past few decades. We here summarize these findings, with emphasis on studies that explicitly tested the involvement of defined regions or residues on the $\text{Na}^+\text{-K}^+$-ATPase $\alpha_1$ polypeptide. We also report new findings on the effect of manipulating $\text{Na}^+\text{-K}^+$-ATPase membrane abundance by targeting one of these defined regions: a dileucine motif of the form [D/E][XXX][L/I]. In this study, opossum kidney cells stably expressing rat $\text{Na}^+\text{-K}^+$-ATPase or a mutant where the motif was disrupted ($\alpha_1\text{-L499V}$) were exposed to 30 min of substrate/coverslip-induced-ischemia followed by reperfusion (I-R). Biotinylation studies suggested that I-R itself acted as an inducer of $\text{Na}^+\text{-K}^+$-ATPase internalization and that surface expression of the mutant was higher than the native $\text{Na}^+\text{-K}^+$-ATPase before and after ischemia. Annexin V/protopidium iodide staining and lactate dehydrogenase release suggested that I-R injury was reduced in $\alpha_1\text{-L499V}$-expressing cells compared with $\alpha_1$-expressing cells. Hence, modulation of $\text{Na}^+\text{-K}^+$-ATPase cell surface abundance through structural determinants on the $\alpha_1$-subunit is an important mechanism of regulation of cellular $\text{Na}^+\text{-K}^+$-ATPase in various physiological and pathophysiological conditions, with a significant impact on cell survival in face of an ischemic stress.

The $\text{Na}^+\text{-K}^+$-ATPase is the membrane-spanning enzyme that both establishes and maintains the electrochemical gradient across the plasma membrane of animal cells by coupling the hydrolysis of ATP to the transport of Na$^+$ and K$^+$ (23, 43). The $\text{Na}^+\text{-K}^+$-ATPase complex consists of two dissimilar $\alpha$- and $\beta$-subunits, which exist as multiple isoforms. The $\alpha$-subunit is the primary contributor to overall catalysis and contains the binding sites for the substrates required by the enzyme. Expression of the $\alpha_1$-isoform is apparently ubiquitous, while the three others ($\alpha_2$–$4$) have increasingly restricted expression patterns (5, 6). Three distinct isoforms of the $\beta$-subunit, which is critical to the structural and functional maturation of $\text{Na}^+\text{-K}^+$-ATPase and regulates its transport properties, have been identified (21). In addition, several members of the FXYD family of accessory proteins have been shown to bind to and regulate $\text{Na}^+\text{-K}^+$-ATPase function in a tissue-specific manner (19, 20).

The $\text{Na}^+\text{-K}^+$-ATPase is also the pharmacological target of endogenous and exogenous cardioioderetic steroids (CTS). CTS have long been known as potent inhibitors of $\text{Na}^+\text{-K}^+$-ATPase ion-pumping function, which is critical to their effect on $\text{Na}^+$-coupled influx of ions, amino acids, or glucose. This inhibitory action on $\text{Na}^+\text{-K}^+$-ATPase ion-pumping function and subsequent modulation of the $\text{Na}^+\text{/Ca}^{2+}$ exchange has been extensively studied in the cardiac positive inotropic action of CTS. In addition, CTS such as ouabain, digoxin, or marinobufogenin, initiate intracellular signaling cascades via stimulation of the $\text{Na}^+\text{-K}^+$-ATPase receptor function (30, 36, 47, 48). The role of this more recently discovered property in the hormone-like function of endogenous CTS and in the therapeutic effect of exogenous CTS in health and diseases is being increasingly recognized. Progress in the understanding of CTS action in the cardiovascular and nervous systems, metabolism, or cell growth and differentiation has been emphasized in recent reviews (1, 2, 40, 42).

**Regulation of $\text{Na}^+\text{-K}^+$-ATPase Cell Surface Abundance and Known Structural Determinants on the $\text{Na}^+\text{-K}^+$-ATPase $\alpha_1$ Polypeptide**

Localization of $\text{Na}^+\text{-K}^+$-ATPase at the cell surface is important to both ion-pumping and receptor functions, and modulation of cellular $\text{Na}^+\text{-K}^+$-ATPase activity through changes in cell surface expression has been reported in response to major physiological or pathophysiological stimuli. Such stimuli include CTS themselves (32, 45), the parathyroid hormone (24), dopamine (4), insulin (3, 12, 18), hypoxia (14), and hypercapnia (46). Over the past 15 years, investigations using heterologous expression systems have focused on the identification of key structural determinants along the $\text{Na}^+\text{-K}^+$-ATPase $\alpha_1$ polypeptide that influence its expression at the cell surface under basal conditions or in response to specific stimuli. Data from such studies are compiled in Table 1. We have recently examined one of these molecular determinants, a dileucine-based motif for recognition by clathrin-coated vesicle (CCV) adaptor proteins of the structure $n(p)_2\_L\_LL$, where $n$ is a negatively charged residue and $p$ is a polar residue (26). The sequence is well conserved among all the known mammalian $\alpha_1$ sequences (Table 2), and our studies revealed that mutations targeting this motif such as L499V or E495S resulted in an increased abundance of $\text{Na}^+\text{-K}^+$-ATPase $\alpha_1$-units at the cell surface (44).
Using a Na\(^{+}\)-K\(^{+}\)-ATPase α1 Structural Determinant of Surface Abundance as a Target for Protection Against Ischemia-Reperfusion Injury

We reckoned that an increased abundance of Na\(^{+}\)-K\(^{+}\)-ATPase pump units at the cell surface could be salutary to cells with critically high levels of intracellular Na\(^{+}\) such as those reported during ischemia-reperfusion (I-R) injury and may result in protection against I-R-induced cell death (34, 35). This hypothesis was tested in opossum kidney (OK) cells stably expressing native or L499V-mutated forms of Na\(^{+}\)-K\(^{+}\)-ATPase α1 polypeptide exposed to substrate/coverslip-induced I-R.

METHODS

Cell Lines

OK cells stably expressing native and L499V-mutated forms of Na\(^{+}\)-K\(^{+}\)-ATPase α1 were used. Details on the experimental procedures related to expression vectors and site-directed mutagenesis, heterologous expression, and initial characterization of Na\(^{+}\)-K\(^{+}\)-ATPase enzyme properties in these cells can be found in Sottejeau et al. (44).

Substrate and Coverslip-Induced Ischemia-Reperfusion

Ischemia was induced by removal of the substrate and placement of a glass coverslip over a portion of the OK cell monolayers, as described previously (38). Briefly, 70% confluent OK cells grown in 100-mm dishes were rinsed once with PBS and incubated in Krebs-Henseleit (KH) buffer containing (in mmol/l) 118.0 NaCl, 4.0 KCl, 1.8 CaCl\(_2\), 1.3 KH\(_2\)PO\(_4\), 1.2 MgSO\(_4\), 0.3 EGTA, 25 NaHCO\(_3\), and 37 D-glucose for 20 min at 37°C. Ischemia was then simulated by removing the LifterSlips and returned to KH buffer at 37°C. For confocal imaging studies, OK cells were grown on square coverslip 22 × 22 mm (Fisher) in six-well plates, and I-R was induced as described above using 18-mm diameter round glass coverslips (Fisher).

Annexin V/Propidium Iodide Staining

At the end of the experiment protocol, OK cells were fixed in 2% paraformaldehyde for 10 min at room temperature after a wash in PBS 1X. Cells were then incubated with Alexa Fluor 488 annexin V and red fluorescent propidium iodide (PI) (Vybrant Apoptosis Assay Kit no. 2, Invitrogen) according to the manufacturer’s recommendations. The coverslips were mounted with ProLong Gold antifade reagent (Invitrogen). Confocal images were captured by sequential scanning with no overlap using a Leica TCS SP5 broadband confocal microscope system coupled to a DMI 6000S inverted microscope equipped with multiple continuous wave lasers and a ×63/1.3 oil objective.

Measurement of Lactate Dehydrogenase Activity

At the end of a 60-min long reperfusion period, the cell incubation buffer was collected and lactate dehydrogenase (LDH) activity was determined colorimetrically using a standard assay (Cytotoxicity Detection Kit, Roche Applied Science), according to the manufacturer recommendations.

Assessment of Na\(^{+}\)-K\(^{+}\)-ATPase α1 Total Protein Abundance and Surface Expression

Total abundance of the introduced rat α1 constructs was determined by electrophoresis and immunoblotting of proteins from cell lysates using anti-NASE antibody as described (44). For total expression, equal loading of the samples among the lanes of the gel was recommended.

Table 1. Summary of domains and sites of posttranslational modifications involved in the regulation of rat Na\(^{+}\)-K\(^{+}\)-ATPase α1 surface expression

<table>
<thead>
<tr>
<th>Structural Determinant</th>
<th>Trigger/Signal Cascade</th>
<th>α1 Surface Expression</th>
<th>Ref. No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine-based domain for AP-binding</td>
<td>Ang II/AT1/AP1</td>
<td>Up</td>
<td>17</td>
</tr>
<tr>
<td>IVYY-255</td>
<td>Dopamine/DR1/AP2</td>
<td>Down</td>
<td>9, 13</td>
</tr>
<tr>
<td>Tyrosine-based domain for AP-binding</td>
<td>Hyoxia</td>
<td>None</td>
<td>44</td>
</tr>
<tr>
<td>537-YLEL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dileucine-based motif for AP-binding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPKHL-499L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorylation/S-18</td>
<td>Dopamine</td>
<td>Down</td>
<td>10</td>
</tr>
<tr>
<td>Phosphorylation/S-18</td>
<td>Hyoxia/PKC</td>
<td>Down</td>
<td>14</td>
</tr>
<tr>
<td>Phosphorylation/S-11</td>
<td>PTH/PKC/ERK/CCV</td>
<td>Down</td>
<td>24</td>
</tr>
<tr>
<td>Ubiquitination/K-16/K-17/K-19/K-20</td>
<td>Hyoxia/ubiquitination</td>
<td>Down</td>
<td>15</td>
</tr>
<tr>
<td>Proline-rich domain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPPPTTP-87</td>
<td>Dopamine</td>
<td>Down</td>
<td>49</td>
</tr>
</tbody>
</table>

ANG II, angiotensin II; AT1, Type 1 angiotensin II receptor; AP1 and AP2, clathrin adaptor protein 1 and 2; DR1, dopamine receptor 1; PKC, protein kinase C; PTH, parathyroid hormone; ERK, extracellular signal-regulated kinase; CCV, clathrin-coated vesicle.

Table 2. Conserved dileucine motif of the form [D/E][X][X][X][L/I] motif in Na\(^{+}\)-K\(^{+}\)-ATPase α1 sequences in various species

<table>
<thead>
<tr>
<th>Species</th>
<th>NCBI Access No.</th>
<th>[D/E][X][X][X][L/I] Motif</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rattus norvegicus (rat)</td>
<td>NM_012504</td>
<td>SIHK497NPNASPEKHL498LVMK</td>
</tr>
<tr>
<td>Homo sapiens (human)</td>
<td>NM_000701</td>
<td>SIHK497NPNASPEKHL498LVMK</td>
</tr>
<tr>
<td>Dario rerio (zebrafish)</td>
<td>NM_131686</td>
<td>SIHK497NPNASPEKHL498LVMK</td>
</tr>
<tr>
<td>Ovis aries (sheep)</td>
<td>NM_001009360</td>
<td>SIHK497NPNASPEKHL498LVMK</td>
</tr>
<tr>
<td>Mus musculus (mouse)</td>
<td>NM_144900</td>
<td>SIHK497NPNASPEKHL498LVMK</td>
</tr>
<tr>
<td>Sus scrofa (pig)</td>
<td>NM_214249</td>
<td>SIHK497NPNASPEKHL498LVMK</td>
</tr>
<tr>
<td>Bos taurus (cattle)</td>
<td>BC123864</td>
<td>SIHK497NPNASPEKHL498LVMK</td>
</tr>
<tr>
<td>Canis familiaris (dog)</td>
<td>NM_001003306</td>
<td>SIHK497NPNASPEKHL498LVMK</td>
</tr>
<tr>
<td>Gallus gallus (chicken)</td>
<td>NM_205521</td>
<td>SIHK497NPNASPEKHL498LVMK</td>
</tr>
</tbody>
</table>

The conserved sequence of amino acid residues that form the dileucine motif appears in boldface.
confirmed by probing with a commercial antibody against actin (Santa Cruz Biotechnology, Santa Cruz, CA). Introduced α1 expressed at the cell surface was detected by biotinylation following the recommended procedures of Gottardi et al. (22), as we have recently reported in detail (44). I-R-induced endocytosis of Na\(^+\)-K\(^-\)\(-ATPase\) α1 units was tested with a pulse–chase strategy as slightly modified from previous studies (27, 44). Briefly, proteins expressed at the cell surface were first biotinylated as described above, quenched with PBS–glycine buffer, and rinsed twice with saline solution. The cells were then incubated in DMEM medium for 20 min at 37°C in 10% CO\(_2\) followed by 30 min of ischemia and 30 min of reperfusion. The remaining surface-bound biotin was then cleaved by treatment with 50 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP) reducing agent for 15 min at 4°C.

**Immunocytochemistry and Fluorescence Imaging**

At the end of the experimental protocol, cells were fixed by 20 min incubation with ice-cold methanol, washed with PBS, and blocked with Signal Enhancer (Invitrogen). The cells were then incubated with a mouse anti-Na\(^+\)-K\(^-\)\(-ATPase\) α1 monoclonal antibody (clone C464.6, Upstate) confirmed by probing with a commercial antibody against actin (Santa Cruz Biotechnology, Santa Cruz, CA). Introduced α1 monoclonal antibody (clone C464.6, Upstate) in PBS containing 1% bovine serum albumin for 2 h at room temperature. After three washes with PBS, cells were exposed to AlexaFluor 488-conjugated anti-mouse secondary antibody for 2 h at room temperature, washed, and mounted onto slides. Image visualization was performed using a Leica TCS SP5 broadband confocal microscope system coupled to a DMI 6000CS inverted microscope.

**Statistical Analysis**

Statistical analysis was conducted using one-way ANOVA followed by Tukey’s multiple comparison post hoc test. \(P < 0.05\) was considered statistically significant.

**RESULTS**

**Decreased Post-I-R Cell Death in Cells Expressing the Na\(^+\)-K\(^-\)\(-ATPase\) α1-L499V Mutant**

In vitro I-R was induced in OK cells by removing the metabolic substrates from the culture medium and by placing coverslips over the monolayer, according to the protocol of Pitts and Tombs (38). Whereas all cells were exposed to substrate depletion for 30 min, it is important to note that the three LifterSlips represented about 57% of the surface of the 100-mm diameter dishes and hence did not cover the entire monolayer. As shown in Fig. 1, this resulted in a significant increase in LDH release in the media over the course of 60 min of reperfusion (IR60), indicative of cell injury. The LDH release by α1-expressing cells was comparable to that observed in nontransfected OK cells (not shown) but was significantly higher than the release measured in α1-L499V-expressing cells exposed to the same I-R protocol. To further assess the I-R-induced decreased cell viability and the comparatively lower expression in the α1-L499V-expressing group, cells grown on coverslips were exposed to control and I-R conditions as detailed in METHODS and stained with Alexa Fluor 488 annexin V to label apoptotic cells and red-fluorescent PI to label late apoptotic/necrotic cells. The representative pictures shown in Fig. 2 present qualitative evidence that 30 min of substrate/coverslip-induced ischemia followed by 60 min of reperfusion resulted in an increase in the annexin V\(^+\) population in α1-expressing cells that was more pronounced than the increase produced in α1-L499V-expressing cells. A few PI\(^+\) cells defined as cells with nuclear PI signal [i.e., overlapping with DAPI fluorescence, which excludes potential “false-positive” with cytoplasmic PI signal only (41)] were detected after I-R. Specifically, 4 PI\(^+\) cells per 100 cells were detected in the α1-group after I-R, and 1 PI\(^+\) cell per 100 cells was detected in the α1-L499V-expressing group.

**Increased Post-I-R Surface Abundance in Cells Expressing the Na\(^+\)-K\(^-\)\(-ATPase\) α1L499V Mutant**

With the use of biotinylation techniques, Na\(^+\)-K\(^-\)\(-ATPase\) surface expression was compared in OK cells stably expressing native and L499V Na\(^+\)-K\(^-\)\(-ATPase\) α1 with or without exposure to 30 min ischemia and 5 min reperfusion (IR5). The data presented in Fig. 3 confirmed our previously reported finding that basal surface expression of Na\(^+\)-K\(^-\)\(-ATPase\) α1-units is significantly higher in the mutant group without change in total expression (44). After 5 min of reperfusion, total expression of Na\(^+\)-K\(^-\)\(-ATPase\) α1 was unchanged (3B), but its surface expression was significantly decreased in both α1- and α1-L499V Na\(^+\)-K\(^-\)\(-ATPase\)-expressing cells compared with their respective controls. The I-R-induced decrease was about 25–30% for both groups. As a result, the post-I-R surface expression in the α1-L499V-expressing cells was comparable to the pre-I-R level in the α1-expressing group.

**I-R Induces Internalization of Na\(^+\)-K\(^-\)\(-ATPase\) Units**

The data collected from the biotinylination studies presented in Fig. 3 suggested that I-R results in decreased abundance of Na\(^+\)-K\(^-\)\(-ATPase\) α1 units at the cell surface. To test whether this was due to an increased internalization of Na\(^+\)-K\(^-\)\(-ATPase\) α1 units during I-R, we compared Na\(^+\)-K\(^-\)\(-ATPase\) removal from the cell surface using a cell surface biotinylation and TCEP treatment (see METHODS) in α1-expressing cells in control conditions (80 min pulse-chase aerobic buffer) or exposed to the IR30 protocol (20 min aerobic buffer, 30 min...
substrate/coverslip ischemia, 30 min aerobic buffer). As shown in Fig. 4A, the amount of Na\(^{+}\)-K\(^{+}\)-ATPase internalized in 80 min was significantly increased in the IR30 group compared with the control (\(P < 0.01\)). Immunofluorescent labeling of Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha_1\) units before and after I-R was consistent with increased intracellular signal after 30 min of reperfusion (Fig. 4B).

**DISCUSSION**

In this article, we review the growing list of physiological and pathological regulators of Na\(^{+}\)-K\(^{+}\)-ATPase surface abundance with emphasis on studies that explicitly tested the involvement of defined regions or residues on the Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha_1\) polypeptide. We also report new findings on the potential protective effect of manipulating Na\(^{+}\)-K\(^{+}\)-ATPase surface abundance during I-R injury by targeting one of these defined regions.

**Regulation of Na\(^{+}\)-K\(^{+}\)-ATPase Cell Surface Abundance and Structural Determinants on the \(\alpha_1\)-Subunit**

The concept that membrane trafficking is an important regulator of Na\(^{+}\)-K\(^{+}\)-ATPase is not a new one. In fact, early studies like those of Lamb and Ogden in HeLa cells pointed to CTS-induced changes in surface expression more than 30 years ago (28). Over the past two decades, this phenomenon has been observed in many other models, and a considerable amount of knowledge has been accumulated on the multiple physiological and pathological regulators of Na\(^{+}\)-K\(^{+}\)-ATPase surface abundance. Based on the results of our in vitro study in OK cells presented in Figs. 3 and 4, we propose that I-R be added to the growing list of those regulators. Studies that identified regulators also provided insights into the cellular pathways and compartments involved, but we are just beginning to understand the role of structural determinants on Na\(^{+}\)-K\(^{+}\)-ATPase enzyme complex in the integrated response to a given stimulus. As shown in Table 1, most of the determinants identified on the \(\alpha_1\) polypeptide are located within the amino terminal part or the large cytoplasmic loop of the molecule. Additional determinants and mechanisms of regulation of surface abundance remain to be identified, and studies like those by Kimura et al. on the regulation of Na\(^{+}\)-K\(^{+}\)-ATPase trafficking by arrestins and spinophilin (25) point to additional roles for the large intracellular loop in particular.

**I-R-Induced Decrease of Na\(^{+}\)-K\(^{+}\)-ATPase Cell Surface Abundance**

The results from this study are consistent with an I-R-induced internalization of Na\(^{+}\)-K\(^{+}\)-ATPase units in OK cells. As shown in Fig. 3, the extent of I-R-induced internalization is
Mechanism of Protection Against I-R-Induced Injury

According to the data presented in Figs. 1 and 2, an increased number of Na\(^{+}-K\(^{-}\)-ATPase units at the cell surface correlates with an increased tolerance to I-R in \(\alpha_1\)-L499V-expressing cells. However, these studies do not reveal the underlying mechanism of protection. Additional Na\(^{+}-K\(^{-}\)-ATPase ion-pumping capacity at the cell surface may help preserve intracellular ion homeostasis during I-R, but studies like our initial characterization of the \(\alpha_1\)-L499V mutant itself (44) or the graded knockdown of \(\alpha_1\) subunit (31) have shown that surface expression does not necessarily correlate with increased ion-pumping function. In fact, other non-ion-pumping functions of Na\(^{+}-K\(^{-}\)-ATPase may be involved, such as survival signaling or preservation of the integrity of intracellular structures (7, 8, 45). Clearly, further investigation in cells exposed to I-R is needed to clarify the relative contribution of Na\(^{+}-K\(^{-}\)-ATPase ion-pumping and non-ion-pumping functions in the protection afforded by increased cell surface expression.

In conclusion, a substantial number of studies have underscored the importance of surface abundance modulation in the

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**Fig. 3.** Surface and total expression of Na\(^{+}-K\(^{-}\)-ATPase units in \(\alpha_1\)- and \(\alpha_1\)-L499V-expressing OK cells exposed to 30 min of substrate/cover-slip-induced ischemia followed by 5 min of reperfusion. \(\alpha_1\), typical immunoblot of biotinylated membrane proteins recovered by affinity purification with streptavidin. Bottom, pooled data relative to basal expression of wild-type \(\alpha_1\) represented as means ± SE (n = 8–10). **P < 0.05 and ***P < 0.001 vs. control \(\alpha_1\); #P < 0.01 vs. \(\alpha_1\)-L499V, and $P < 0.01 vs. IR5 \(\alpha_1\). B: total expression. Top, typical immunoblots of cell lysates probed with antibodies specific for rat \(\alpha_1\) and actin. Bottom, pooled data relative to basal expression of wild-type \(\alpha_1\), represented as means ± SE (n = 8–10). No significant difference was found. IR5, exposed to 30 min coverslip-induced ischemia followed by 5 min of reperfusion.

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**Fig. 4.** Internalization of Na\(^{+}-K\(^{-}\)-ATPase units in cells exposed to 30 min of substrate/cover-slip-induced ischemia followed by 30 min of reperfusion. After treatment, assessment of the amount of endocytosed Na\(^{+}-K\(^{-}\)-ATPase \(\alpha_1\) units and immunofluorescent staining of Na\(^{+}-K\(^{-}\)-ATPase \(\alpha_1\) were performed as described in Methods. A: endocytosed Na\(^{+}-K\(^{-}\)-ATPase. Inset, typical immunoblot of biotinylated endocytosed Na\(^{+}-K\(^{-}\)-ATPase \(\alpha_1\) recovered by affinity purification with streptavidin. Graph depicts the pooled data relative to control represented as means ± SE (n = 4). **P < 0.01 vs. control. B: pictures are representative of 6 independent experiments for each condition. C, control (60 min without ischemia); IR30, 30 min substrate/cover-slip-induced ischemia followed by 30 min of reperfusion. Scale bar = 10 \(\mu\)M.
regulation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase function. In addition, a number of structural determinants have been identified on the α-polypeptide, with variable degree of divergence among various α isoforms and between different species. The exact role of these variations in tissue- and species-specific response to various stimuli and diseases remains to be established. The data presented here suggest that modulation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase cell surface abundance by targeting structural determinants on the α-subunit has a significant impact on I-R-induced cell injury.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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Perspectives

MOLECULAR DETERMINANTS OF NA\textsuperscript{+}-K\textsuperscript{+}-ATPASE CELL SURFACE ABUNDANCE

C47


