Reactive oxygen species and the regulation of renal Na\(^+\)-K\(^+\)-ATPase in opossum kidney cells

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ROS function as important second messengers to modulate many downstream signaling molecules, such as protein tyrosine phosphatases, protein tyrosine kinases, transcription factors, mitogen-activated protein kinases, and ion channels, and they have been shown to have the potential to alter the transcription of a number of genes, most notably those of transcription factors: nuclear factor-κB and activator protein 1 (4, 9, 13, 24). Although the molecular mechanisms underlying ROS increase and age-related disease are not clearly understood, it has been reported that the use of antioxidant enriched-diets protects against age-associated conditions and some types of hypertension (2, 7, 11, 13, 18, 25).

Aging-induced changes in kidney function are among one of the most dramatic of any human organ or organ system. In kidney proximal tubules, Na\(^+\)-K\(^+\)-ATPase plays an important role in the control of Na\(^+\) reabsorption, and it is well known, that aging induces alterations in kidney Na\(^+\)-K\(^+\)-ATPase.

Several studies have documented that in old Fischer 344 rats, dopamine failed to inhibit renal Na\(^+\)-K\(^+\)-ATPase activity due to a reduced number of dopamine receptors and defective coupling with G proteins. Recently, Asghar and Lokhandwala (2) demonstrated that the protective coupling of dopamine receptors with G proteins was due to phosphorylation of the receptor induced by an increase in ROS and that treatment with antioxidants restored the signaling cascade.

Previous work from our laboratory demonstrated that opossum kidney (OK) cells, which retain characteristics of kidney proximal tubular cells, were endowed with increased activity and expression of Na\(^+\)-K\(^+\)-ATPase when aged in vitro by serial passaging, and this paralleled with increases in H\(_2\)O\(_2\) production (20). ROS may play a role in the age-induced changes in Na\(^+\)-K\(^+\)-ATPase regulation by interfering with Na\(^+\)-K\(^+\)-ATPase mRNA abundance, protein abundance, and Na\(^+\)-K\(^+\)-ATPase activity due to a reduced number of dopamine receptors and defective coupling with G proteins. Recent work from our laboratory demonstrated that the protective coupling of dopamine receptors with G proteins was due to phosphorylation of the receptor induced by an increase in ROS and that treatment with antioxidants restored the signaling cascade.

THE INCREASE IN FREE RADICAL AND reactive oxygen species (ROS) has been related to cellular aging and aging-associated conditions such as brain dysfunction, cancer, diabetes, and cardiovascular and renal diseases (3, 11, 22–24). ROS encompass a series of oxygen intermediates that include the superoxide anion (O\(_2^−\)) hydrogen peroxide (H\(_2\)O\(_2\)), hydroxyl radical (HO), and hypochlorous acid. Xanthine oxidase, NADPH oxidase (NOX), mitochondrial oxidative phosphorylation, lipoxygenase, cytochrome P-450 monooxygenase, and heme-oxgenase 1 are sources of ROS in the organism. Despite the existence of several sources of ROS, NOX appears to be especially important for the redox-signal (5, 9). This enzyme produces ROS with rapid kinetics of activation and inactivation, which allow a tight regulation of intracellular ROS levels within the short time required for signal transduction.
**METHODS**

**Cell culture.** OK cells (ATCC 1840 CRL; batch number 2129181), obtained from American Type Culture Collection (Rockville, MD) at passage 36 of culture, were maintained in a humidified atmosphere of 5% CO2-95% air at 37°C. Cells were grown in Minimum Essential Medium (Sigma Chemical, St. Louis, MO) supplemented with 10% FBS (Sigma), 100 U/ml penicillin G, 0.25 μg/ml amphotericin B, 100 μg/ml streptomycin (Sigma), and 25 mM HEPE (Sigma). The medium was changed every 2 days, and cells reached confluence 3–5 days after initial seeding. For subculturing, the cells were dissociated with 0.05% trypsin-EDTA (Sigma), split 1:5, and subcultured in a 21 cm² growth area (Costar, Badhoevedorp, The Netherlands). Twenty-four hours before experiments, the cell medium was free of fetal bovine serum. For electrophysiology studies, the cells were seeded onto polycarbonate filter supports (Snapwell, Costar) at a density of 13,000 cells per well, and experiments were performed 4 days after initial seeding. For experiments performed with apocynin, cells were grown for 4 days in the presence of the drug, and the medium was changed daily.

**Ion transport experiments.** All transport experiments were conducted under short-circuit conditions. OK cells grown on polycarbonate filters (Snapwell, Costar) were mounted in Ussing chambers (window area, 1 cm²), equipped with water-jacketed gas lifts bathed on both sides with 10 ml of Krebs-Henseleit solution, gased with 95% O₂:5% CO2, and maintained at 37°C. The standard composition of the apical and basolateral bathing Krebs-Henseleit solution was (in mM) 118 NaCl, 4.7 KCl, 25 NaHCO3, 1.2 KH2PO4, 2.5 CaCl2, and 1.2 MgSO4; pH was adjusted to 7.4 after gasing with 5% CO2-95% O2. The apical bathing Krebs-Henseleit solution contained mannitol (10 mM) instead of glucose (10 mM) to avoid entry of apical Na⁺ through the Na⁺-dependent glucose transporter. After 5 min stabilization, monolayers were continuously voltage clamped to zero potential differences by application of external current, with compensation for fluid resistance, by means of an automatic voltage current clamp (DVC 1000: World Precision Instruments, Sarasota, FL). Cells were allowed to stabilize for further 25 min before permeabilization of apical membrane with amphotericin B (1 μg/ml); this period was also used for exposure of cells to the relevant drug treatments. Under these conditions, the resulting Iₑ is due to Na⁺ transport across the basolateral membrane by Na⁺/K⁺-ATPase (6). The voltage current-clamp unit was connected to a computer via a BIOPAC MP1000 data acquisition system (BIOPAC Systems, Goleta, CA). Data analysis was performed using AcqKnowledge 2.0 software (BIOPAC Systems).

**Western blot analysis.** OK cells grown for 4 days in 21 cm² plates were rinsed twice with cold PBS and lysed by the addition of radioimmunoprecipitation assay (RIPA) buffer [150 mM NaCl, 50 mM Tris·HCl pH 7.4, 1 mM EDTA, 1% NP-40 (IGEPAL), 0.1% sodium deoxycholate] containing protease inhibitors: 1 mM PMSF, 1 μg/ml leupeptin, and 1 μg/ml aprotinin; and phosphatase inhibitors: 1 mM Na3VO4 and 1 mM NaF. Cells were scraped, briefly sonicated, incubated on ice for 1 h, and centrifuged (13,000 rpm for 45 min). Supernatants free from nucleus and intact cells were used fresh or stored at −70°C. Proteins were mixed with 1:6 sample buffer (0.35 M Tris·HCl, 4% SDS, 30% glycerol, 9.5% DTT, pH 6.8, 0.01% bromophenol blue) and warmed at 37°C for 15 min. Equal amounts of total protein were separated on a 7.5% SDS-polyacrylamide gel and electrottransferred to a nitrocellulose membrane in Tris-Glycine transfer buffer containing 20% methanol. Membranes were blocked in 3% nonfat dry milk in PBS for 1 h and then incubated overnight at 4°C with specific primary antibody to α1-subunit Na⁺/K⁺-ATPase, β₁-subunit Na⁺/K⁺-ATPase, NOX1, NOX2, SOD1, SOD2, SOD3, and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The immunoblots were subsequently washed and incubated with the respective fluorescently labeled secondary antibody (goat anti-mouse from LI-COR Biosciences (Lincoln, NE), goat anti-rabbit from Rockland or donkey anti-goat from Rockland) for 1 h at room temperature and protected from light. Membranes were washed and imaged by scanning at 700 or 800 nm with the Odyssey Infrared System (LI-COR Biosciences). Data were normalized to the expression of β-actin and are shown as a percentage of the mean density of the control group.

**In-cell Western blot analysis.** OK cells grown for 4 days in 96-well plates, were rinsed twice with cold PBS and immediately fixed with 4% formaldehyde in PBS for 20 min at room temperature. Cells were permeabilized with 0.1% Triton X-100 in PBS and blocked in 3% nonfat dry milk in PBS for 1.5 h. Incubation with primary antibodies was performed for 2 h at room temperature. Wells were subsequently washed and incubated with the respective fluorescently labeled secondary antibody for 1 h at room temperature and protected from light. Wells were washed and imaged by scanning at 700 or 800 nm with the Odyssey Infrared System (LI-COR Biosciences).

**Real-time PCR.** Gene expression profile was determined by real-time RT-PCR. Standards of α₁-subunit Na⁺/K⁺-ATPase, GAPDH, and β-tubulin were obtained by conventional RT-PCR amplification, using Platinum TaqPCR DNA Polymerase (Invitrogen), and the following specific primers for opsonum: α₁-subunit Na⁺/K⁺-ATPase, sense 5'-GAG CGG CTT TTC TTT GAA TAA-3'; antisense 5'-GCT TCA GAA TGG GTA GAT TT-3'; GAPDH, sense 5'-GGC ATG GTG GAA GGG CTT ATG AC-3'; antisense 5'-ATG CCA GTG AGC TTC CGG TTC AGC-3'; β-tubulin, sense 5'-AAA GGT CTG GGA GGT CAG C-3'; antisense 5'-CGA ACA GAG TCC ATG GTC C-3'. PCR products were gel purified with Qiagen II (Qiagen, Mannheim, MD) and quantified by spectrophotometry at 260 nm. The concentration was determined, and the DNA diluted accordingly in serial steps. Real-time RT-PCR was carried out using a LightCycler (Roche, Mannheim, Germany). Each RT-PCR reaction mixture (50 μl) included reverse transcription products corresponding to 50 ng of total RNA or standard DNA, 1 × SYBR Green I master mix (LightCycler FastStart DNA MasterPLUS SYBR Green I, Roche), 0.5 μM of each forward and reverse primers. Cycling conditions were optimized, and amplification specificity was checked using melting curves following the manufacturer’s instructions. In addition, PCR products were separated by electrophoresis in a 2% Tris–borate-EDTA (TBE) agarose gel to confirm band sizes. Results were analyzed with LightCycler Software v3.5 (Roche Applied Sciences, Mannheim, Germany) using the second derivate maximum method. Quantification was performed using standard curves. Data were normalized to the expression the GAPDH and β-tubulin gene.

**Cell viability.** Cell viability was measured using calcein-AM (Molecular Probes, Eugene, OR), as previously described (17). The membrane permeant calcein-AM, a nonfluorescent dye, is taken up and converted by intracellular esterases to membrane-impermeant calcein, which emits green fluorescence. After treatment, cells were washed twice with Hanks’ medium (in mM: 137 NaCl, 5 KCl, 0.8 MgSO4, 0.33 NaH2PO4, 1.0 MOPS, 0.25 Calcium, 0.15 Tris/HCl, and sodium butyrate 1.0, pH 7.4) and loaded with 2 μM calcein-AM in Hanks’ medium, at room temperature for 30 min. Fluorescence was measured at 485-nm excitation and 530-nm emission wavelengths in a multiplate reader (Spectromax Gemini, Molecular Devices, Sunnyvale, CA). Six wells were treated with ethanol 30 min before calcein-AM addition, and the readings obtained were used for background correction.

H₂O₂ production and accumulation in extracellular medium. H₂O₂ was measured fluorometrically using the Amplex Red Hydrogen Peroxide Assay Kit (Molecular Probes, Eugene, OR), as previously described (17). Amplex Red is a fluorogenic substrate with very low background fluorescence that reacts with H₂O₂ with a 1:1 stoichiometry to produce a highly fluorescent reagent (15). Measurement of H₂O₂ accumulation was followed in extracellular medium. Fluorescence intensity was measured in a multplate reader (Spectromax Gemini, Molecular Devices) at an excitation wavelength of 530 nm and emission wavelength of 590 nm, at room temperature. The
concentration of H$_2$O$_2$ was calculated using a resorufin-H$_2$O$_2$ standard calibration curve.

**Data analysis.** Arithmetic means are given with SE and geometric means with 95% confidence values. Statistical analysis was carried out with a one-way ANOVA followed by Newman-Keuls test for multiple comparisons. A P-value less than 0.05 was assumed to denote a significant difference.

**RESULTS**

**H$_2$O$_2$ production and expression of NOX and SOD isoforms.** As previously shown (20), serial passage of OK cells was accompanied by increases in H$_2$O$_2$ levels in extracellular medium when measured over a 24-h period (Fig. 1). In this set of experiments, we evaluated whether H$_2$O$_2$ accumulation in extracellular medium correlated with the rate of H$_2$O$_2$ production in OK cells cultured for 40 and 80 passages. As shown in Fig. 1B, the rate of H$_2$O$_2$ production in OK cells with 80 passages in culture (7.37 ± 0.02 nM/min) was twice that in cells with 40 passages in culture (5.21 ± 0.02 nM/min). One of the main sources of H$_2$O$_2$ in the kidney is NOX. The isoforms NOX1 and NOX2 have been shown to be altered in animal models of induced and spontaneous hypertension (9, 25). As revealed by Western blot analysis, NOX1 expression in cells with 80 passages was significantly increased when compared with OK cells with less time in culture (Fig. 2A). NOX2 isoform expression was not affected by passage number (Fig. 2B). Because alterations in the oxidative stress of cells may result from altered balance between NOX and SOD, we have also evaluated the expression of three isoforms of SOD (SOD1, SOD2, and SOD3). OK cells were found to express all three SOD isoforms (Fig. 3). Serial cell passaging of OK cells induced increases of 66.7 ± 21.6%, 204.0 ± 7.0%, and 109.1 ± 8.1% in SOD1, SOD2, and SOD3 protein expression, respectively (Fig. 3). However, the most pronounced change was observed with SOD2 (Fig. 3B).

Effect of apocynin on H$_2$O$_2$ production and Na$^+$/K$^+$-ATPase activity and expression. To evaluate whether increases in rate of H$_2$O$_2$ production might contribute to the increased expression and activity of Na$^+$/K$^+$-ATPase, OK cells were treated with the antioxidant apocynin. Apocynin was used to decrease H$_2$O$_2$ production in OK cells, due to the capacity of inhibiting NOX activity. Apocynin was added to the culture medium for 4 days and was first evaluated on its ability to affect cell viability and then on the ability to alter H$_2$O$_2$ production. As shown in Fig. 4A, OK cells grown in the presence of 30, 100, and 300 μM apocynin for 4 days did not show alterations in viability. H$_2$O$_2$ accumulation was significantly decreased at the concentrations of 100 and 300 μM apocynin (Fig. 4B). Apocynin 300 μM also induced a decrease in H$_2$O$_2$ production (Fig. 4C) and lipid peroxidation (Fig. 4D) in OK cells.

OK cells in culture over 40 passages were found to overexpress α$_1$-subunit and β$_1$-subunit Na$^+$/K$^+$-ATPase when using in-cell Western blot technique (Fig. 5A), as previously observed with classic Western immunoblotting (20). To evaluate the role of oxidative stress in the modulation of Na$^+$/K$^+$-ATPase activity and expression, OK cells with 80 passages were treated with apocynin 300 μM for 4 days and Na$^+$/K$^+$-ATPase activity and expression were assayed. Apocynin treatment induced a decrease of 25.9 ± 6.7% in Na$^+$/K$^+$-ATPase activity, as measured by changes in Na$^+$ short-circuit current, induced by apical amphotericin B permeabilization (Fig. 5, B and C). This decrease in the enzyme activity was accompanied by a decrease in the relative abundance of α$_1$-subunit Na$^+$/K$^+$-ATPase but not β$_1$-subunit Na$^+$/K$^+$-ATPase (Fig. 6, A and B). The levels of NOX1 protein expression were not altered by apocynin treatment (data not shown).

Na$^+$/K$^+$-ATPase α$_1$ subunit mRNA expression. To investigate further the differential expression of α$_1$-subunit of Na$^+$/K$^+$-ATPase in OK cells maintained in culture over 40 passages, we analyzed the abundance of its mRNA levels and two house-keeping genes, GAPDH, and tubulin, by real-time RT-PCR analysis. The abundance of α$_1$-subunit Na$^+$/K$^+$-ATPase transcript was increased over time in culture. Because GAPDH mRNA expression, although not attaining statistical significance, increased with the increasing number of cell passages,
tubulin was used to normalize mRNA expression (Fig. 7A). Independently of the normalization used, α1-subunit Na\(^+\)-K\(^+\)-ATPase mRNA levels were significantly increased in OK cells with increased number of cell passages (Fig. 7B). Though apocynin treatment reverted increases in Na\(^+\)-K\(^+\)-ATPase activity and α1-subunit Na\(^+\)-K\(^+\)-ATPase protein expression, treatment with apocynin (300 μM) for 4 days failed to decrease Na\(^+\)-K\(^+\)-ATPase α1 subunit mRNA abundance (Fig. 7C).

DISCUSSION

We had previously shown that OK cells kept in culture for several passages overexpressed the α1-subunit Na\(^+\)-K\(^+\)-ATPase and were endowed with increased Na\(^+\)-K\(^+\)-ATPase

Fig. 2. Protein expression of NADPH-oxidase (NOX) in OK cells with 40 and 80 passages. A: relative abundance of NOX1. B: relative abundance of NOX2. Representative immunoblots are depicted on top of the bar graphs. Columns represent the mean of four independent immunoblots; vertical lines show means ± SE. Significantly different from corresponding values of passage 40 (*P < 0.05).

Fig. 3. Protein expression of superoxide dismutase (SOD) in OK cells with 40 and 80 passages. A: relative abundance of SOD1. B: relative abundance of SOD2. C: relative abundance of SOD3. Representative immunoblots are depicted on top of the bar graphs. Columns represent the mean of four independent immunoblots; vertical lines show means ± SE. Significantly different from corresponding values of passage 40 (*P < 0.05).
activity, which paralleled with increases in their ability to produce H$_2$O$_2$ (20). The results presented here strongly suggest that increases in the ability to produce H$_2$O$_2$ in cells after serial passaging (40 passages) may be related to overexpression of NOX1 and SOD1, SOD2, and SOD3 isoforms. In agreement with this view is the finding that apocynin, a NADPH inhibitor, markedly attenuated the increased in H$_2$O$_2$ production and significantly decreased Na$^+$/H$_3$O$^+$ and K$^+/H_2$O$_2$-ATPase activity and Na$^+$/H$_3$O$^+$-ATPase 1-subunit expression. Increases in Na$^+$/H$_3$O$^+$-ATPase mRNA abundance were also observed in OK cells after serial passaging, but apocynin did not affect transcript abundance.

Several studies have demonstrated that ROS can interfere with protein regulation, including the activity of renal transporters (14, 16, 21). In the kidney, one of the main sources of ROS is NOX, and its upregulation has been described to be responsible for the development of hypertension (1, 5, 8, 10, 14, 25). In the kidney, three isoforms of NOX enzymes have been described, NOX1, NOX2, and NOX4. NOX isoforms have been shown to be upregulated by ANG II, serum, and a high-salt diet (10, 5, 23). In OK cells, prolonged serial passaging is accompanied by increases in NOX1 without changes in the expression of NOX2, supporting the view that serum present in the culture medium could be responsible for changes observed in oxidative stress levels of OK cells.

O$_2^-$ in the cells is dismutated into H$_2$O$_2$ spontaneously or by SOD. SOD is expressed mainly in three isoforms: SOD1, SOD2, and SOD3, all expressed in the kidney (24). Both increases in O$_2^-$ and H$_2$O$_2$ appear to contribute to the development of hypertension, in part, due to its effects on the kidney and heart. In OK cells, after 80 passages in culture, all SOD

Fig. 4. Apocynin incubation in OK cells with 80 passages. A: concentration-dependent effect of apocynin for 4 days on cell viability. B: hydrogen peroxide accumulation during 24 h in extracellular medium. C: rate of hydrogen production. D: thiobarbituric acid reactive substances (TBARS) in OK cells treated with apocynin during 4 days. TBARS were determined measuring malondialdehyde (MDA) levels and normalized for total protein expression. Significantly different from corresponding control values (*P < 0.05).

Fig. 5. A: relative abundance of $\alpha_1$-subunit and $\beta_1$-subunit of Na$^+$/K$^+$-ATPase protein expression in OK cell monolayers with 40 and 80 passages. Protein expression was normalized for DNA per well. Representative wells are depicted on top of the bar graphs. Columns represent the mean of four independent wells; vertical lines indicate means ± SE. Significantly different from values of passage 40 (*P < 0.05). B: Na$^+$/K$^+$-ATPase activity measured under short-circuit current in OK cell monolayers with 80 passages pretreated with apocynin for 4 days. Representative traces of the effect of amphotericin B (1.0 μg/ml) upon increases in basolateral sodium currents (INa, A/cm$^2$). C: Mean values of amphotericin B (0.6 and 1.0 μg/ml) induced increases in basolateral sodium currents (ΔINa, μA/cm$^2$). Columns represent the mean of eight experiments per group; vertical lines indicate means ± SE. Significantly different from corresponding control values (*P < 0.05).
isoforms were overexpressed, SOD2 being the most affected. NOX and SOD isoforms have been shown to be altered in animals affected by several pathologies such as hypertension, diabetes, and obesity, and treatment with antioxidants such as apocynin, tempol, and a combination of tempol and catalase are helpful in improving ROS-related conditions (2, 7, 12, 18, 19, 25). To address the role of ROS production in Na\(^{+}\)/H\(^{+}\)-ATPase activity in OK cells after prolonged serial passaging, we used apocynin to inhibit the formation of the functional NOX complex. Apocynin inhibits NOX by preventing the transfer of the soluble cytosolic subunits to the membrane complex. OK cells grown in the presence of apocynin have reduced ROS production, as indicated by quantification of 24 h H\(_2\)O\(_2\) accumulation in extracellular medium and decreased rate of H\(_2\)O\(_2\) production. The parallel decrease in Na\(^{+}\)/K\(^{+}\)-ATPase activity and rate of H\(_2\)O\(_2\) production in OK cells treated with apocynin strongly suggests that ROS may play an important role as mediators in the regulation of Na\(^{+}\)/K\(^{+}\)-ATPase in renal epithelial cells. The results obtained by Western blot analysis showed that prolonged serial passaging of OK cells results in

![Fig. 6. α1-subunit and β1-subunit of Na\(^{+}\)/K\(^{+}\)-ATPase (NKA) protein expression in OK cell monolayers with 80 passages treated with apocynin. A: relative abundance of α1-subunit and β1-subunit of Na\(^{+}\)/K\(^{+}\)-ATPase protein expression in OK cells determined by Western blot analysis and normalized for β-actin. B: Relative abundance of α1-subunit and β1-subunit of Na\(^{+}\)/K\(^{+}\)-ATPase protein expression in OK cell determined by in-cell Western blot analysis and normalized with DNA. Representative wells are depicted on top of the bar graphs. Columns represent the mean of four independent wells; vertical lines show means ± SE. Significantly different from corresponding control values (*P < 0.05).](http://ajpregu.physiology.org/)

![Fig. 7. α1-subunit of Na\(^{+}\)/K\(^{+}\)-ATPase mRNA in OK cells. A: expression of α1-subunit of Na\(^{+}\)/K\(^{+}\)-ATPase mRNA and the housekeeping genes GAPDH and tubulin in OK cells with 40 and 80 passages. B: relative expression of α1-subunit Na\(^{+}\)/K\(^{+}\)-ATPase mRNA in OK cells with 40 and 80 passages. C: relative expression of α1-subunit of Na\(^{+}\)/K\(^{+}\)-ATPase mRNA in OK cells with 80 passages treated with apocynin. Columns represent the means of 6–9 experiments per group; vertical lines indicate SE. Significantly different from corresponding control values (*P < 0.05).](http://ajpregu.physiology.org/)
overexpression of α₁-subunit of Na⁺-K⁺-ATPase and that treatment with apocynin results in a decrease in α₁-subunit of Na⁺-K⁺-ATPase abundance. It is suggested that increased ROS production might be responsible for an increase in protein abundance.

Because in OK cells, there was an increase in α₁-subunit Na⁺-K⁺-ATPase total protein, it was felt worthwhile to investigate the relative abundance of α₁-subunit Na⁺-K⁺-ATPase mRNA. The fact that OK cells treated with apocynin have no changes in the relative expression of α₁-subunit Na⁺-K⁺-ATPase mRNA suggests that the changes observed at the protein level may be due to alterations in protein recycling or in protein translation.

In vitro studies using vascular smooth muscle cells showed that NOX1 is upregulated by growth factors such as PDGF and ANG II and that the study of the redox signaling in vascular smooth muscle cells may be essential for a better understanding of the molecular events taking place in vascular disease. However, the downstream effects of NOX1 upregulation were not addressed in the present study. OK cells with high passage number represent a model of increased ROS production and may provide new insights into the regulation of kidney membrane transporters during oxidative stress. These findings are of considerable importance, as it further supports previous knowledge that increases in oxidative stress have a profound effect on renal function and are associated with the development of diseases such as hypertension, obesity-associated hypertension, and diabetes, as well as during the aging process. Future work intends to elucidate the intracellular mechanisms through which an increase in ROS production leads to increases in α₁-subunit Na⁺-K⁺-ATPase protein expression.

In conclusion, overexpression of NOX1 and SOD contribute to the increased production of H₂O₂ in OK cells cultured over time. The increased availability of H₂O₂ in the intracellular milieu plays an important role in the long-term regulation of Na⁺-K⁺-ATPase expression and activity. It is suggested that these cells may constitute an interesting in vitro model to study events related to oxidative stress in which adaptation to increased exposure to H₂O₂ had progressively adapted in a more natural manner.

GRANTS

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