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Hydrogen Peroxide-induced Alterations in Na^+-phosphate Cotransport in Renal Epithelial Cells

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This study was undertaken to examine the effect of oxidants on membrane transport function in renal epithelial cells. Hydrogen peroxide (H_2O_2) was used as a model oxidant and the membrane transport function was evaluated by measuring Na^+-dependent phosphate (Na^+-Pi) uptake in opossum kidney (OK) cells. H_2O_2 inhibited Na^+-Pi uptake in a dose-dependent manner. The oxidant also caused loss of cell viability in a dose-dependent fashion. However, the extent of inhibition of the uptake was larger than that in cell viability. H_2O_2 inhibited Na^+-dependent uptake without any effect on Na^+-independent uptake. H_2O_2-induced inhibition of Na^+-Pi uptake was prevented completely by catalase, dimethylthiourea, and deferoxamine, suggesting involvement of hydroxyl radical generated by an iron-dependent mechanism. In contrast, antioxidants Trolox, *N,N'*-diphenyl-*p*-phenylenediamine, and butylated hydroxyanisole did not affect the H_2O_2 inhibition. Kinetic analysis indicated that H_2O_2 decreased Vmax of Na^+-Pi uptake with no change in the Km value. Phosphonoformic acid binding assay did not show any difference between control and H_2O_2-treated cells. H_2O_2 also did not cause degradation of Na^+-Pi transporter protein. Reduction in Na^+-Pi uptake by H_2O_2 was associated with ATP depletion and direct inhibition of Na^+-K^+-ATPase activity. These results indicate that the effect of H_2O_2 on membrane transport function in OK cells is associated with reduction in functional Na^+-pump activity. In addition, the inhibitory effect of H_2O_2 was not associated with lipid peroxidation.

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I. Introduction

Reactive oxygen species (ROS) have been implicated in the pathogenesis of a number of renal diseases including ischemic and nephrotoxicant-induced acute renal failure (Baliga *et al*, 1999). The cell injury by ROS involved alterations in physical integrity of the cell membrane, such as rigidity and permeability, and functional integrity, such as membrane transport and enzymes functions (Gardes-Albert *et al*, 1993). ROS cause DNA damage (Schraufstatter *et al*, 1986), rapid depression of intracellular ATP (Andreoli *et al*, 1993), a fast rise of intracellular Ca^{2+} (Hyslop *et al*, 1986), oxidation of susceptible amino acids in proteins (Aruoma *et al*, 1989), and gross perturbations to the cytoskeleton and plasma membrane (Andreoli *et al*, 1993). All these processes occur before loss of plasma membrane integrity, as measured by vital dye staining (Hyslop *et al*, 1986; Schraufstatter *et al*, 1986) or loss of preloaded ^{51}Cr (Andreoli and Mallett, 1997). In renal epithelial cells, most of the studies on acute oxidative stress have focused on the physical integrity of the cell membrane (Schnellmann, 1988; Andreoli and McAteer,

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1990; Walker and Shah, 1991; Sheridon *et al*, 1996) Many reports and propose that lipid peroxidation plays a critical role in oxidant-induced cell death (Salahudeen, 1995; Sheridan *et al*, 1996; Schnllmann, 1998).

In vivo studies have shown that ischemia and various nephrotoxicants produce significant changes in structure and function of the proximal tubule, a major site where exhibits many energy-dependent, specialized functions including reabsorption of solutes such as phosphate, glucose, amino acids. Although a recent study shows that H_2O_2 inhibits phosphate transport through a lipid peroxidation-independent mechanism in opossum kidney (OK) cells (Min *et al*, 2000), the mechanism by which H_2O_2 alters the transport function in renal epithelial cells remains to be identified. Therefore, the present study was carried out to examine the mechanism by which H_2O_2 alters membrane transport function by measuring Na^+-Pi uptake in renal proximal tubular epithelial cells.

II. Materials and Methods

1. Chemicals

[^{32}P]-phosphate and [^{14}C]-phosphonoformic acid were obtained from Amersham international (Amersham, UK). H_2O_2, deferoxamine (DFO), 3-aminobenzamide (AB), catalase, Tolox, dimethythiourea (DMTU), *N,N*'-diphenyl-p-phenylenediamine (DPPD), butylated hydroxyanisole (BHA), and malondialdehyde tetraethylacetal were purchased from Sigma-Aldrich Chemical (St. Louis, MO). All other chemicals were of the highest commercial grade available.

2. Culture of OK cells

OK cells were obtained from ATCC and maintained by serial passages in 75-cm^2 culture flasks (Costar, Cambridge, MA). The cells were grown in Dulbecco's modified Eagle's medium/Ham's F12 (DMEM/F12, Sigma Chemical Co. St. Louis USA) containing 10% fetal bovine serum at 37℃ in 95% air/5% CO_2 incubator. When the cultures reached confluence, subculture was prepared using a 0.02% EDTA-0.05% trypsin solution. The cells were grown on 24-well tissue culture plates in DMEM/F12 medium containing 10% fetal bovine serum. All experiments started 3~4 days after plating when a confluent monolayer culture was achieved.

3. Induction of oxidant injury

Cells were treated with H_2O_2 of the indicated concentration in Hanks' balanced salt solution (HBSS) containing the following; 115 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 2 mM NaH_2PO_4, 1 mM $MgSO_4$, 1 mM $CaCl_2$, and 5 mM glucose (pH 7.4) for 120 min at 37℃. Following oxidant stress, uptakes of solutes and ATP content were measured as described below.

4. Uptake studies

The uptake of solutes was determined in cell monolayers grown on 24 well plates. After an exposure to oxidant stress, the reaction buffer was removed and washed twice with the uptake buffer containing the following; 137 mM NaCl, 5.4 mM KCl, 2.8 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 10 mM Hepes (pH 7.4). The cells were incubated for 30 min at 37℃ in the uptake buffer containing 5 μM [^{32}P]-phosphate. For kinetic studies, the cells were incubated for 20 min at 37℃ in the uptake buffer containing [^{32}P]-phosphate of various concentrations (0.005-1 mM). For measurement of Na^+-independent phosphate uptake, NaCl was replaced by 137 mM *N*-methyl-D-glucamine (NMG). At the end of the incubation period, the cells were washed three times with ice-cold uptake buffer and solubilized in 0.5 mL of 0.2 % Triton X-100. Aliquots of each sample were transferred to scintillation vials and the radioactivity was counted in a liquid scintillation counter (TRI-CARB 2100TR, Packard, USA). Protein was measured by the method of Bradford (Bradford, 1976).

5. [^{14}C]PFA binding studies

The binding of [^{14}C]PFA was measured in cell monolayers grown on 24 well plates. After an exposure to oxidant stress, the reaction buffer was removed and washed twice with the uptake buffer described above. The cells were incubated for 30 min at 37℃ in the uptake buffer containing 1 mM [^{14}C]PFA. At the end of the incubation period, the cells were washed three times with ice-cold buffer and solubilized in 0.5 mL of 0.2 % Triton X-100. Aliquots of each sample were transferred to scintillation vials and the radioactivity was counted in a liquid scintillation counter as described above

6. Electrophoresis and Immunoblotting

The intrinsic type II Na+-dependent phosphate transporter protein (NaPi-4) in OK cells was analyzed as described by Pfister et al. (1997). Cells were grown to confluency in Φ 10 cm Petri dishes. After treatment with H2O2, the cells were scraped off the dish and washed twice with TBS (0.9% NaCl and 10 mM Tris-HCl, pH 7.4). The scraped cells were homogenized in solution containing 10 mM NaCl, 1 mM EGTA, 1 mM EDTA, 20 mM Tris-HCl (pH 7.4), and 1% Triton X-100. The homogenate was centrifuged at 2000 rpm for 10 min at 4℃. The supernatant was saved and centrifuged at 16,000 rpm for 40 min at 4℃. The pellet corresponding to a crude membrane preparation was resuspended in 100 μL of 50 mM mannitol and 10 mM Hepes-Tris (pH 7.2).

All samples were prepared by heating to 100℃ for 10 min in SDS gel-loading buffer. A 30 μg of total protein was used for SDS-polyacrylamide gel electrophoresis (10%) and subsequent transfer to nitrocellulose membrane. To confirm identical loading, nitrocellulose membrane was stained with Ponceau-S. Nonspecific binding was then blocked by incubating the nitrocellulose at room temperature for 1 hr in TBS containing 5% nonfat dry milk and 1% Triton X-100. Expression of the NaP_i-4 protein was estimated using a affinity pure polyclonal antibody (Alpha Diagnostic, Inc.) raised against the C-terminal 12 amino acids of the published NaPi-4 sequence (antibody dilution, 1/100). Incubation with the primary antibody took place overnight at room temperature. The nitrocellulose was washed four times with TBS containing 1% Triton X-100. The nitrocellulose was then incubated with a 1:10,000 dilution of an anti-rabbit IgG labeled with horseradish peroxidase (Amersham Life Science, Inc.) for 1 hr at room temperature. Blots were developed by ECL kit.

7. Measurement of cell viability

The cell viability was estimated by a trypan blue exclusion assay. Cells were grown to confluence in 24-well dishes, treated with H_2O_2, and then harvested using 0.025% trypsin. Cells were incubated with 4% trypan blue solution. Cells failing to exclude the dye were considered nonviable, and the data are expressed as percentage of nonviable cells.

8. Measurement of ATP content

ATP levels were measured on OK cells with a luciferin-luciferase assay. After an exposure to oxidant stress, the cells were solubilized with 500 μL of 0.5 % Triton X-100 and acidified with 100 μL of 0.6 M perchloric acid and placed on ice. Cell suspension was then diluted with 10 mM potassium phosphate buffer containing 4 mM $MgSO_4$ (pH 7.4), and 100 μL of 20 mg/ml luciferin-luciferase was added to 10 μL of diluted sample. Light emission was recorded at 20 sec with a luminometer (MicroLumat LB96P, Berthold, Germany). Protein content was determined on a portion of the cell sample.

9. Measurement of Na^+-K^+-ATPase activity

The Na^+-K^+-ATPase activity was measured in the microsomal fraction prepared from OK cells. For the preparation of microsomal fraction, cells were grown to confluence in 100 mm dish, scraped from the dish in 10 mM mannitol and 2 mM Tris/HCl (pH 7.1) at 4℃, and briefly sonicated. Then, the cell lysate was centrifuged for

2 min at 2,000×g to remove unbroken cells and the supernatant was saved, centrifuged for 12 min at 15,000 ×g. Pale-pink layer on top of pellet was removed and resuspended in 10 mM mannitol and 2 mM Tris/HCl (pH 7.1). Microsomal fraction was treated with ethanol for 60 min at 37℃ and Na^+-K^+-ATPase was measured.

The ATPase activity was determined by measuring inorganic phosphate (Pi) released by ATP hydrolysis during incubation of microsome with an appropriate medium containing 3 mM ATP (Sigma) as the substrate. The total ATPase activity was determined in the presence of 100 mM Na^+, 20 mM K^+, 3 mM Mg, 2 mM EDTA, and 40 mM imidazole (pH 7.4). The Mg^{2+}-ATPase activity was determined in the absence of K^+ and in the presence of 1 mM ouabain. The difference between the total and the Mg^{2+}-ATPase activities was taken as a measure of the Na^+-K^+-ATPase activity. At the end of a 10-min incubation, the reaction was terminated by the addition of ice-cold 6% perchloric acid. The mixture was then centrifuged at 3,500×g, and Pi in the supernatant fraction was determined by the method of Fiske and SubbaRow (Fiske and SubbaRow, 1925).

10. Statistical analysis

Data are expressed as mean ± SEM. Comparisons between two groups were made using the unpaired *t* test. Multiple group comparisons were done using one-way analysis of variance followed by the Turkey *post hoc* test. $P < 0.05$ was considered statistically significant.

III. Results

The concentration dependent effect of H_2O_2 on Na^+-Pi uptake in OK cells was examined. Based on preliminary studies, the uptake was determined at 30 min following exposure of cells to various concentrations of H_2O_2 for 120 min (Fig. 1A). When cells were exposed to 0, 0.1, 0.2, 0.3, 0.5, and 1 mM, Na^+-Pi uptake was reduced by

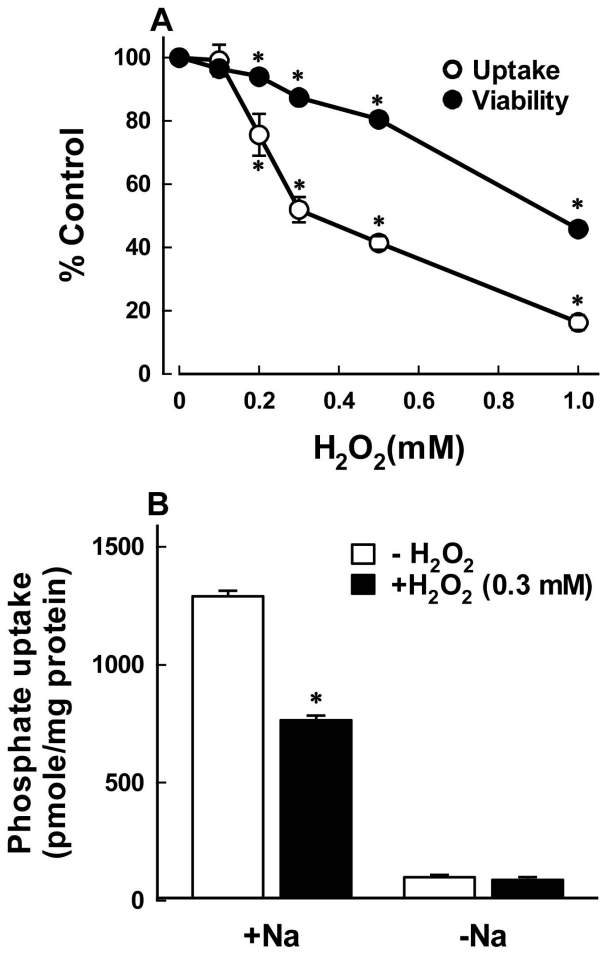

Fig. 1. (A) Concentration dependent effect of H_2O_2 on phosphate uptake and cell viability in OK cells. Cells were exposed to various concentrations of H_2O_2 for 120 min, and the uptake and cell viability were estimated. The uptake was measured for 30 min and cell viability was evaluated by a trypan blue exclusion assay. Data are mean ± SE of four experiments. *P<0.05 compared with the absence of H_2O_2 (control). (B) Effect of H_2O_2 on Na^+-dependent and ‾independent phosphate uptake in OK cells. Cells were exposed to 0.3 mM H_2O_2 for 120 min and the uptake was measured for 30 min in a buffer with or without Na^+ (replaced by NMG). Data are mean SE of four experiments. *P<0.05 compared with control.

H_2O_2 in a dose-dependent manner, with an IC_{50} (the inhibitor concentration for 50% inhibition) of 0.33 mM.

In order to determine whether the inhibition of Na^+-Pi uptake was attributed to irreversible cell injury, the effect of H_2O_2 on cell death as measured by trypan blue

exclusion was examined. As shown in Fig. 1A, H_2O_2 caused cell death in a dose-dependent manner similar to the inhibition of uptake. However, the extent of cell death was much less than that of the uptake.

Phosphate uptake in the presence of Na^+ was significantly reduced by 0.3 mM H_2O_2, whereas the uptake in the absence of Na^+ was not substantially altered by H_2O_2 (Fig. 1B). These data suggest that H_2O_2 inhibits the active phosphate uptake driven by the gradient of Na^+ without any effect on Na^+-independent passive uptake. The uptake in control cells in the absence of Na^+ was 99.04 ± 10.62 pmole/mg/30 min, which was approximately 7.7% of the total uptake (1289.40 ± 24.79 pmole/mg/30 min). Na^+-independent uptake was therefore not routinely measured.

H_2O_2 is converted into hydroxyl radical, a more potent oxidant, in the presence of iron via the Fenton/ Haber-Weiss reactions. To confirm the role of H_2O_2 and hydroxyl radical in mediating the inhibition of Na^+-Pi uptake, effects of the H_2O_2 scavenger catalase and the hydroxyl radical scavenger DMTU were examined. H_2O_2-induced alteration in Na^+-Pi uptake was nearly completely prevented by these scavengers (Fig. 2A). A similar protection was also obtained with the iron chelator deferoxamine (Fig. 2B). These results suggest that hydroxyl radicals are responsible for the uptake inhibition. Since hydroxyl radicals are a potent initiator of lipid peroxidation, H_2O_2-induced inhibition of Na^+-Pi uptake could be resulted from lipid peroxidation. To test this hypothesis the effect of Trolox on the inhibition of Na^+-Pi uptake was examined. The antioxidant Trolox (0.5 mM) did not prevent H_2O_2-induced inhibition of Na^+-Pi uptake, indicating that the effect of H_2O_2 on Na^+-Pi uptake is not mediated by lipid peroxidation. Similarly, the other lipophilic antioxidants DPPD and BHA at 20 and 50 μM, respectively, did not protect against H_2O_2-induced inhibition of Na^+-Pi uptake (data not shown). These results are consistent with those of previous studies (Min *et al*, 2000). The concentrations of antioxidants used in the present study was similar to or higher than concentrations that have completely prevent oxidant-induced cell injury and lipid peroxidation (Chen and Stevens, 1991; Kim and Kim, 1996; Robb and Connor, 1998; Lin and Ho, 2000).

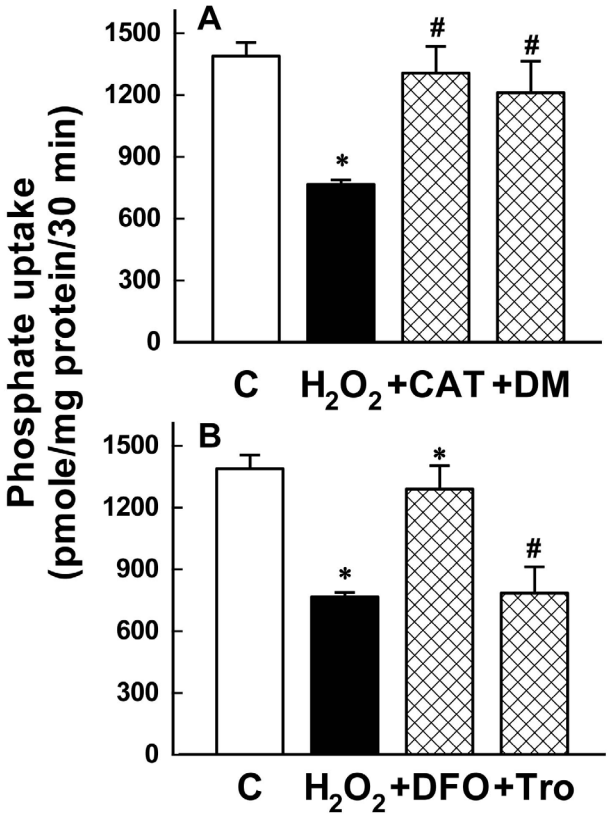

Fig. 2. Effects of radical scavengers (A) and antioxidants (B) on H_2O_2-induced inhibition of Na^+-Pi uptake in OK cells. Cells were exposed to 0.3 mM H_2O_2 for 120 min in the presence of 500 units/mL catalase (CAT), 30 mM dimethythiourea (DEMTU), 2 mM deferoxamine (DFO), and 1 mM Trolox (Tro). The uptake was measured for 30 min. Data are mean SE of five experiments. *P<0.05 compared with control (C). #P<0.05 compared with H_2O_2 alone.

In an attempt to gain insight into the mechanisms by which H_2O_2 modulates Na^+-Pi uptake, the effect of H_2O_2 on the kinetics of Na^+-Pi uptake was examined. The time course of Na^+-Pi uptake was linear up to 30 min incubation in cells with or without H_2O_2 treatment (data not shown). Based on these findings, the effect of H_2O_2 on the kinetics of Na^+-Pi uptake was analyzed by

measuring the initial velocity (20 min) of Na^+-Pi uptake as a function of phosphate concentration in the presence or absence of external Na^+. The results are summarized in Fig. 3. Total phosphate uptake measured in the presence of external Na^+ increased curvilinearly as the external phosphate concentration increased in control and H_2O_2-treated cells, whereas the uptake in the absence of Na^+ increased linearly with increasing phosphate concentration in both groups. H_2O_2 inhibited the total uptake without a significant change in the Na^+-independent uptake (Fig. 3A). The Na^+-dependent uptake, computed by subtracting the uptake in the absence of Na^+ from the total uptake in each group are illustrated in Fig. 3B. An Eadie-Hofstee transformation of the Na^+-dependent uptake shows that the relationship between the initial rate of phosphate uptake (V) and V/[phosphate] was linear in both control and H_2O_2-treated cells (Fig. 3C). This indicates that in both cases the Na^+-dependent phosphate uptake follows a simple Michaelis-Menten kinetics, i.e., V = Vmax × [S]/(Km + [S]), where Vmax is the maximal uptake, [S] is the substrate concentration, and Km is the apparent Michaelis constant indicating the concentration of phosphate for ½Vmax. H_2O_2 caused a significant reduction in the Vmax for Na^+-dependent phosphate uptake (5.41 ± 0.50 vs. 12.01 ± 0.03 pmole/mg/20 min in control cells), with no change in the apparent Km (0.050 ± 0.004 vs. 0.042 ± 0.009 mM in control cells).

PFA acts as a specific, competitive, and reversible inhibitor of Na^+-Pi cotransport across the renal brush-border membrane and has been employed as a probe for studies of this transport system (Szczepanska *et al*, 1986; Szczepanska *et al*, 1989). Therefore, we examined the effect of H_2O_2 on PFA binding in OK cells. The results depicted in Fig. 4A indicated that PFA binding was not different between control and H_2O_2-treated cells. These data suggest that H_2O_2-induced reduction in Vmax of Na^+-Pi uptake is not attributed to a decrease in the number of Na^+-Pi cotransporters.

Expression of the NaP_i-4 protein in OK cells was also

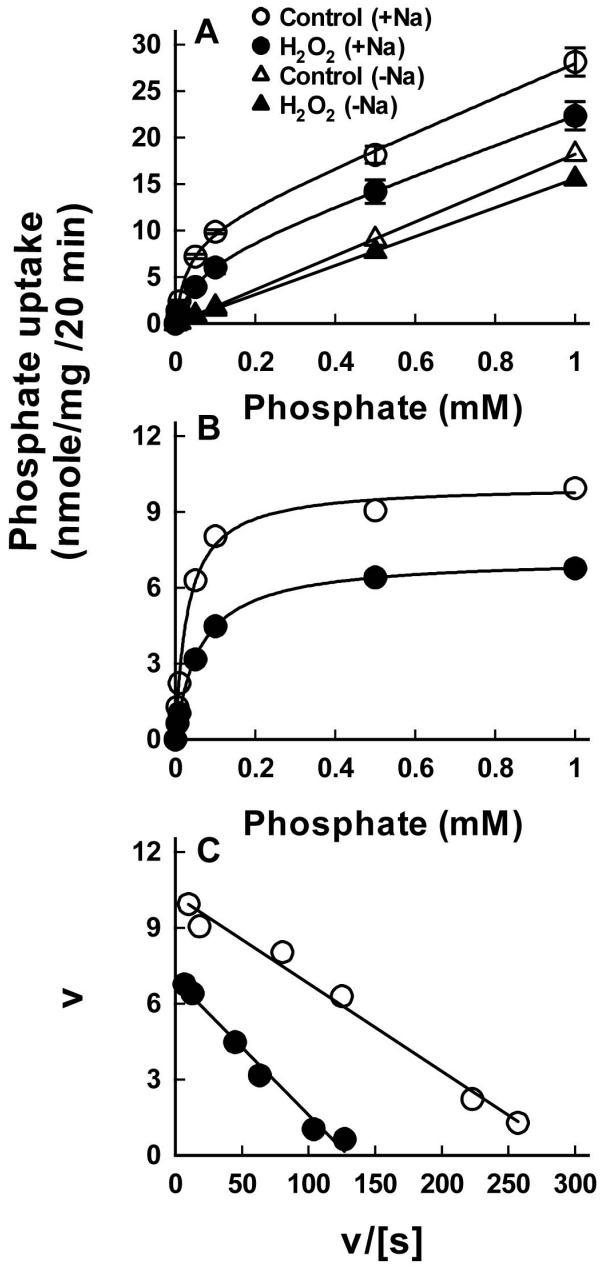

Fig. 3. Initial rate of phosphate uptake in control and H_2O_2-treated cells as a function of phosphate concentrations. (A) Cells were exposed to 0.3 mM H_2O_2 for 120 min and the uptake was measured for 20 min in a buffer with or without Na^+ (replaced by NMG). (B) Na^+-dependent uptake was calculated by subtracting the uptake in the absence of Na^+ from the total uptake. Data are mean ± SE of eight experiments. (C) Data of the Na^+-dependent uptake presented according to Eadie-Hofstee transformation of Michaelis-Menten equation. In this plot, the intercept of the line with Y-axis represents Vmax and the slope indicates the Km for phosphate.

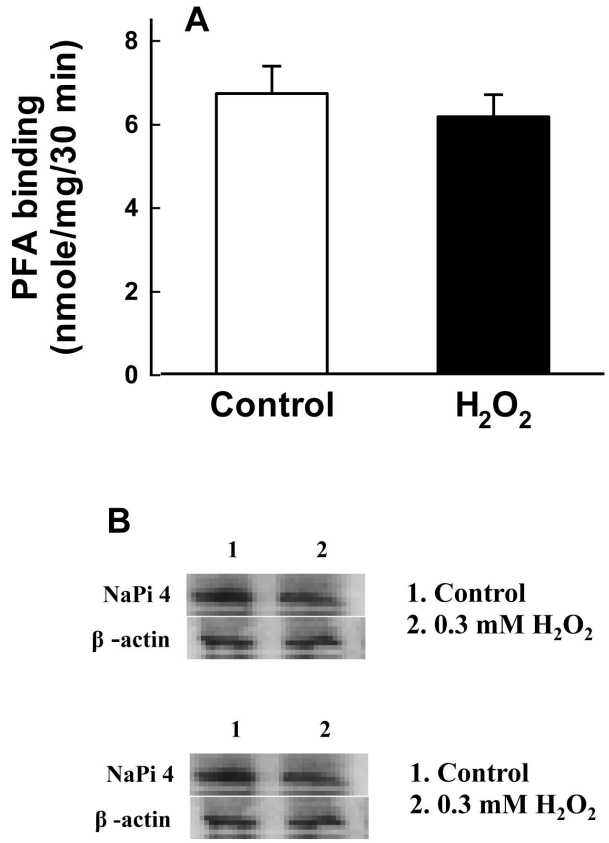

Fig. 4. (A) Effect of H_2O_2 on phosphonoformic acid (PFA) binding in OK cells. Cells were exposed to 0.3 mM H_2O_2 for 120 min, and PFA binding was measured for 30 min. Data are mean ± SE of five experiments. (B) Effect of H_2O_2 on expression of the Na^+-dependent phosphate transporter (NaPi-4) protein. Cells were exposed to 0.3 mM H_2O_2 for 120 min, and the expression of NaPi-4 protein was analyzed by immunoblotting using a affinity pure polyclonal antibody.

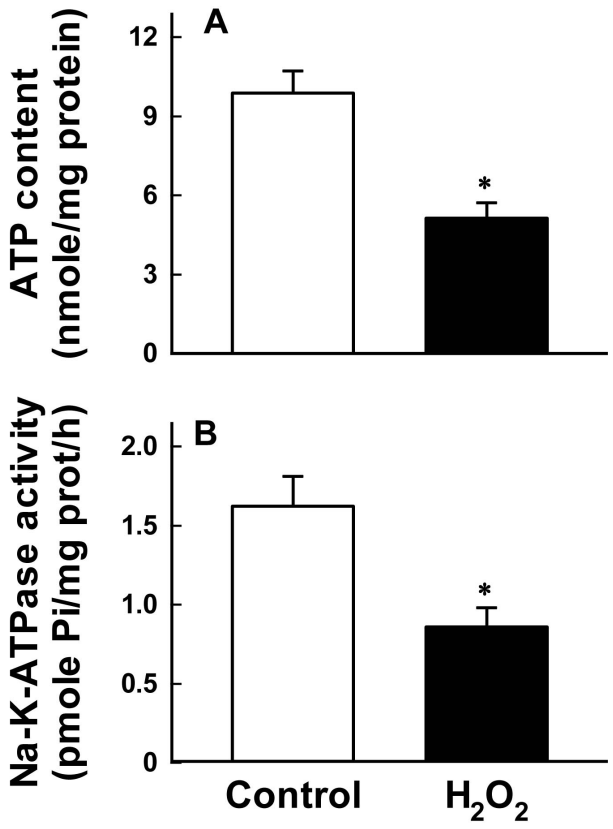

Fig. 5. Effect of H_2O_2 on ATP depletion in whole cells (A) and Na^+-K^+-ATPase activity in disrupted cells (B). Cells were exposed to 0.3 mM H_2O_2 for 120 min, and ATP content and the enzyme activity were measured. Data are mean SE of five experiments. *P<0.05 compared with control.

analyzed by immunoblotting. As shown in Fig. 4B, treatment with H_2O_2 did not caused any change in NaP_i-4 protein. Together with data from kinetic analysis and PFA binding assay, these results suggest that H_2O_2-induced inhibition in Na^+-Pi uptake is not a result of a decrease in the number of the transporters.

Since maintenance of intracellular Na^+ gradient needed to drive Na^+-dependent cotransport requires normal activity of the Na^+-pump, H_2O_2 treatment may reduce Na^+-Pi uptake through the inhibition of Na^+-pump activity. Reduction in Na^+-pump activity could be resulted from ATP depletion and/or direct inhibition of Na^+-K^+-ATPase in whole cells. To test the possibility, we measured ATP content in whole cells and Na^+-K^+-ATPase activity in disrupted cells. Both ATP content and Na^+-K^+-ATPase activity were decreased approximately 50% of control in cells treated with H_2O_2 (Fig. 5).

IV. Discussion

The mechanism by which oxidants alter membrane transport functions in renal proximal tubular cells is not clearly defined. Previous studies have shown that oxidants

inhibit Na^+-dependent solute transport by lipid peroxidation and direct damage of the transport protein (Jourd' heuil *et al*, 1993) and disruption of normal ion gradients resulting from ATP depletion and inactivation of Na^+-K^+-ATPase (Andreoli *et al*, 1993).

In the present study, we demonstrate that membrane transport function such as Na^+-Pi transport is inhibited in response to oxidant stress. The extent of the inhibition was much larger than that in cell death, suggesting that most of the uptake inhibition is not attributed to irreversible cell death. H_2O_2-induced inhibition of Na^+-Pi uptake was completely prevented by the H_2O_2 scavenger catalase, the hydroxyl radical scavenger DMTU, and the iron chelator deferoxamine. These data indicate that the H_2O_2 inhibition is mediated by the intracellular generation of an iron-dependent hydroxyl radical.

Because hydroxyl radicals are a potent initiator of lipid peroxidation, the inhibition of Na^+-Pi uptake induced by H_2O_2 could be resulted from lipid peroxidation. However, the H_2O_2 inhibition was not altered by antioxidants. Therefore, it is likely that H_2O_2 inhibits Na^+-Pi uptake through a lipid peroxidation-independent mechanism..

Effects of oxidants on kinetic analysis of membrane transport were studied in Na^+-glucose uptake by brush-border membrane vesicles (Jourd'heuil *et al*, 1993) and organic anion uptake by renal proximal tubular cells (Takeda *et al*, 2000). They found that oxidants cause a significant reduction in Vmax of these transport systems without any change in Km. The effect of oxidants on the kinetics of Na^+-Pi uptake in OK cells has not been explored until now. In the present study, the Vmax of Na^+-Pi uptake was significantly reduced in cells treated with H_2O_2, whereas the Km value remained unchanged. In the kinetic analysis of carrier-mediated transport, the Vmax is determined by two factors: firstly, the capacity of the carrier system and, secondly, the proportion of adsorbed molecules which dissociate in a forward direction in unit time. The former depends mainly on the number of carrier sites per unit area of membrane. The latter depends on (1) the probability of a substrate molecule to dissociate from a carrier site in a given time, and (2) the rate of turnover of carrier across the membrane. Since the Km for phosphate in the present study was not changed, it is unlikely to have altered carrier-substrate dissociation. Therefore, the decrease in Vmax could be attributed to reduction in the number of functional carrier units or the rate of turnover. Assuming that PFA binding provides an accurate estimate of the number of Na^+-Pi transport (Szczepanska *et al*, 1987), the results of PFA binding studies (Fig. 4A) indicate that the decrease in Vmax may be mediated by a decrease in the turnover rate but by not the number of functional carrier units. This assumption was supported by an immunoblotting assay (Fig. 4B).

The present study demonstrates that the principal mechanism of H_2O_2-induced modulation in Na^+-Pi uptake is the decrease in Na^+-pump activity resulting from ATP depletion and direct inhibition of Na^+-K^+-ATPase (Fig. 5), consistent with previous studies in LLC-PK1 cells (Andreoli *et al*, 1993). It is not certain, however, whether H_2O_2-induced ATP depletion was attributed to impairment of ATP synthesis resulting from mitochondrial damage and/or to activation of poly (ADP-ribose) polymerase (PARP). Since PARP catalyzes the transfer of ADP-ribose from NAD to protein with the concomitant release of nicotinamide, the activation of this enzyme results in depletion of NAD and a consequent reduction in ATP. In previous study, H_2O_2 produces activation of poly (ADP-ribose) polymerase (Min *et al*, 2000). Such a reduction in functional Na^+-pump activity may contribute to the decrease in Vmax.

In conclusion, H_2O_2 reduced Na^+-Pi uptake through the inhibition of function Na^+-pump activity. Such effects are mediated by iron-dependent hydroxyl radical generation, but not attributed to lipid peroxidation or mostly irreversible cell death.

Acknowledgments

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