Superoxide enhances Na-K-2Cl cotransporter activity in the thick ascending limb

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Juncos, Ramiro, and Jeffrey L. Garvin. Superoxide enhances Na-K-2Cl cotransporter activity in the thick ascending limb. Am J Physiol Renal Physiol 288: F982–F987, 2005; doi:10.1152/ajprenal.00348.2004.—Superoxide (O$_2^-$) enhances Na reabsorption by the thick ascending limb (THAL). Na absorption in this segment involves the Na-K-2Cl cotransporter, K channel, and Na-K-ATPase. We hypothesized that O$_2^-$ stimulates NaCl absorption primarily by enhancing Na-K-2Cl cotransport. First, we measured steady-state intracellular Na (Na$_i$) and chloride (Cl$_i$) using the radiolabeled sodium flux technique. Next, we tested cotransport activity by measuring the initial rate of increase in Na$_i$ caused by changing luminal Na$_i$-Cl$_i$-K$_i$ from 50/0/0 to 140/143/4 mM. During the control period, the initial rate of increase was 0.03 ± 0.02 arbitrary units (AU)/min. After treatment with O$_2^-$, it rose to 20.9 ± 3.3 mM, a 71% increase (P < 0.01). Cl$_i$ also increased (P < 0.01). Neither XO nor HX alone had a significant effect on Na$_i$ or Cl$_i$. Next, we tested cotransport activity by measuring the initial rate of increase in Na$_i$ caused by changing luminal Na$_i$-Cl$_i$-K$_i$ from 50/0/0 to 140/143/4 mM. During the control period, the initial rate of increase was 0.13 ± 0.02 arbitrary units (AU)/min. After treatment with O$_2^-$, it rose to 22 ± 0.04 AU/min (P < 0.025), a 69% increase. Neither XO nor HX alone had a significant effect. Fluoreseimide completely blocked the increase in intracellular Na$_i$ in the control and O$_2^-$ treatment periods. Next, we studied K channel activity by measuring the depolarization caused by increasing luminal K from 1 to 25 mM using a voltage-sensitive dye. During the control period, Na$_i$ was 12.2 ± 1.9 mM. After treatment with O$_2^-$, it rose to 20.9 ± 3.3 mM, a 71% increase (P < 0.01). Cl$_i$ also increased (P < 0.01). Neither XO nor HX alone had a significant effect on Na$_i$ or Cl$_i$. Next, we tested cotransport activity by measuring the initial rate of increase in Na$_i$ caused by changing luminal Na$_i$-Cl$_i$-K$_i$ from 50/0/0 to 140/143/4 mM. During the control period, the initial rate of increase was 0.13 ± 0.02 arbitrary units (AU)/min. After treatment with O$_2^-$, it rose to 22 ± 0.04 AU/min (P < 0.025), a 69% increase. Neither XO nor HX alone had a significant effect. Fluoreseimide completely blocked the increase in intracellular Na$_i$ in the control and O$_2^-$ treatment periods. Next, we studied K channel activity by measuring the depolarization caused by increasing luminal K from 1 to 25 mM using a voltage-sensitive dye. During the control period, maximum depolarization was 7.31 ± 0.77 AU. After O$_2^-$ treatment, it was 6.18 ± 0.90 AU (P < 0.05), a 15% decrease. Finally, we assessed the effects of O$_2^-$ on Na-K-ATPase activity in THAL suspensions by measuring ATP hydrolysis. $V_{\text{max}}$ and K$_{\text{1/2}}$ for Na were not affected by O$_2^-$. We concluded that O$_2^-$ stimulates THAL NaCl absorption primarily by enhancing Na entry via Na-K-2Cl cotransport.

reactive oxygen species; transport; Na-K-ATPase; K channels; NKCC2

SUPEROXIDE (O$_2^-$) HAS BEEN implicated as one of the primary factors in salt-sensitive hypertension (22). This is, in part, because O$_2^-$ enhances Na and water reabsorption by the kidney (18). Zou et al. (22) reported that O$_2^-$ production in the renal medulla exerts a vasoconstrictor as well as antiuretic and antinatriuretic action. Majid and Nishiyama (10) found that infusion of a superoxide dismutase inhibitor into the renal artery in anesthetized dogs decreased urine flow and fractional Na excretion without significantly affecting glomerular filtration rate. These latter data suggest that O$_2^-$ has a direct effect on nephron transport but do not clarify which segments are involved.

The thick ascending limb (THAL) plays an important role in maintenance of NaCl homeostasis. This segment reabsorbs 25–30% of the filtered NaCl load and generates the corticomedullary osmotic gradient necessary for urine concentration. Recent data from our laboratory showed that O$_2^-$ enhances net NaCl absorption by the THAL both indirectly and directly. Endogenous O$_2^-$ was shown to indirectly augment transport by decreasing the bioavailability of nitric oxide (NO), a molecule that inhibits NaCl absorption (12). Hypoxanthine (HX) and xanthine oxidase (XO) also increased NaCl absorption by isolated, perfused THALs in the absence of NO. Because this effect was eliminated by superoxide dismutase, we concluded that the effect was due to O$_2^-$ (4, 13). However, the specific transporters affected were not addressed.

NaCl absorption in the THAL can be described as a two-step process. First, Na and Cl enter the cell via the luminal Na-K-2Cl cotransporter while additional Na can enter via the Na/H exchanger (6). Then, Na is extruded across the basolateral membrane via Na-K-ATPase (20). Cl exits primarily via Cl channels (11, 20), although KCl cotransport may also be involved. K is recycled via luminal membrane K channels and this process is required for full activity of the cotransporter (8). We hypothesize that O$_2^-$ stimulation of net NaCl absorption in the THAL is due to enhanced Na entry via Na-K-2Cl cotransport, resulting in an increase in intracellular Na that augments Na-K-ATPase activity.

METHODS

Animals. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 120–150 g were fed a diet containing 0.22% sodium and 1.1% potassium (Purina, Richmond, IN) for at least 7 days. On the day of the experiment, rats were anesthetized with ketamine (100 mg/kg body wt ip) and xylazine (20 mg/kg body wt ip).

Isolation and perfusion of rat THALs. Once a rat was anesthetized, the abdominal cavity was opened, and the kidney was bathed in ice-cold saline and removed. Coronial slices were placed in oxygenated physiological saline containing (in mM) 130 NaCl, 2.5 NaH$_2$PO$_4$, 4 KCl, 1.2 MgSO$_4$, 6-1-histidine, 1 Na$_2$ citrate, 5.5 glucose, 2 Ca$_2$-ethylenediaminetetraacetic acid, and 10 HEPES (pH 7.4). THALs were dissected from the outer medulla under a stereomicroscope at 10°C and transferred to a temperature-regulated chamber, where they were perfused using a concentric glass pipet at 37 ± 1°C as described previously (3, 14).

Measurement of intracellular Na and Cl. Tubules were perfused and bathed with physiological saline. THAL cells were loaded by bathing the tubules for 15 min at 37 ± 1°C with physiological solution containing 1 μM sodium green tetraacetate in 0.01% Pluronic or 5 μM 6-methoxy-N-[3-sulfopropyl]quinolinium (SPQ; Molecular Probes, Eugene, OR) for measurements of intracellular Na and Cl concentration (Na$_i$ and Cl$_i$), respectively. Once the cells were loaded, they were washed with physiological saline for 15 min. Samples were taken at a rate of 1 sample every 30 s for 5 min. These values were averaged to obtain a value for the control period. Sodium green was excited at 488 nm and SPQ at 360 nm. Emitted fluorescence was collected after passing through either a 510- or 400-nm dichroic mirror and either a 515- or 435-nm long-pass filter, respectively. Then XO (0.75 mM/ml) and HX (125 μM) were added to the bath via

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separate infusion lines for 15 min to enhance \( \text{O}_2^- \) levels. Sampling continued at a rate of 1 sample every 30 s for 15 min. Five samples around the maximum change in fluorescence were averaged for the treatment period. In situ calibration was performed for \( \text{Na}^+ \), using different \( \text{Na}^+ \) calibration solutions and the ionophore gramicidin (Sigma, St. Louis, MO). Two solutions were mixed to obtain the appropriate \( \text{Na}^+ \) concentration for a calibration solution. Solution A contained (in mM) 0 NaCl, 70 KCl, 80 NMDG-Cl, 10 HEPES, 1.2 MgSO\(_4\), and 1 CaCl\(_2\); and solution B contained 50 NaCl, 20 KCl, 80 NMDG-Cl, 10 HEPES, 1.2 MgSO\(_4\), and 1 CaCl\(_2\). Gramicidin was prepared daily as a 5-\( \mu \)M stock and diluted in the calibration solutions before use. No calibration was performed for Cl\(^-\) measurements. SPQ is quenched by Cl\(^-\), so that a decrease in fluorescence represents an increase in Cl concentration. Osmolarity of all solutions was adjusted to 290 ± 3 mosmol/kgH\(_2\)O.

Measurement of Na-K-2Cl cotransporter activity. Tubules were perfused at 37°C in a chamber on the stage of a Nikon Diaphot inverted microscope with a solution designed to prevent Na-K-2Cl cotransporter activity, containing (in mM) 28 NaCl, 2.5 NaH\(_2\)PO\(_4\), 1.2 MgSO\(_4\), 6 l-alanine, 1 Na\(_2\) citrate, 5.5 glucose, 2 Ca dilactate, 10 HEPES, 20 Na gluctose, and 217 mannitol. THAL cells were loaded by bathing them for 45 min in physiological saline containing 4.4 \( \mu \)M Na-sensitive fluorescent dye benzofuran isothialate-acetoxymethyl ester (SBFI-AM; Molecular Probes) in 0.015% Pluronic for ratiometric measurements of \( \text{Na}^+ \). Loading was followed by a 30-min wash with physiological saline. SBFI-AM was prepared daily. SBFI-AM-loaded tubules were excited alternately at 340/380 nm. Emitted fluorescence first passed a 400-nm dichroic mirror and then a 435-nm long-pass filter. An Image One Metafluor system was used to record fluorescence. Aliquots were added to a reaction mixture containing 200 \( \mu \)M of the membrane potential-sensitive dye 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetrathyl benzimidazolyl carbocyanine-tetrachloro-1,1′/H\(_2\)O (JC-1; Molecular Probes) in 0.1% Pluronic for 30 min at 37°C. Then, the luminal membrane. The high-K solution had the same composition as the low-K solution except that NaCl was reduced by 24 mM and KCl increased by the same amount. Five measurements around the maximum change in fluorescence were averaged to obtain the maximum depolarization. The change in voltage is a measure of K channel activity (14). The luminal solution was then switched back to 1 mM K, and XO and HX were added to the bath to raise \( \text{O}_2^- \) levels. After 15 min, measurements were recorded, taking samples every 3 s. After a baseline was established, luminal K was again increased. The percent change in fluorescence was calculated for both control and experimental periods. Control experiments were performed according to the same protocol, except that either XO or HX was added alone before the second period.

Measurement of the effects of superoxide on the Na-K-ATPase pump. Kidneys were perfused retrograde via the aorta with physiological saline (pH 7.0) with 0.1% collagenase (Sigma) and 100 U heparin. The inner stripe of the outer medulla was cut from coronal slices of the kidney, minced, and incubated in 1% collagenase for 30 min at 37°C. The tissue was pelleted via centrifugation at 140 \( g \) (Sorvall, Asheville, NC), resuspended in cold solution, and stirred on ice for 30 min to release the tubules. The suspension was filtered through a 250-\( \mu \)m nylon mesh and centrifuged at 148 \( g \). The pellet was rinsed twice with 1 ml cold physiological saline. The THALs were resuspended in 1 ml physiological saline and divided among four Eppendorf tubes (250 \( \mu \)l each). They were spun to pellet and then resuspended by adding 250 \( \mu \)l physiological saline to tubes 1 and 2 (control) and 250 \( \mu \)l of physiological saline plus HX and XO to tubes 3 and 4 (treatment). Then, they were incubated for 20 min at 37°C and rinsed three times with 500 \( \mu \)l of 150 mM NaCl. After the last rinse, THALs were resuspended in 50 \( \mu \)l distilled H\(_2\)O and freeze-thawed three times. Then, 350 \( \mu \)l of prewarmed solution C containing (in mM) 1 Na\(_2\)EGTA, 5 MgCl\(_2\), 100 imidazole, and 54 KOH (pH 7) were added to tubes 1 and 3. A total of 350 \( \mu \)l of prewarmed solution D containing (in mM) 1 Na\(_2\)EGTA, 5 MgCl\(_2\), 100 imidazole, 49 NaOH, and 2 ouabain were added to tubes 2 and 4. The tubes were incubated for 10 min at 37°C, after which 350 \( \mu \)l of 5% trichloroacetic acid was added to stop the reaction. Then, tubes were centrifuged to pellet the protein. Aliquots of the supernatant were used to determine P, Pi, and lactate. Aliquots were added to a reaction mixture containing 200 \( \mu \)M of 5% trichloroacetic acid, 300 \( \mu \)l of incubation buffer, 150 \( \mu \)l of acid-molybdate, and 0.4 \( \mu \)l of Fiske and Subbarow reductor (Sigma). After 10 min, absorbance was recorded at 600 nm (2). Then, 200 \( \mu \)l of a solution containing 0.04% SDS and 0.05 mM NaOH were added to the pellet to dissolve the protein. Aliquots of the suspension were used to determine protein concentration using Coomassie protein assay reagent (Pierce, Rockford, IL). Phosphate and protein were measured using a UV-visible spectrophotometer (UV 160 U, Shimadzu, Columbia, MD). The difference between ATPase activity in solutions C and D was taken as Na-K-ATPase activity.

Statistics. Results are expressed as mean ± SE. Data were evaluated with Student’s paired t-test. \( P < 0.05 \) was considered significant.

RESULTS

To investigate how \( \text{O}_2^- \) affects NaCl absorption by the THAL, we first tested whether an exogenous \( \text{O}_2^- \)-generating system alters \( \text{Na}^+ \). During the control period, intracellular \( \text{Na}^+ \) was 12.2 ± 1.9 mM. After treatment with XO (0.75 mU/ml) and HX (125 \( \mu \)M) to stimulate \( \text{O}_2^- \) levels, \( \text{Na}^+ \) rose to 20.9 ± 3.4 mM, a 71% increase (\( P < 0.01; n = 5 \)). Neither XO, HX, nor time alone had any significant effect on steady-state Na, (Fig. 1). In the THAL, Na and Cl entry are coupled through the Na-K-2Cl cotransporter. Therefore, we tested whether \( \text{O}_2^- \) could also increase Cl\(^-\). In isolated, perfused THALs, addition of XO and HX to the bath decreased SPQ fluorescence by 28% (\( P < 0.004; n = 5 \); Fig. 2). Because we previously showed that \( \text{O}_2^- \) increases net JCl, these data likely indicate that \( \text{O}_2^- \) increases NaCl entry.

Because \( \text{O}_2^- \) increased both Na\(_2\) and Cl\(^-\), the effects of \( \text{O}_2^- \) on the THAL are probably due to stimulation of ion entry through Na-K-2Cl cotransport. Therefore, we tested whether \( \text{O}_2^- \) could increase Na-K-2Cl cotransport activity by measuring the initial...
rate of Na entry through the cotransporter in the absence and presence of $O_2^-$. Figure 3A shows a representative experiment. Figure 3B shows the mean data. During the control period, the initial rate of increase in Na was $0.13 \pm 0.02$ arbitrary units (AU)/min. After treating tubules with $O_2^-$, the initial rate was $0.22 \pm 0.04$ AU/s ($P < 0.025; n = 8$), a $69\%$ increase. Similar increases were measured after administering dimethylamiloride to inhibit the Na/H exchanger, so the data were pooled. Control experiments with either XO or HX alone showed no significant effect. Differences in initial rates could not be attributed to initial starting ratios, as these values were not different in control and $O_2^-$ treatment periods. Taken together, these data indicate that $O_2^-$ stimulates Na-K-2Cl cotransport.

Na-K-2Cl cotransport could be stimulated either directly or by an increase in apical K permeability. Therefore, we tested whether $O_2^-$ stimulates K channel activity by measuring the depolarization of the luminal membrane caused by increasing luminal K from 1 to 25 mM in the absence and presence of $O_2^-$. During the control period, depolarization was $73.1 \pm 7.7$ AU/s. After treating the tubules with $O_2^-$, it was $61.8 \pm 9.0$ AU/s, a $15\%$ decrease ($P < 0.05$; Fig. 4). Control experiments with either XO or HX alone showed no significant effect. These data indicate that $O_2^-$ inhibits rather than stimulates K channel activity.

Although Na increases after treatment with XO and HX, pump activity could be stimulated, albeit less than cotransport. Therefore, we next investigated the effects of $O_2^-$ on Na-K-ATPase activity measured by ATP hydrolysis. First, we measured Na-K-ATPase activity in the presence of 60 mM Na to determine whether the $K_m$ for Na is affected by $O_2^-$. We found that during the control period, pump activity was $0.35 \pm 0.07$ mmol·µg protein$^{-1}$·min$^{-1}$. After we treated the tubules with $O_2^-$, it was $0.35 \pm 0.05$ mmol·µg protein$^{-1}$·min$^{-1}$. These data suggest that $O_2^-$ has no effect on the $K_m$ of the Na-K-ATPase for Na (Fig. 5A). To determine whether $V_{max}$ is affected by $O_2^-$, we measured Na-K-ATPase activity in the presence of 60 mM Na. We found that during the control period, pump activity was $1.08 \pm 0.21$ P$_i$ mmol·µg protein$^{-1}$·min$^{-1}$. After we treated tubules with $O_2^-$, it was $0.91 \pm 0.07$ P$_i$ mmol·µg protein$^{-1}$·min$^{-1}$. These data suggest that $O_2^-$ has no effect on the $V_{max}$ of the Na-K-ATPase (Fig. 5B).

**DISCUSSION**

$O_2^-$ has been shown to stimulate NaCl absorption by the THAL. Consequently, we investigated which transporters are involved in this process. To do this, we first measured the effect of $O_2^-$ on intracellular Na and Cl. Our data indicate that
O₂⁻ generated by XO and HX increased steady-state intracellular Na from 12 to 21 mM, a 71% increase. Fluorescence of the Cl-sensitive dye SPQ decreased by 28%, indicating that intracellular Cl also increased. These increases could be caused by either an increase in Na and Cl influx or a decrease in efflux. Because O₂⁻ increases net NaCl absorption, it is likely that the increase in intracellular NaCl represents enhanced influx.

Na enters THAL cells via two luminal membrane transporters: the Na-K-2Cl cotransporter (NKCC2) and the Na/H exchanger (NHE3). Cl only enters the cell via NKCC2. NKCC2 accounts for ~80% of absorbed Na, whereas NHE3 accounts for 20% (6). Because O₂⁻ increased both intracellular Na and Cl, and NKCC2 accounts for most of the reabsorbed Na, we next tested whether O₂⁻ stimulates NKCC2 activity. NKCC2 activity was studied by measuring the rate at which intracellular Na increased in response to switching the luminal perfusion solution from 50/0/0 to 140/134/4 mM Na-Cl-K. We found that O₂⁻ stimulated the initial rate of Na influx by ~70%. To show that the increase in intracellular Na in these experiments was in fact due to the Na-K-2Cl cotransporter, we tested the ability of furosemide to block the increase. Furosemide added to the luminal perfusate completely blocked the increase in intracellular Na caused by switching the luminal solution from 50/0/0 to 140/134/4 Na-Cl-K in both the control and superoxide treatment periods. These data indicate that O₂⁻ increases intracellular NaCl primarily by enhancing cotransport activity.

It is unlikely that NHE3 contributed significantly to Na influx under the conditions of our experiments. First, NHE3 accounts for only 20% of Na influx under normal circumstances. Second, the composition of the luminal solutions was chosen to maximize changes in NKCC2 activity while minimizing changes in NHE3 activity. In the absence of Cl and K, no matter what the Na concentration might be, NKCC2 will be inactive whereas in the presence of 140/134/4 mM Na-Cl-K it will be fully active. In contrast, in the presence of 50 mM Na NHE3 will be 83% active and in the presence of 140 mM Na it will be fully active. This small difference in activity is unlikely to cause a measurable change in Na. Finally, to ensure that our measurements were not influenced by changes in Na/H exchange activity, we performed experiments in the presence of the NHE inhibitor dimethyl amiloride. Under these conditions, O₂⁻ enhanced Na entry at a rate similar to controls.

Na-K-2Cl cotransport may be enhanced by O₂⁻ via an effect on NKCC2 itself or on luminal K channels, because active K channels are necessary for full cotransport activity. To study whether K channels are affected by O₂⁻, we measured the depolarization caused by changing luminal K concentration from 1 to 25 mM. K-induced depolarization is a measure of luminal K permeability and thus K channel activity. We found that O₂⁻ decreased luminal K permeability by 15%. These data suggest that O₂⁻ does not enhance Na entry via NKCC2 by activating luminal K channels. It should be noted, however, that our experiments were only designed to reveal a primary effect of O₂⁻ on K channel activity due to a change in either number or conductance. These experiments cannot account for enhanced recycling of K that would occur due to increased intracellular K subsequent to Na-K-2Cl cotransport activation.

For O₂⁻ to stimulate net NaCl absorption, it must increase Na exit as well as entry either directly or indirectly. To test whether O₂⁻ also stimulates ion exit directly, we measured its effect on Na-K-ATPase activity. Our data suggest that O₂⁻ does not affect the K₁/₂ of Na-K-ATPase for Na or the maximum rate at which Na is extruded from the cell. Thus it appears unlikely that O₂⁻ directly alters Na efflux via Na-K-ATPase. If O₂⁻ does not directly enhance Na efflux, then it must indirectly increase pump activity. Based on the steady-state intracellular Na concentration in the presence of O₂⁻ and published values for the K₁/₂ for Na (17), we calculated that the expected rise in pump activity caused by the increase in intracellular Na would
be ~25%. This value is in agreement with the measured increase in net NaCl absorption (13).

Previously, we reported that O$_2^-$ increased net chloride absorption by ~30% (13), whereas here we report that Na-K-2Cl cotransport was increased by 70%. Given the good agreement between the increase in intracellular Na, calculated Na-K-ATPase activity and reported net NaCl fluxes, one might well ask why the O$_2^-$-induced changes in NC2K2 activity appear to be much greater. The explanation for this apparent discrepancy is that NC2K2 activity was measured under different conditions. Thus the Na gradient, which provides energy for transport, was maximal under the experimental conditions of our NC2K2 measurements, compared with the steady-state conditions under which net fluxes were measured.

The XO/HX system will increase O$_2^-$, but it will also increase H$_2$O$_2$. We assumed that the effects of XO and HX reported here are due to O$_2^-$ rather than H$_2$O$_2$ for a number of reasons. Previously, we showed that the effect of XO and HX on net NaCl absorption was not mediated by H$_2$O$_2$ (13). Adding H$_2$O$_2$ alone did not affect Cl transport when added at concentrations higher than those found in the kidney (5). Additionally, superoxide dismutase completely prevented the stimulatory effect of XO and HO. In contrast, catalase had no effect. Superoxide dismutase decreases O$_2^-$ and increases H$_2$O$_2$, whereas catalase decreases H$_2$O$_2$. Thus we assumed that our results can only be explained by O$_2^-$ rather than H$_2$O$_2$ being the biologically active species in these experiments.

Under some circumstances, increasing extracellular O$_2^-$ could cause an increase in intracellular O$_2^-$. To test for this possibility, we measured O$_2^-$ levels using the fluorescent dye dihydroethidium. We found no evidence that when XO/HX was added to the bath to increase extracellular O$_2^-$, intracellular O$_2^-$ was altered (data not shown). Thus our results cannot be due to an increase in intracellular O$_2^-$ levels, and so extracellular O$_2^-$ must stimulate some intracellular signaling pathway by directly acting on a receptor or nonenzymatically producing a product such as isoprostanes that occupies a receptor.

Our finding that O$_2^-$ stimulates Na-K-2Cl cotransport in the THAL may provide an explanation for in vivo results demonstrating that O$_2^-$ reduces urinary volume and Na excretion. Majid and Nishiyama (10) reported that infusion of a superoxide dismutase inhibitor into the renal artery of anesthetized dogs decreased urinary flow and fractional Na excretion without significantly affecting glomerular filtration rate. These data suggest that O$_2^-$ stimulates NaCl reabsorption by the nephron, although the specific segment was not identified. Similarly, Zou et al. (22) reported that increasing and decreasing O$_2^-$ levels in the renal medulla by infusion of either a superoxide dismutase inhibitor or O$_2^-$ scavenger diminished or enhanced urinary Na excretion, respectively.

In other cells O$_2^-$ has been shown to both stimulate and inhibit other transporters. In the epithelium of the ear (9) and respiratory tract (7), O$_2^-$ increases transepithelial Na transport. These data indicate that it stimulates a rate-limiting transport step in the overall process. O$_2^-$ has also been shown to activate ATP-sensitive K channels in cardiocytes (19, 21). Although O$_2^-$ stimulates a number of transporters, it has also been shown to reduce the activity of others. For instance, reactive oxygen species have been shown to inhibit Na-K-ATPase. In lung epithelial cells, O$_2^-$ inhibits the pump (7) as it does in coronary arteries (1). In red blood cell membranes, free radicals also inhibit the pump and Ca ATPase (15). Similar to Na-K-ATPase, O$_2^-$ also inhibits Cl channels in rabbit gastric parietal cells (16).

In contrast to what has been reported for ATP-sensitive K channels and Na-K-ATPase in other cells, we found that O$_2^-$ had no direct effect on Na-K-ATPase and inhibited luminal K channels in THALs. The explanation for these differences is unclear but may be due to differences in concentration, duration of exposure, tissue type, or specific type of K channel.

In summary, we found that O$_2^-$ produced by XO and HX in the basolateral bath enhances luminal membrane Na-K-2Cl cotransport but inhibits luminal K$^+$ channels. This reactive oxygen species did not alter the affinity of the basolateral Na-K-ATPase for Na or its maximum rate of transport. These data indicate that O$_2^-$ stimulates net NaCl absorption by the THAL primarily by enhancing Na entry via the Na-K-2Cl cotransporter.

REFERENCES


