Superoxide Anions Are Involved in Mediating the Effect of Low K Intake on c-Src Expression and Renal K Secretion in the Cortical Collecting Duct*

We previously demonstrated that low K intake stimulated the expression of c-Src and that stimulation of protein tyrosine kinase inhibited ROMK channel activity (Wei, Y., Bloom, P., Lin, D. H., Gu, R. M., and Wang, W. H. (2001) Am. J. Physiol. 281, F206–F212). Decreases in dietary K content significantly increased O$_2^-$ levels and the phosphorylation of c-Jun, a transcription factor, in renal cortex and outer medulla. The role of O$_2^-$ and related products such as H$_2$O$_2$ in stimulating the expression of protein tyrosine kinase is suggested by the observation that addition of 50–200 μM H$_2$O$_2$ increased the phosphorylation of c-Jun and the expression of c-Src in M1 cells, a mouse collecting duct principal cell line. The effect of H$_2$O$_2$ on c-Src expression was completely abolished with cyclohexamide or actinomycin D. The treatment of animals on a K-deficient (KD) diet with tempol significantly increased renal K excretion measured with metabolic cages in Sprague-Dawley rats (6–8 weeks, either sex) were purchased from Taconic Farms (Germantown, NY). Rats were housed in metabolic cages for 7 days to study urinary K excretion. After 3 days of training in the cage, rats were divided into three groups: 1) control group in which animals were kept on a normal K (1.1%) diet and had a daily intraperitoneal injection of saline (15 mg/kg) for 1 week; 2) the low K group in which rats were maintained on a K-deficient (KD) diet and received a daily intraperitoneal injection of saline (10 μl) for 7 days; and 3) the tempol-treated group in which rats were also fed with KD diet and had a daily intraperitoneal injection of tempol (10 μl/kg) for 1 week. Data regarding the 24-h food intake, body weight, and urine output were recorded. Urinary Na and K concentrations were measured by a flame photometer, and daily Na and K excretion were calculated as mEq/24 h. Animals were anesthetized with pentobarbital (60 mg/kg), and blood samples were drawn from the heart to measure the plasma K and Na concentrations. Rats were then killed, and the abdomens were opened to remove the kidneys.

EXPERIMENTAL PROCEDURES

Animals—Sprague-Dawley rats (6–8 weeks, either sex) were purchased from Taconic Farms (Germantown, NY). Rats were housed in metabolic cages for 7 days to study urinary K excretion. After 3 days of training in the cage, rats were divided into three groups: 1) control group in which animals were kept on a normal K (1.1%) diet and had a daily intraperitoneal injection of saline (15 mg/kg) for 1 week; 2) the low K group in which rats were maintained on a K-deficient (KD) diet and received a daily intraperitoneal injection of saline (10 μl) for 7 days; and 3) the tempol-treated group in which rats were also fed with KD diet and had a daily intraperitoneal injection of tempol (10 μl/kg) for 1 week. Data regarding the 24-h food intake, body weight, and urine output were recorded. Urinary Na and K concentrations were measured by a flame photometer, and daily Na and K excretion were calculated as mEq/24 h.

Animals were anesthetized with pentobarbital (60 mg/kg), and blood samples were drawn from the heart to measure the plasma K and Na concentrations. Rats were then killed, and the abdomens were opened to remove the kidneys.
supernatant was collected. Protein concentrations were measured in duplicate using a Bio-Rad DC protein assay kit.

Preparation of M1 Cells—M1 cells, a mouse CCD line, were purchased from the American Type Culture Collection (Manassas, VA) and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum. Before H$_2$O$_2$ treatment, the cells were cultured in medium containing 1% fetal bovine serum for 16 h, followed by incubation for an additional 30 min in a solution containing 22 mM HEPES (pH 7.4), 124 mM NaCl, 5 mM KCl, 1 mM MgCl$_2$, 1.5 mM CaCl$_2$, 0.16 mM HPO$_4$$_2$, 0.4 mM H$_2$PO$_4$, 5 mM NaHCO$_3$, 5.6 mM H$_2$O$_2$(50–200 μM final concentration) was added to the cells in HEPES buffer for 30–120 min. The viability of M1 cells treated with H$_2$O$_2$ as determined by the Trypan Blue dye exclusion method was ~90% of the corresponding control cells. After treatment with H$_2$O$_2$, the cells were washed with ice-cold phosphate-buffered saline twice and incubated for 30 min in radioimmune precipitation assay lysis buffer.

Immunoprecipitation and Western Blot—The corresponding antibody was added to the protein samples (500 μg) harvested from kidneys at a ratio of 1:10 ml phenylmethysulfonyl fluoride and 10 μl of protease inhibitor mixture/ml. The agarose pellet was resuspended in 25 μl of 2x SDS sample buffer containing 4% SDS, 100 mM Tris-HCl (pH 6.8), 20% glycerol, 200 mM dithiothreitol, 0.2% bromphenol blue. After boiling the sample for 5 min, proteins were separated by electrophoresis on 10% SDS-polyacrylamide gels and transferred to Immuno-Blot polyvinylidene difluoride membrane (Bio-Rad). The membrane was blocked with 5% nonfat dry milk in Tris-buffered saline (TBS) and incubated overnight with the primary antibody at 4 °C. The membrane was washed 3x × 15 min with TBS containing 0.05% Tween 20 followed by incubation for 30 min on a rocker. Secondary antibody horseradish peroxidase conjugate. ECL Plus (Amersham Biosciences) was used to detect the protein bands; the intensity of the bands of interest was determined using Alpha DigiDoc 1000 (Alpha Innotech, San Leandro, CA).

Measurement of Superoxide Anion—The method for measurement of O$_2^-$ has been previously described (7). Briefly, renal cortex and outer medulla were isolated from rats on a normal K diet or KD diet for 7–10 days. The tissue (total 100 mg) was cut into small pieces with a sharp blade and suspended in air-equilibrated MOPS-sucrose buffer (pH 7.4) containing 5 μM lucigenin. The chemiluminescence elicited in the presence of lucigenin was measured in a liquid scintillation counter with a single active photomultiplier tube positioned in out-of coincidence mode. Blanks were subtracted from the average level of chemiluminescence signal.

Preparation of CCDS for Patch Clamping—Single CCDS were isolated, placed on a 5 × 5-mm coverglass coated with polylysine and transferred to a chamber (1000 μl) mounted on a inverted Nikon microscope. The CCDS were superfused with HEPES-buffered NaCl solution containing (in mM) 140 NaCl, 5 KCl, 1.5 CaCl$_2$, 1.8 MgCl$_2$, and 5 HEPES (pH 7.4). The pipette solution was composed of (in mM) 140 KCl, 1.8 MgCl$_2$, and 5 HEPES (pH 7.4). The temperature of the chamber was maintained at 37 °C by circulating warm water around the chamber. The CCD was cut open with a sharpened micropipette to expose the apical membrane.

Patch Clamp Technique—We followed the methods described previously to dissect the CCD from rats on a normal K and KD diet and on tempol-treated rats on a KD diet (1). An Axon200A patch clamp amplifier was used to record channel current. The current was low-pass filtered at 1 kHz by an 8-pole Bessel filter (902LPF; Frequency Devices, Haverhill, MA) and digitized with Axon interface (Digidata 1200). Data were analyzed using the pClamp software system 6.04 (Axon Instruments, Burlingame, CA). Channel activity was defined as NPo, which represents secondary antibody horseradish peroxidase conjugate. ECL Plus (Amersham Biosciences) was used to detect the protein bands; the intensity of the bands of interest was determined using Alpha DigiDoc 1000 (Alpha Innotech, San Leandro, CA).

RESULTS

Low K intake has been shown to increase the generation of O$_2^-$ anions in rabbit arteries (5). Thus, we investigated whether low K intake also increased O$_2^-$ production in the kidney. We used the lucigenin enhanced chemiluminescence method described elsewhere (7, 8) to determine O$_2^-$ production in the renal cortex and outer medulla from rats on a KD diet and on a normal K diet. Fig. 1 summarizes results from seven measurements demonstrating that concentrations of O$_2^-$ in the renal cortex and outer medulla increased by ~110 ± 5% in the rats on a KD diet in comparison with those on a normal chow.

Because O$_2^-$ anion and related products such as H$_2$O$_2$ have been demonstrated to activate c-Jun, a transcription factor, in endothelial cells (6), we speculated that high O$_2^-$ concentrations induced by low K intake may be responsible for increased expression of c-Src in the kidney through activation of c-Jun. Therefore, we used the antibody recognizing the phosphorylated c-Jun on serine residue 73, an indication of c-Jun activation (9), to determine the effect of low K intake on c-Jun phosphorylation. Fig. 2 is a Western blot showing that phosphorylation of c-Jun on serine residue 73 is 95 ± 5 and 110 ± 5% (n = 7 rats) higher in the renal cortex and outer medulla from rats on a KD diet than those from animals on a normal K diet, respectively.

To further determine the role of O$_2^-$ in stimulating c-Jun phosphorylation, we investigated the effect of H$_2$O$_2$ on c-Jun phosphorylation in M1 cells, a mouse CCD cell line (10–12). M1 cells were incubated in 200 μM H$_2$O$_2$-containing medium for 30, 60, and 120 min, and c-Jun phosphorylation was examined with Western blot. Fig. 3 is a Western blot demonstrating that incubation of M1 cells in 200 μM H$_2$O$_2$-containing medium for 60 and 120 min significantly increased phospho-c-Jun by 45 ± 5 and 110 ± 20% (n = 5) in comparison to the control value, respectively. We also studied the dose response curve of c-Jun phosphorylation to the stimulation of H$_2$O$_2$. Fig. 4A is a Western blot showing the effect of H$_2$O$_2$ on c-Jun phosphorylation on serine residue 73. Data summarized in Fig. 4B demonstrate that 100 and 200 μM H$_2$O$_2$ significantly stimulated the phosphorylation of c-Jun by 90 ± 10 (n = 4) and 120 ± 10%, respectively. Thus, we used 200 μM H$_2$O$_2$ in the following experiments.

We examined the effect of H$_2$O$_2$ on c-Src expression in M1 cells to determine whether H$_2$O$_2$ increases the expression of c-Src, a representative member of Src family PTK (13). M1 cells were incubated in 200 μM H$_2$O$_2$-containing medium for 30, 60,
and 120 min, followed by incubation in the control medium for an additional 3 h. Fig. 5A is a Western blot demonstrating that incubation of M1 cells in H$_2$O$_2$-containing medium for 120 min significantly increased c-Src by 190 ± 20% (n = 7) in comparison to the control value. Moreover, the effect of H$_2$O$_2$ on c-Src was completely blocked by actinomycin D (5 ng/ml) and cyclohexamide (20 ng/ml) (Fig. 5B). In addition, in the presence of actinomycin D, the c-Src expression level decreased progressively. The decrease was most likely because of the degradation of c-Src.

To further examine the role of O$_2^*$ and related products in mediating the effect of low K intake on PTK expression, we employed tempol, an agent that has been used to decrease O$_2^*$ formation (14). We measured O$_2^*$ levels in rats on a normal K (1.1%) diet, KD diet, and KD diet plus tempol treatment. Data summarized in Fig. 6 demonstrate that tempol treatment significantly decreased O$_2^*$ levels by 70 ± 10% (n = 5) in the renal cortex and outer medulla in comparison with that without tempol treatment, whereas low K intake significantly increased O$_2^*$ production by 150 ± 20% (n = 5). We also examined the effect of low K intake on c-Jun phosphorylation in the tempol-treated and untreated rats. Fig. 7 is a Western blot showing that low K intake increased c-Jun phosphorylation by 90 ± 10% (n = 4). In contrast, the c-Jun phosphorylation in rats on KD diet plus tempol was not significantly different from the control value. Thus, suppression of O$_2^*$ production abolished the effect of low K intake on c-Jun activation.
We have previously shown that low K intake increased the expression of Src family PTK (1). If O$_2^-$ is responsible for mediating the effect of low K on PTK expression, decreases in O$_2^-$ production are expected to suppress the low K intake-induced increases in Src family PTK. Thus, we examined whether tempol treatment inhibits the effect of low K intake on the expression of c-Src as a representative member of Src family PTK. Fig. 8 is a Western blot showing that low K intake significantly increased the expression of c-Src by 220% (n = 4) in comparison with the control. In contrast, tempol treatment abolished the stimulatory effect of low K intake on c-Src expression because c-Src expression in rats on KD diet plus tempol was not significantly different from those on the control K diet.

After showing that tempol treatment suppressed the effect of low K intake on O$_2^-$ levels, c-Jun phosphorylation, and c-Src expression, we examined the effect of tempol on ROMK tyrosine phosphorylation. We confirmed the previous finding that low K intake stimulates the tyrosine phosphorylation of ROMK (15). However, the stimulatory effect of low K intake on tyrosine phosphorylation of ROMK was almost absent in tempol-treated rats (Fig. 9A). Data summarized in Fig. 9B show that low K intake stimulates the tyrosine phosphorylation of ROMK by 150% ± 10% (n = 6), whereas the tyrosine phosphorylation of ROMK in rats on KD diet plus tempol was not significantly different from the control value.

Because stimulation of tyrosine phosphorylation of ROMK1 has been shown to decrease ROMK channel activity in the CCD (16), tempol treatment-induced inhibition of ROMK tyrosine phosphorylation is expected to increase ROMK channel activity in the CCD. Thus, we used the patch clamp technique to examine the ROMK-like SK channels in the CCD from rats on a control (1.1%) or KD (1.1% < 0.001%) diet and in rats on KD diet plus tempol. Data summarized in Fig. 10A show that SK channel activity in the CCD from rats on KD diet (NPo = 0.5 ± 0.1, n = 11) was significantly lower than that (NPo = 1.37 ± 0.2, n = 10) in the CCD from rats on a control K diet. However, SK channel activity in the CCD from rats on a KD diet plus tempol was significantly higher than those (NPo = 1.1 ± 0.1, n = 10) in rats on KD diet. This value is not significantly different from the control value.

Because ROMK-like SK channels are responsible for K secretion, increases in channel activity were expected to stimulate K secretion. We measured renal K excretion in rats on KD diet plus tempol for 7 days with metabolic cage. Results summarized in Fig. 10B show that renal K excretion decreased from 3.5 ± 0.3 mEq/day in rats on a control K diet (n = 7) to 0.03 ± 0.01 mEq in rats on a KD diet (n = 7). Tempol treatment significantly increased renal K excretion to 0.5 ± 0.1 mEq (n = 7). This increase in K excretion leads to a severe hypokalemia in rats on KD diet plus tempol treatment. Plasma K concentration decreased from 3.8 ± 0.3 mEq in animals on the control K diet to 2.8 ± 0.2 mEq in rats on a KD diet. However, tempol treatment further lowered plasma K (to 2.2 ± 0.1 mEq) in rats on KD diet.

**DISCUSSION**

The main findings of the present study are that low K intake increases O$_2^-$ levels in the kidney and that O$_2^-$ and the related products mediate the inhibitory effect of low K intake on ROMK channel activity in the CCD. The notion that O$_2^-$ may play a role in suppressing renal K excretion induced by low K intake is supported by several lines of evidence. First, low K intake significantly increased O$_2^-$ production in the kidney. Second, application of H$_2$O$_2$ mimicked the effect of low K intake on c-Jun phosphorylation and the expression of c-Src in M1 cells. Third, decreasing O$_2^-$ levels induced by tempol significantly suppressed c-Jun phosphorylation, expression of c-Src, and tyrosine phosphorylation of ROMK. Finally, tempol treatment abolished the inhibitory effect of low K intake on the ROMK-like SK channel activity and increased renal K excretion. Therefore, increases in renal O$_2^-$ production from K-re-
stricted rats play an important role in mediating the effect of low K intake on ROMK channel activity and renal K excretion.

It is well established that a low K intake suppresses renal K excretion (2). This is achieved by both inhibition of renal K secretion in the connecting tubule and the CCD and stimulation of K absorption through K-H-ATPase in the outer medullary collecting duct (3). The inhibition of K secretion in the CCD induced by low K intake is at least in part the result of decreases in the apical K conductance. Two types of K channels are present in the apical membrane of the CCD (17): Ca\(^{2+}\)-dependent maxi K and ROMK-like SK channel. Although maxi K channels may be involved in K secretion in the CCD when tubule flow rate is high (18, 19), ROMK-like SK channels play an important role in mediating K secretion.
during normal tubule flow. Thus, alterations in ROMK channel activity in the CCD could affect renal K secretion in the CCD.

We previously demonstrated that low K intake significantly increased the expression of Src family PTK such as c-Src and c-Yes (1). Furthermore, we have shown that ROMK1 was a substrate of PTK and that tyrosine phosphorylation of ROMK increased during K depletion and decreased by high K intake (15). The role of PTK in regulating ROMK channel activity in the CCD is further established by the observation that inhibition of PTK increases (1), whereas inhibition of protein tyrosine phosphatase decreases (20), ROMK channel activity. The inhibitory effect of PTK on ROMK1 is the result of stimulation of the ROMK internalization. In the present study, we have demonstrated that the ROMK channel activity was significantly lower in the CCD from rats on KD diet than those on a control K diet. However, we observed previously that the SK channel activity was not significantly different in the CCD of rats on normal K diet (0.7%) from that on KD diet (1). The discrepancy may be because a 0.7% K-containing rat chow was used as a control K diet in previous experiments and the content of K was 1.1% in the present experiment. Thus, it is conceivable that a physiological variation of dietary K intake could have an effect on ROMK channel activity in the CCD.

Although tempol treatment increased ROMK channel activity in the CCD from rats on a KD diet to a similar extent observed in the CCD from the control animal, renal K excretion in tempol-treated rats was still significantly lower than that of control animals. It is possible that stimulation of K absorption in the outer medullary collecting duct during K restriction plays an important role in preserving K (3). However, the observation that suppressing O$_2^-$ production with tempol significantly increased renal K excretion in comparison with rats on a KD diet alone strongly indicates the role of O$_2^-$ in mediating the effect of low K on renal K secretion. We hypothesized that low K intake stimulates O$_2^-$ production, which stimulates transcription factors such as c-Jun and increases PTK expression. As a consequence, tyrosine phosphorylation of ROMK increased and channel activity decreased. The notion that O$_2^-$ and the related products are involved in mediating the effect of low K intake on tyrosine phosphorylation of ROMK is supported by the observation that suppressing O$_2^-$ production by tempol significantly attenuated the tyrosine phosphorylation of ROMK in rats on a KD diet in comparison with those without tempol treatment. There are at least two possibilities by which O$_2^-$ and related products can stimulate the tyrosine phosphorylation of ROMK: 1) O$_2^-$ stimulates the expression of Src family PTK, which in turn increases the tyrosine phosphorylation of ROMK; 2) O$_2^-$ and the related products facilitate the tyrosine phosphorylation of ROMK by directly modulating the activity of PTK and protein tyrosine phosphatase. O$_2^-$ and H$_2$O$_2$ have been demonstrated to modulate the activity of a variety of protein kinases and phosphatases (21–26). H$_2$O$_2$ has been shown to inhibit protein tyrosine phosphatase (24, 27) and activate several members of Src family PTK, such as Lck and Fyn (25, 26). Thus, it is possible that O$_2^-$ can stimulate the tyrosine phosphorylation of ROMK channels by increasing PTK expression and activity. O$_2^-$ generation increases by activation of enzymes such as NAD(P)H oxidases and xanthine oxidase (28). Superoxide dismutases convert O$_2^-$ to H$_2$O$_2$, which is then metabolized to water by catalase. Thus, increases in O$_2^-$ are expected to raise H$_2$O$_2$ concentrations. O$_2^-$ and H$_2$O$_2$ have initially been identified to be involved in the regulation of immune response and programmed cell death. However, a large body of evidence has supported the notion that O$_2^-$ and H$_2$O$_2$ may play an important role in mediating a variety of cell functions (28). O$_2^-$ has been shown to mediate the effect of nerve growth factor (NGF) in neuronal cells (29) and epidermal growth factor (EGF) in human epidermoid carcinoma cells (30). Stimulation of NGF and EGF receptors results in transient increases in O$_2^-$ and H$_2$O$_2$ concentrations. Moreover, elimination of H$_2$O$_2$ by catalase has been demonstrated to inhibit EGF and NGF receptors. Stimulation of insulin receptors has been shown to augment the formation of O$_2^-$ (31), and low concentrations of H$_2$O$_2$ can potentiate the insulin effect in insulin-responsive tissues (32). Moreover, high concentrations of H$_2$O$_2$ can induce insulin-like effects in the absence of insulin via stimulation of the insulin-independent tyrosine phosphorylation of the insulin receptor (33). H$_2$O$_2$ mediates the stimulatory effect of angiotensin II on nitric oxide production in endothelial cells (34). In addition, H$_2$O$_2$ stimulates cGMP generation and causes the transient relaxation of calf coronary arteries (35). Thus, it is possible that O$_2^-$ and the related products play a physiological role in the regulation of renal K secretion.

The mechanism by which low K intake increases O$_2^-$ production is not known. Several hormones such as growth factor and angiotensin II have been shown to stimulate NAD(P)H and increase O$_2^-$ production (29, 30, 34). K depletion has been shown to increase the concentration of growth factors such as insulin-like growth factor (36, 37). Thus, it is possible that growth factors and angiotensin II may be involved in O$_2^-$ generation during low K intake. Further experiments are required to test these speculations. We conclude that low K intake increases O$_2^-$ and the related products, which in turn stimulate the expression of PTK, and that increases in PTK activity stimulate tyrosine phosphorylation of ROMK channels and inhibit ROMK channel activity.

REFERENCES

1. Wei, Y., Bloom, P., Lin, D. H., Gu, R. M., and Wang, W. H. (2001) *Am. J. Physiol.* **281**, F206–F212.
2. Giebish, G. (1998) *Am. J. Physiol.* **274**, F817–F833.
3. Wingo, C. S., and Cain, B. D. (1993) *Ann. Rev. Physiol.* **55**, 323–347.
4. Wang, W. H. (2004) *Annu. Rev. Physiol.* **66**, 547–569.
5. Yang, B. C., Li, D. Y., Weng, Y. F., Lynch, J., Wingo, C. S., and Wehta, J. L. (1999) *Am. J. Physiol.* **277**, H1955–H1961.
6. Cook, K., Vita, J. A., Berk, B. C., and Keaney, J. F., Jr. (2001) *J. Biol. Chem.* **276**, 16045–16050.
7. Mohazzab, H. M., and Wolin, M. S. (1994) *Am. J. Physiol.* **267**, L815–L822.
8. Munzel, T., Asnas‘ev, I. G., Kleschev, A. L., and Harrison, D. G. (2002) *Arterioscler. Thromb. Vasc. Biol.* **22**, 1761–1768.
9. Karin, M. (2002) *J. Biol. Chem.* **270**, 16483–16486.
10. Korbmacher, C., Segal, A. S., Fejes-Toth, G., Giebish, G., and Boulpaep, E. L. (1993) *J. Gen. Physiol.* **102**, 761–769.
11. Ahmad, I., Korbmacher, C., Segal, A. S., Cheung, P., Boulpaep, E. L., and Barnstable, C. J. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 10262–10266.
12. Letz, B., Ackermann, A., Canessa, C. M., Rossier, B. C., and Korbmacher, C. (1995) *J. Membr. Biol.* **148**, 127–141.
13. Thomas, S. M., and Brugge, J. S. (1997) *Ann. Rev. Cell Biol.* **13**, 513–589.
14. Krishna, M. C., Grahame, D. A., Samuni, A., Mitchell, J. B., and Russo, A. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 5557–5561.
15. Lin, D. C., Sterling, H., Lerea, K. M., Welling, P., Jin, L., Giebish, G., and Wang, W. H. (2002) *Am. J. Physiol.* **283**, F671–F677.
16. Sterling, H., Lin, D. H., Gu, R. M., Dong, K., Hebert, S. C., and Wang, W. H. (2002) *J. Biol. Chem.* **277**, 4317–4323.
17. Wang, W. H., Hebert, S. C., and Giebish, G. (1997) *Ann. Rev. Physiol.* **59**, 413–436.
18. Woda, C. B., Leite, M., Jr., Rohatgi, R., and Satlin, L. M. (2002) *Am. J. Physiol.* **283**, F437–F446.
19. Woda, C. B., Bragin, A., Kleyman, T., and Satlin, L. M. (2001) *Am. J. Physiol.* **280**, F786–F793.
20. Wei, Y., Bloom, P., Gu, R. M., and Wang, W. H. (2000) *J. Biol. Chem.* **275**, 20502–20507.
21. Guyton, K. Z., Liu, Y., Gerose, M., Xu, Q., and Holbrook, N. J. (1996) *J. Biol. Chem.* **271**, 4138–4144.
22. Lo, Y. C., Wong, J. M. S., and Cruz, T. P. (1996) *J. Biol. Chem.* **271**, 15703–15709.
23. Baas, A. S., and Berk, B. C. (1995) *Circ. Res.* **77**, 29–36.
24. Mahadev, K., Zilbering, A., Zhu, L., and Goldstein, B. J. (2001) *J. Biol. Chem.* **276**, 21938–21942.
25. Brumell, J. H., Burkhardt, A. L., Bolen, J. B., and Grinstein, S. (1996) *J. Biol. Chem.* **271**, 1455–1461.
26. Nakamura, H., Hori, T., Sato, N., Sugie, K., Kawakami, T., and Yodoi, J. (1993) *Oncogene* **8**, 3133–3139.
27. Dunn, J. M., and Tanner, K. G. (1998) *Biochemistry* **37**, 5633–5642.
Effect of $H_2O_2$ on Renal K Secretion

28. Droge, W. (2002) Physiol. Rev. 82, 47–95
29. Suzukawa, K., Miura, K., Mitsushita, J., Resau, J., Hirao, K., Crystal, R., and Kamata, T. (2000) J. Biol. Chem. 275, 13175–13178
30. Bae, Y. S., Kang, S. W., Seo, M. S., Baines, I. C., Tekle, E., Chock, P. B., and Rhee, S. G. (1997) J. Biol. Chem. 272, 217–221
31. Mahadev, K., Wu, X., Zilbering, A., Zhu, L., Lawrence, J. T. R., and Goldstein, B. J. (2001) J. Biol. Chem. 276, 48662–48669
32. Schmid, E., Hotz-Wagenblatt, A., Hack, V., and Droge, W. (1999) FASEB J. 13, 1491–1500
33. Hayes, G. R., and Lockwood, D. H. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 8115–8119
34. Cai, H., Li, Z., Dikalov, S., Holland, S. M., Hwang, J., Jo, H., Dudley, S. C., Jr., and Harrison, D. G. (2002) J. Biol. Chem. 277, 48311–48317
35. Mohazzab-H., K. M., Kaminski, P. M., Fayngersh, R. P., and Wolin, M. S. (1996) Am. J. Physiol. 270, H1044–H1053
36. Hsu, F. W., Tsao, T., and Rabkin, R. (1997) Kidney Int. 52, 363–370
37. Evan, E. P., Henry, D. P., Connors, B. A., Summerlin, P., and Lee, W. H. (1995) Kidney Int. 48, 1517–1529