The kidney medulla is exposed to very high interstitial osmolality leading to the activation of mitogen-activated protein kinases (MAPK). However, the respective roles of increased intracellular osmolality and of cell shrinkage in MAPK activation are not known. Similarly, the participation of MAPK in the regulatory volume increase (RVI) following cell shrinkage remains to be investigated. In the rat medullary thick ascending limb of Henle (MTAL), extracellular hypertonicity produced by addition of NaCl or sucrose increased the phosphorylation level of extracellular signal-regulated kinase (ERK) and p38 kinase and to a lesser extent c-Jun NH2-terminal kinase with sucrose only. Both hypertonic solutions decreased the MTAL cellular volume in a dose- and time-dependent manner. In contrast, hypertonic urea had no effect. The extent of MAPK activation was correlated with the extent of MTAL cellular volume decrease. Increasing intracellular osmolality without modifying cellular volume did not activate MAPK, whereas cell shrinkage without variation in osmolality activated both ERK and p38. In the presence of 600 mosmol/liter NaCl, the maximal cell shrinkage was observed after 10 min at 37 °C and the MTAL cellular volume was reduced to 70% of its initial value. Then, RVI occurred and the cellular volume progressively recovered to reach about 90% of its initial value after 30 min.

SB203580, a specific inhibitor of p38, almost completely inhibited the cellular volume recovery, whereas inhibition of ERK did not alter RVI. In conclusion, in rat MTAL: 1) cell shrinkage, but not intracellular hyperosmolality, triggers the activation of both ERK and p38 kinase in response to extracellular hypertonicity; and 2) RVI is dependent on p38 kinase activation.

During diuresis and antidiuresis, the kidney medulla is exposed to large fluctuations of interstitial osmolality (1), which challenge cell volume constancy. Cells of the medullary thick ascending limb of Henle (MTAL) are of special interest, since they are the major contributor to the generation of the renal cortico-papillary osmotic gradient allowing urinary concentration in terrestrial animals. The first adaptive process occurring in response to extracellular hypertonicity-induced cell shrinkage is a regulatory cell volume increase (RVI). The RVI results from the stimulation of ion transporters which increase the intracellular ion content within minutes and partially restore the cellular volume from the initial cell shrinkage (2, 3). A second adaptive mechanism, in mammalian cells, is the induction of genes encoding proteins involved in the accumulation of intracellular “compatible osmolytes” within hours and days. These osmoprotective proteins are either enzymes, i.e. aldose reductase generating sorbitol from glucose, or organic osmolytes transporters, i.e. myo-inositol, taurine, glycero-phosphocholine, and betaine (4). The intracellular signaling pathways mediating these adaptive mechanisms, especially the role of MAP kinases, are still incompletely understood.

Mitogen-activated protein (MAP) kinase cascades are important intracellular signal-transduction pathways activated in response to changes in osmolality. MAP kinases are serine/threonine kinases activated via a cascade of kinases involving a sequential phosphorylation of two kinases (MAP kinase kinase and MAP kinase kinase), which activates a MAP kinase via a dual phosphorylation on threonine and tyrosine residues (5). In mammalian cells, the MAP kinase family contains three major subgroups responding to distinct extracellular stimuli: extracellular signal-regulated kinases 1 and 2 (ERK) principally activated by growth factors, integrin-matrix interaction, and hormones or neurotransmitters with serpine receptors (6–12); c-Jun NH2-terminal kinases (JNK, also known as stress-activated protein kinases 1); and p38 kinases (also known as stress-activated protein kinases 2) strongly activated by inflammatory cytokines, ultraviolet light, and hypertonic stress (13–18). To date, the effects of MAP kinases have been mostly attributed to the control of gene transcription via phosphorylation of nuclear transcription factors (8, 13). However, non-genomic effects of MAP kinases are increasingly recognized. ERKs may control glucose metabolism through activation of phospholipase A2, which phosphorylates and inhibits glycogen synthase kinase 3 (19, 20). In addition, ERKs might be involved in the control of cAMP-specific phosphodiesterase activity (21). On the other hand, p38 kinase might be implicated in stress- and growth factor-induced cytoskeleton reorganization (22, 23) and in the stimulation of glucose transport by insulin (24).

Activation of the three families of MAP kinases by extracellular hypertonicity has been shown in different cell lines in culture (18, 25–29), as well as in native rat MTAL cells (30), but...
the mechanisms of their activation by extracellular hypertonicity need to be clarified. Neither the respective role of intracellular hypertonicity versus cell shrinkage in the activation of MAP kinases by extracellular hyperosmolality nor the relationship between the MAP kinase activation and the regulatory cell volume increase have been investigated. We have undertaken this study: 1) to examine the effects of different solutes on the activation of MAP kinases in MTAL cells by hypertonicity, and 2) to analyze the relationship between MAP kinase activation and cell volume variations.

**EXPERIMENTAL PROCEDURES**

**Preparation of Single MTALs**—Male Wistar rats weighing 150–200 g were anesthetized with pentobarbital sodium (5 mg/100 g body weight, intraperitoneally) and left kidney was immediately removed after perfusion with ice-cold solution (120 mM NaCl, 5 mM KCl, 4 mM NaHCO$_3$, 1 mM CaCl$_2$, 1 mM MgSO$_4$, 0.2 mM Na$_2$HPO$_4$, 0.15 mM Na$_3$VO$_4$, 1 mM EDTA, 30 mM NaF, 30 mM Na$_4$O$_7$P$_2$, 2 mM Na$_3$VO$_4$, 1 mM AEBSF, 10 µg/ml leupeptin, 4 µg/ml aprotinin, 1% Triton X-100, pH 7.4). After measurement of protein content by the DC protein assay (Pierce), equal amounts of protein were separated by 10% SDS-PAGE and transferred on a polyvinylidene difluoride membrane. The two kidneys were perfused with Tris-buffered saline (50 mM Tris, 150 mM NaCl) with 0.2% (w/v) Nonidet P-40 (TBS-Nonidet P-40) and 5% (w/v) nonfat dry milk for 1 h at room temperature and then incubated for 2 h at room temperature with first antibody diluted in TBS-Nonidet P-40 with 5% of milk. After preincubation in isotonic incubation solution, MTALs were microdissected under stereomicroscopic control in oxygenated (95% O$_2$, 5% CO$_2$) incubation solution.

**Preparation of Suspensions of MTALs**—The two kidneys were perfused with ice-cold incubation solution without collagenase. The inner stripes of the outer medulla were excised and minced on ice, and fragments of medullary tubules were obtained by gentle pressure through nylon filters with pore size decreasing from 150 to 100 µm. After centrifugation, the pellet was resuspended in ice-cold oxygenated (95% O$_2$, 5% CO$_2$) incubation solution. As controlled under stereomicroscope, MTALs account for about 90% of the tubule fragments in this preparation. Therefore, it will be referred to as MTAL suspension.

**Immunoblots**—After pre-incubation in isotonic incubation solution, MTALs were incubated in isotonic or hypertonic incubation solutions with or without addition of drugs. Incubation was stopped by cooling and centrifugation before addition of ice-cold lysis buffer containing 20 mM Tris-HCl, 2 mM EGTA, 2 mM EDTA, 30 mM NaF, 30 mM Na$_2$PO$_4$, 2 mM Na$_3$VO$_4$, 1 mM AEBSF, 10 µg/ml leupeptin, 4 µg/ml aprotinin, 1% Triton X-100, pH 7.45. After measurement of protein content by the BCA protein assay (Pierce), equal amounts of protein were separated by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane. 0.05% (w/v) collagenase. After incubation at 30 °C for 20 min in incubation solution containing 0.05% (w/v) collagenase, kidney slices were stored at 4 °C.
Activation of MAP Kinases by Hyperosmolarity

RESULTS

Hypertonic NaCl and Sucrose but Not Urea Increased ERK and p38 Kinase Phosphorylation in MTAL Cells—MTAL suspensions, as well as microdissected MTALs were incubated for 15 min at 37°C in isotonic or hypertonic incubation solution. Osmolarity was raised up to 600 mosmol/liter by addition of hypertonic NaCl or sucrose. Fig. 1 shows that hypertonic NaCl and sucrose increased the phosphorylation level of ERK (as percentage of control ± S.E.; NaCl: 295 ± 60%, p < 0.005; sucrose: 975 ± 327%, p < 0.005) and p38 kinase (NaCl: 317 ± 32%, p < 0.01; sucrose: 320 ± 124%, p < 0.005) in microdissected MTALs (Fig. 1A) as well as in MTAL suspensions (Figs. 1, C and D). As depicted in Fig. 1B, the total amounts of ERK and p38 kinase were identical in both preparations, suspensions or microdissected MTALs (Fig. 1, A and C). Thus all subsequent experiments were performed in MTAL suspensions.

Dose Dependence of MAP Kinase Phosphorylation—The dose dependence of the effect of extracellular osmolarity on MAP kinases phosphorylation was determined by a 15-min incubation of MTAL suspension in solution with osmolarity increasing from 300 to 700 mosmol/liter by addition of NaCl, sucrose, or urea. As shown in Fig. 2, NaCl-induced extracellular hypertonicity produced a dose-dependent increase of the phosphorylation level of ERK. This effect reached a maximum at 600 mosmol/liter (as percentage of control ± S.E.: 295 ± 60%, p < 0.005). Similarly, the phosphorylation level of p38 kinase was maxi-
Figure 5. Effect of incubation time with hypertonic NaCl on the phosphorylation level of ERK, JNK, and p38 kinase. Suspensions of MTAL were incubated for 30 min at 37 °C. Controls were incubated in isotonic medium (300 mosmol/liter), and the other samples were incubated in a medium containing 600 mosmol/liter NaCl for 1–30 min. Phosphorylated MAP kinases were detected by immunoblot with phosphospecific antibodies. A, representative immunoblot showing the phosphorylation level of ERK, JNK, and p38 kinase in response to 600 mosmol/liter NaCl for various times. B, densitometric quantitation of the effect of extracellular osmolarity on the phosphorylation level of ERK (filled squares), JNK (filled triangles), and p38 kinase (filled circles). Results were expressed as a percentage of the control and are means ± S.E. from five independent experiments (*, p < 0.05; **, p < 0.01; ****, p < 0.005).

Hypertonic NaCl decreased MTAL cellular volume in a dose-dependent manner at 500 mosmol/liter NaCl (334 ± 45%, p < 0.001). By contrast, the phosphorylation level of JNK was not altered by hypertonic NaCl.

Hypertonic sucrose induced a dose-dependent increase in ERK phosphorylation with a maximum at 600 mosmol/liter (975 ± 327%, p < 0.005) and in p38 kinase phosphorylation with a maximum at 500 mosmol/liter (320 ± 99%, p < 0.005), respectively. In contrast with NaCl, hypertonic sucrose increased JNK phosphorylation with a maximum at 600 mosmol/liter (273 ± 59%, p < 0.005) (Fig. 3).

Finally, hypertonic urea did not significantly alter the phosphorylation level of MAP kinases (Fig. 4).

Time Dependence of MAP Kinase Phosphorylation—To determine the time course of the effect of extracellular osmolarity on phosphorylation of MAP kinases, MTAL suspensions were incubated for a total period of 30 min at 37 °C including adjuncction (experimental) or not (control) of a prewarmed hypertonic NaCl or sucrose up to 600 mosmol/liter incubation solution for periods varying from 1 to 30 min. Exposure to hypertonic NaCl solution produced an increase in the phosphorylation level of ERK which peaked at 3 min (as percentage of control ± S.E.: 271 ± 61%, p < 0.005) and then progressively decreased to the control level after 30 min (Fig. 5). Similarly, phosphorylation of p38 kinase increased progressively during the first 5 min of incubation to reach a maximum of 404 ± 116% of the control level (p < 0.005). Phosphorylation of p38 kinase then gradually decreased and reached a plateau of 183 ± 84% of the control value after 30 min of incubation. The phosphorylation level of JNK was not significantly altered during the 30-min incubation with hypertonic NaCl.

With hypertonic sucrose, the ERK phosphorylation increased progressively during the incubation and reached a maximum of 1034 ± 397% of the control level (p < 0.005) at 5 min (Fig. 6). Phosphorylation of p38 kinase increased within the same time course and reached a maximum of 609 ± 237% of the control level (p < 0.005). By contrast with NaCl, we did not observe a progressive decrease in phosphorylation level of both ERK and p38 kinase, which was sustained for 30 min. The phosphorylation level of JNK increased progressively in response to hypertonic sucrose and reached a maximum after 30 min of incubation (as percentage of control ± S.E.: 251 ± 68%, p < 0.05).

Hypertonic NaCl and Sucrose but Not Urea Decreased the Volume of Single MTALs in a Dose- and Time-dependent Manner—The effects of extracellular hyperosmolarity on the MTAL cellular volume were determined by the following experiments. After a 15-min preincubation period at 37 °C, single MTALs were incubated for 15 min at 37 °C in iso-osmotic or hyperosmotic incubation solutions. Osmolarity was raised up to 600 mosmol/liter by addition of hypertonic NaCl, sucrose, or urea. Fig. 7 shows that hypertonic NaCl and sucrose decreased the MTAL cellular volume. In contrast, urea did not alter MTAL cellular volume.

As shown in Fig. 8A, increasing osmolality with hypertonic NaCl decreased MTAL cellular volume in a dose-dependent
manner with a maximal reduction of 30.64 ± 2.01% of the initial volume (p < 0.001) (Fig. 8A). Similarly, sucrose solution induced a dose-dependent decrease in MTAL cellular volume reaching a 34.92 ± 3.04% reduction from the initial volume (p < 0.05). Over the range of extracellular osmolarity studied, urea did not alter MTAL cellular volume.

For time-course experiments, single MTALs were incubated for a total period of 30 min at 37 °C including adjunction (experimental) or not (control) of a prewarmed hypertonic (NaCl or sucrose up to 600 mosmol/liter) incubation solution for periods varying from 1 to 30 min. Fig. 8B shows the time-dependent change in MTAL cellular volume. Increasing incubation time with hypertonic NaCl decreased MTAL cellular volume with a maximal decrease to 68.98 ± 2.21% of the initial volume (p < 0.001) after 10 min of incubation. Increasing incubation time with hyperosmotic NaCl was associated with a progressive recovery of tubule volume that peaked after 30 min of incubation (89.14 ± 2.24%, p < 0.01). Incubation with hypertonic sucrose gave similar results, although the decrease in MTAL cellular volume was slightly more pronounced than with NaCl, reaching 65.47 ± 5.94% of the initial volume (p < 0.001) after 5 min and it was sustained for 15 min. After 30 min of incubation with hypertonic solution, a partial recovery of initial MTAL cellular volume was observed to a lesser extent than with NaCl (81.81 ± 5.94%, p < 0.05).

The Phosphorylation Level of ERK/p38 Kinase Correlated with the MTAL Cellular Volume—To assess the possibility of a relationship between the phosphorylation level of ERK/p38 kinase and the tubule volume, the changes in phosphorylation level of ERK and p38 kinase were plotted as a function of the variations in MTAL cellular volume.

Fig. 9A, drawn from the data presented in Figs. 2, 3, 5, 6, and 8, shows the linear relationship existing between the phosphorylation level of ERK and the decrease in MTAL cellular volume (r = 0.75). This linear relationship (r = 0.88) was even stronger for p38 kinase (Fig. 9B).

Change in Cell Volume Rather than in Osmolarity Was Responsible for the Alteration in Phosphorylation Level of ERK and p38 Kinase—The following experiments were designed to analyze separately the effects of intracellular hyperosmolarity and cell shrinkage on the increase in phosphorylation level of ERK and p38 kinase.

To induce intracellular hypertonicity without cell volume variation, suspensions or single MTALs were incubated for 9 min in the presence of 10−4 M nystatin (Sigma) in ice-cold isotonic incubation solution (300 mosmol/liter) in which NaCl was substituted for KCl. After three washes in ice-cold isotonic KCl incubation solution, tubules were incubated first in ice-cold hypertonic KCl medium (600 mosmol/liter) for 5 min and then in prewarmed (37 °C) hypertonic NaCl medium (600 mosmol/liter) for 5 additional min. Control experiments have shown that MTAL cellular volume was 94.6 ± 2.1% of its initial value after this procedure. Fig. 10 (A and B) shows that when the MTAL cellular volume decreased, hypertonicity increased the phosphorylation level of ERK and p38 kinase by 269 ± 37% (p < 0.005) and by 179 ± 32% (p < 0.05), respectively.

FIG. 7. Effect of hyperosmolarity on the cellular volume of single MTALs. Microdissected single MTALs were incubated for 10 min at 37 °C either under isotonic conditions (C; 300 mosmol/liter) or after addition of NaCl (N), sucrose (S), and urea (U) up to 600 mosmol/liter. Tubules were visualized with an inverted microscope, and photographs were taken before (upper photographs) and after (lower photographs) incubation. A–D, representative photographs showing the effect of incubation in isotonic or hypertonic media on cellular volume. E, quantitation of the effect of extracellular osmolarity on cellular volume. Results are expressed as a percentage of control (initial volume) and are means ± S.E. from four to six independent experiments (**, p < 0.01; ***, p < 0.005).
contrast, when the MTAL cellular volume was maintained constant, intracellular hypertonicity did not alter the phosphorylation level of ERK and p38 kinase.

A decrease in MTAL cellular volume at constant intracellular osmolality was obtained by 20-min incubation of tubules at 37 °C with 10^{-2} M nystatin in isotonic incubation solution in which NaCl was substituted for sucrose. This isotonic cell shrinkage was reversed by three washes in isotonic sucrose incubation solution without nystatin followed by a 5-min incubation at 37 °C. Control experiments have shown that MTAL cellular volume was 71.1 ± 0.2% of its initial value after incubation with isotonic sucrose plus nystatin, and returned to 102.1 ± 2.0% of its initial value after removal of nystatin and incubation with isotonic NaCl. As depicted in Fig. 10 (A and B), decreasing MTAL cellular volume under isotonic condition increased the phosphorylation level of ERK and p38 kinase by 239 ± 41% (p < 0.001) and 166 ± 20% (p < 0.05), respectively. This effect was reversible upon recovery of initial cellular volume.

**DISCUSSION**

The present study shows that cell shrinkage induced by extracellular hypertonicity activates MAP kinases in rat MTAL. In addition, our results indicate that, among the MAP kinases, p38 kinase appears to play a major role in the early cell protective response to extracellular hypertonicity, i.e. the RVI.
Extracellular hypertonicity has been shown to activate MAP kinases in various animal cell lines (5), including MDCK cells (26, 27, 31) that share some properties with mammalian distal nephron cells and mIMCD cells (8, 29, 32). Our study, taken with the results of Watts et al. (30), extends this finding to native rat MTAL cells, which are exposed to extracellular osmolarities from 300 to 600 mosmol/liter under water diuresis and antidiuresis, respectively (1). In the present study, the level of phosphorylation of MAP kinases was taken as an index of their activation since only phosphorylated MAP kinases are active (5) and the increase in phosphorylation parallels the activity of MAP kinases in MTAL cells (30). Our results, taken together with a recent study showing that water restriction activates MAP kinases in the rat renal inner medulla (33), support the physiological relevance of the activation of MAP kinases by extracellular hypertonicity.

The major role of MTAL cell shrinkage in the hypertonicity-induced MAP kinases activation is supported by the following observations. 1) Hypertonic urea, which did not alter cellular volume, did not activate MAP kinases; 2) hypertonic sucrose, which induced a larger decrease in cellular volume, was a more powerful activator of ERK and p38 kinases than hypertonic NaCl; 3) cellular volume was inversely correlated with the activation level of ERK and p38 kinases; 4) increased intracellular osmolarity without alteration in cellular volume did not activate ERK and p38 kinase, which was reversible upon return to the initial cell volume. These results strongly suggest that cell shrinkage activates the MAP kinases in rat MTAL cells. A role for cell volume variations in the regulation of cellular signaling pathways has already been demonstrated in human polymorphonuclear cells (34).

Since hyperosmolarity activates MAP kinases through changes in cellular volume, the hypothesis that components of the cytoskeleton may be the primary sensor should be considered. Moreover, extracellular hypertonicity drives water movement outside the cells leading to an increase in cytoplasmic fluid.
The RVI is an essential mechanism of cellular protection against osmotic stress. After the initial cell shrinkage, MTAL cells exhibit a RVI response that involves the activation of ion transporters (2). This process drives sodium influx coupled to secondary water movements into the cells and allows the partial recovery of the initial cell volume (2). Our results confirm that MTAL cells undergo RVI after an hypertonic challenge (3).

The RVI is an essential mechanism of cellular protection against osmotic stress. After the initial cell shrinkage, MTAL cells exhibit a RVI response that involves the activation of ion transporters (3). This process drives sodium influx coupled to secondary water movements into the cells and allows the partial recovery of the initial cell volume (2). Our results confirm that MTAL cells undergo RVI after an hypertonic challenge (3).

acknowledgment—We thank dr. s. gonin for critical reading of the manuscript.

References
1. Knepper, M. A., and Rector, F. C., Jr. (1996) in The Kidney (Brenner, B. M., ed) 5th Ed., pp. 532–570, W. B. Saunders Co., Philadelphia
2. Parker, J. C. (1993) Am. J. Physiol. 265, C1191–C1200
3. Sun, A. M., Saltzberg, S. N., Kikkeri, D., and Hebert, S. C. (1996) Kidney Int. 49, 1019–1029
4. Burg, M. B. (1995) Am. J. Physiol. 268, F983–F996
5. Wollmann, C., Gibson, S., Jarpe, M. B., and Johnson, G. L. (1999) Physiol. Rev. 79, 143–180
6. Wu, J., Dent, P., Jelinek, T., Wolfman, A., Weber, M. J., and Sturgill, T. W. (1993) Science 262, 1065–1072
7. Graves, L. M., Bornfeld, K. E., Raines, E. W., Potts, B. C., Macdonald, S. G., Ross, R., and Krebs, E. G. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 10300–10304
8. Ferby, I. M., Waga, I., Sakanaka, C., Kume, K., and Shimizu, T. (1994) J. Biol. Chem. 269, 30485–30488
9. Morino, N., Mimura, T., Hamaaki, K., Toke, K., Ueki, K., Kikuchi, K., Takehara, K., Kadowaki, T., Yazaki, Y., and Nijima, Y. (1995) J. Biol. Chem. 270, 269–273
10. Fordellis, C. S., Bergeraud, M., Gouache, P., Barbu, V., Gavrias, H., Handy, D. E., Bertaizt, G., and Masliah, J. (1996) J. Biol. Chem. 271, 3491–3494
11. Crep, C., Xu, N., Simmonds, W. S., and Gutkind, J. S. (1994) Nature 369, 418–420
12. Lian, D.-F., Monia, B., Dean, N., and Berk, B. C. (1997) J. Biol. Chem. 272, 6146–6150
13. Ip, T., and Davis, R. J. (1998) Curr. Opin. Cell Biol. 10, 205–219
14. Freshney, N. W., Rawlinson, L., Guesdon, F., Jones, E., Cowley, S., Huan, J., and Saklatvala, J. (1994) Cell 78, 1093–1049
15. Sluss, H. K., Barrett, T., Derijard, B., and Davis, R. J. (1994) Mol. Cell. Biol. 14, 8376–8384
16. Matsuda, S., Kawakami, H., Morigni, T., Gotoh, Y., and Nishida, E. (1995) J. Biol. Chem. 270, 12781–12786
17. Chen, Y.-R., Wang, X., Templeton, D., Davis, R. J., and Tan, T.-H. (1996) J. Biol. Chem. 271, 31929–31936
18. Rossetto, C., and Karin, M. (1996) Science 274, 1194–1197
19. Eldar-Finkelman, H., Seger, R., Vandenheede, R., and Krebs, E. G. (1995) J. Biol. Chem. 270, 987–990
20. Chang, P.-Y., Le Marchand-Brustel, Y., Cheatham, L. A., and Moller, D. E. (1995) J. Biol. Chem. 270, 29928–29935
21. Liu, H., and Maurice, D. H. (1999) J. Biol. Chem. 274, 10557–10565
22. Huot, J., Houle, F., Rousseau, S., Deschesnes, R. G., Shah, G. M., and Landry, R. G. (1995) J. Cell Biol. 143, 1361–1373
23. Matsumoto, T., Yokote, K., Tamura, K., Takehara, K., Moriguchi, T., Kadowaki, T., Kume, K., and Shimizu, T. (1995) J. Biol. Chem. 270, 30485–30488
24. Sweeney, G., Somwar, R., Ramal, T., Volchuk, A., Uyama, A., and Klip, A. (1999) J. Biol. Chem. 274, 10071–10078
25. Galacheva-Gargova, Z., Derijard, B., Wu, L.-H., and Davis, R. J. (1994) Science 265, 806–811
26. Ishi, T., Yamauchi, A., Miyai, A., Yokoyama, K., Kamada, T., Ueda, N., and Fujikawa, Y. (1994) J. Clin. Invest. 93, 2387–2392
27. Terada, Y., Tomita, K., Hotta, M., Noguchi, H., Yang, T., Yamada, T., Yuasa, Y., Krebs, E. G., Sasaki, S., and Marumo, F. (1994) J. Biol. Chem. 270, 31296–31301
28. Zhang, Z., and Cohen, D. M. (1996) Am. J. Physiol. 271, F1234–F1238
29. Berli, T., Sirwardana, G., Ao, L., Butterfield, L. M., and Heasly, L. E. (1997) Am. J. Physiol. 272, F305–F313
30. Watts, B. A. I., Di Mari, J. F., Davis, R. I., and Good, D. W. (1998) Am. J. Physiol. 275, F478–F486
31. Sheikh-Hamad, D., Di Mari, J., Suki, W. N., Safirstein, R., Watts, B. A. I., and Rouse, D. (1998) J. Biol. Chem. 273, 1832–1837
32. Kult, D., Madhany, S., and Burg, M. B. (1998) J. Biol. Chem. 273, 13645–13651
33. Wojtaszek, P. A., Heasley, L. E., and Berli, T. (1998) J. Clin. Invest. 102, 1874–1881
34. Krump, E., Nikitas, K., and Grinstein, S. (1997) J. Biol. Chem. 272, 10230–10211
35. Kapus, A., Sza´szi, K., Sun, J., Rizoli, S., and Rotstein, O. D. (1999) J. Biol. Chem. 274, 8093–8102