Differential Regulation of Sphingomyelinase and Ceramidase Activities by Growth Factors and Cytokines

IMPLICATIONS FOR CELLULAR PROLIFERATION AND DIFFERENTIATION*

(Received for publication, May 26, 1995, and in revised form, July 21, 1995)

Emmanuel Coroneos, Michael Martinez, Siobhan McKenna, and Mark Kester‡

From the Departments of Medicine and Physiology/Biophysics, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106

Sphingosine is a product of sphingolipid metabolism that has been linked to a protein kinase C-independent mitogenic response. In previously published data, utilizing an in vitro model system for platelet-derived growth factor (PDGF)-induced vascular smooth muscle proliferation, we have demonstrated that sphingosine is increased at the expense of a concomitant decrease in ceramide formation, implicating an altered ceramidase activity. To explore mechanisms of growth factor-stimulated sphingosine formation, we have developed and investigated a cell-free model system assessing ceramidase activity. We now report that an alkaline, membrane-associated, ceramidase activity in the rat glomerular mesangial cell, a smooth muscle-like pericyte, is up-regulated by growth factors, apparently via a tyrosine kinase phosphorylation mechanism. PDGF also stimulated sphingomyelinase activity which generates sufficient substrate to drive the subsequent ceramide reaction. Inflammatory cytokines, including interleukin-1, and tumor necrosis factor-α, stimulated sphingomyelinase but not ceramidase activity, a result consistent with the cellular accumulation of the ceramide, apoptotic, differentiating second messenger. Mitogenic vasoconstrictor peptides such as endothelin-1 stimulated neither sphingomyelinase nor ceramidase activities. An inhibitor of ceramidase activity, N-oleylethanolamine, reduced PDGF- but not endothelin-1-stimulated proliferation. Thus, we conclude that, in mesangial cells, growth factors but not vasoconstrictor peptides or cytokines induce mitogenesis, in part, through ceramidase-mediated sphingosine formation.

Sphingolipids are complex ubiquitous lipids that have been relegated to serving a structural role in membranes. Sphingolipids are characterized as consisting of a long chain amino dialcohol base (sphingoid), an amide-linked fatty acyl group, and a polar or glycosidic head group. Over the last few years, sphingolipid derivatives have been identified as endogenous membrane signal-transducing molecules. Sphingomyelin, the major membrane sphingolipid, can be hydrolyzed by sphingomyelinase to form ceramide, a second messenger which stimulates differentiation, inhibits proliferation, and has been associated with apoptosis (1, 2). Several cytokines and steroids have been shown to stimulate sphingomyelinase and form ceramide.

Tumor necrosis factor-α (TNF-α), interleukin-1β, γ-interferon, and 1α,25-dihydroxy vitamin D3 activate sphingomyelinase in hematopoietic cell lines (3–5). The sphingomyelin signaling pathway stimulated by TNF-α and interleukin-1β can be reconstituted in cell-free extracts (4, 6) and may be mediated by arachidonic acid (7). Ceramides are themselves substrates for ceramidases that form the promitogenic lipid, sphingosine (8). Our laboratory and the laboratory of Spiegel have recently reported that platelet-derived growth factor (PDGF) stimulates mitogenic, sphingolipid-derived, second messengers including sphingosine and sphingosine-1-phosphate by degrading ceramide (9, 10). These data are consistent with the hypothesis that growth factors, but not inflammatory cytokines, activate alkaline ceramidase, a membrane-associated enzyme that has only been partially characterized (11). A synthetic analog of sphingosine, N-oleylethanolamine, has been shown to be a potent inhibitor of ceramidase in vitro (11). Utilizing this inhibitor, we investigate the contribution of ceramidase activation to growth factor-induced proliferation in MC. MC are a useful model to explore proliferative responses and phenotypic changes in a contractile, smooth muscle-like cell. Ligands for both tyrosine kinase-linked receptors (PDGF) and G protein-linked receptors (ET-1) are potent mitogens for MC (12). However, it is not completely understood how the biochemical events initiated by either PDGF- or ET-1-binding results, within hours, in DNA replication and cell division. Cytokines, including IL-1α, are, at best, poor co-mitogens for smooth muscle cell types (13). In fact, IL-1α induces a phenotypic change in MC, switching these cells from a contractile, promitogenic state to a secretory, nonproliferating phenotype (13). Thus, PDGF- or ET-1-treated MC can be used as in vitro models for a myogenic, mitogenic, phenotypic state while IL-1α-treated MC can serve as an in vitro model for an inflammatory, secretory phenotypic state. In the present study, we correlate sphingomyelinase-activated ceramide formation and ceramidase-stimulated sphingosine generation with growth factor-, vasoconstrictor peptide-, and cytokine-induced cellular proliferation and differentiation.

MATERIALS AND METHODS

Growth factors and cytokines were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY) and ET-1 was purchased from Peptide Institute (Tokyo, Japan). Genistein and all required culture media were obtained from Life Technologies, Inc. [3H]Thymidine was obtained from DuPont NEN. All other reagents were obtained from Sigma.

* This work was supported by American Heart Association, Northeast Ohio Affiliate, Inc., Grant 4818. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed. Fax: 216-368-1249.

‡To whom correspondence should be addressed. Fax: 216-368-1249.

The abbreviations used are: TNF-α, tumor necrosis factor-α; IL-1α, interleukin-1α; PDGF, platelet-derived growth factor; ET-1, endothelin-1; CAPSO, 3-cyclohexylamino)-2-hydroxy-1-propanesulfonic acid; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate; MC, mesangial cell(s); Bicine, N,N-bis(2-hydroxyethyl)glycine; Me2SO, dimethyl sulfoxide.
Mesangial Cell Culture—As previously reported, primary rat MC strains were cultured from isolated glomerular explants partially digested with bacterial collagenase to form glomerular cores (14). Cell passages 3–10 were maintained in RPMI 1640 medium supplemented with 17% fetal bovine serum, 100 μg/ml penicillin, 100 μg/ml streptomycin, 5 μg/ml each of insulin and transferrin, and 5 ng/ml sodium selenite at 37°C in 5% CO2 and 95% air. For the present experiments, MC at 90% confluence were rendered quiescent (G0) by holding them for 2 days in supplemented serum- and insulin-free RPMI.

Cell-free Ceramidase Assay—The method of Sugita et al. (11), as modified by us, was used to assess membrane-associated ceramidase activity. MC, pretreated with 1 mM sodium vanadate for 1 h to inhibit phosphatase activity, were stimulated with selected agonists for 1 h (37°C, pH 7.4). The cells were then placed on ice, washed twice with ice-cold phosphate-buffered saline, and scraped into a buffer containing either 0.1 mM CAPSO (pH 9.0), 0.1 mM citric acid/phosphate buffer (pH 4.5), or 0.1 mM Bis (pH 7.4). These buffers were supplemented with 1 mM CaCl2, 1 mM phenylmethylsulfonylfluoride, 1 mM benzamidine, and 20 μg/ml of protease inhibitors leupeptin, pepstatin, and aproatinin, as well as 1 mM sodium vanadate. The cells were sonicated three times for 15-s bursts. The lysates were then centrifuged at 480 g for 5 min to remove whole cells and nuclei and then ultracentrifuged at 70,000 g at 4°C to pellet the membrane fraction. The membrane pellet was resuspended in the appropriate ceramidase buffer supplemented with 1% Triton X-100 and 1% CHAPS. After resuspension in the above buffer, the membrane fraction was continuously shaken at 37°C for 1 h with 0.2 μCi of [3H]oleoyl-di-O-sphingosine (2.0 × 106 cpm/nmol). Ten μl of this ceramidase substrate were prepared for solubilization in an additional 1 μl of Tween 20, 2.5 μl of Triton X-100, 170 μl of chloroform, and 85 μl of methanol. The ceramide mixture was vortexed, dried under nitrogen, and then reconstituted in 275 μl of double-distilled water containing 4 mg of sodium cholate. After sonication, 25-μl aliquots of the ceramide substrate were added to 200 μl of MC membrane preparation, and the reaction was initiated. To terminate the reaction, 10 μl of 5 mg/ml oleic acid were added, and the reaction vials were placed on ice. One ml of chloroform/methanol (2:1, v/v) was added, and then 1 ml of Dole’s solution (78% isopropyl alcohol, 20% heptane, 2% H2SO4) was added to the reaction mixture. After 10 min, 400 μl of heptane and 600 μl of water were added. The top layer, containing the hydrolyzed [3H]oleic acid, was extracted and evaporated under N2. The labeled oleic acid, resuspended in 100 μl of heptane, was applied to Silica Gel 60 TLC plates and developed in a mobile phase consisting of 94% chloroform, 5% acetic acid, and 1% methanol. Unlabeled oleic acid was used as internal and external standards. The oleic acid spot, visualized with 2-(p-toluidino)naphthalene-6-sulfonic acid spray, was scraped into a scintillation vial for liquid scintillation analysis. The assay is linear with respect to time (0–2 h) and concentration of substrate (0.1–2 mg of protein). In addition, in previously reported data (11), the K_m for ceramide for the acidic ceramidase assay utilizing human kidney was 3.6 × 10^-4 M, and we used a substrate concentration of oleoylsphingosine consistent with this value. Our acidic ceramidase data for vehicle-treated MC correspond to previously reported values from these autopsied human kidneys (3.5 nmol/mg of protein/h (11)). Also, potential inhibitors of ceramidase were investigated and only N-oleylethanolamine (K_i, 7 × 10^-4 M) was found to be a potent inhibitor (11). In contrast, oleic acid, sphingosine, and ceramide glucoside all were far less effective inhibitors with K_i > 10^-3 M.

Cell-free Sphingomyelinase Assay—MC sphingomyelinase activity (pH 7.4) was assessed by the method of Rao and Spence (15), as modified by us, utilizing [14C]sphingomyelin labeled in the polar head group. In contrast to the ceramidase assay, these MC preparations were not pretreated with vanadate. After stimulation with agonists for 1 h at 37°C, MC were washed twice with phosphate-buffered saline and then scraped into a buffer consisting of 50 mM Tris (pH 7.4), 0.25% Triton X-100, 0.5 mM EDTA, 10 mM MgCl2, and freshly prepared protease inhibitors leupeptin, pepstatin, and aproatinin (20 μg/ml). After 1 h on ice, the suspension was centrifuged at 480 g for 5 min and the supernatant lysate was removed and continuously shaken with 0.1 μCi of [14C]sphingomyelin (1.7 × 10^6 cpm/nmol) at 37°C for 1 h. The reaction was stopped by the addition of heptane/isopropyl alcohol/H2O (2.5:2:5.2, v/v/v). The top phase was aspirated, and the bottom aqueous phase containing the hydrolyzed [14C]phosphorylcholine was concentrated utilizing a Speed-Vac concentrator. The samples were applied to Silica Gel 60W TLC plates (0.25 mm). External and internal standards included phosphorylcholine, C18:1 ceramide, and sphingosine. The elution system consisted of methanol, 0.5% NaCl, ammonia (100:100:2, v/v/v), and the phosphorylcholine spots were visualized with acid molybdate spray. A 0.01 dilution of 30% H2O2 solution was used to bleach the silica gel before liquid scintillation analysis.

[3H]Thymidine Incorporation into DNA—As described previously in MC (16) and in A1f5 VSM (9), DNA synthesis was assessed in PDGF-BB-, ET-3-, or sphingosine-treated cultures containing RPMI 1640 supplemented with 0.5% bovine serum albumin for 24 h. Sphingoines was added in RPMI 1640 media containing 5% MeSO and 0.5% bovine serum albumin for the full 24 h. During the last 6 h, [3H]thymidine (0.5 μCi/ml) was added for pulse-labeling newly synthesized DNA. Cells were placed on ice and washed four times with ice-cold 5% trichloroacetic acid, which fixed the cells to the culture plate. Cells were then solubilized in 1 ml of 0.1 N NaOH from which acid-insoluble counts were quantitated by liquid scintillation counting. In contrast to our previous studies (9, 16), mitogenesis was now assessed in the absence of any added fetal bovine serum or insulin.

Statistical Analysis—Levels of significance were determined by independent t tests (parametric) or by the Mann-Whitney U test (nonparametric). When applicable, the p values of the individual comparisons were adjusted for multiple comparisons by the Bonferroni method.

2 E. Coroneos, M. Martinez, S. McDenna, and M. Kester, unpublished data.
RESULTS AND DISCUSSION

We and others have previously demonstrated that PDGF stimulates [3H]sphingosine at the expense of [3H]ceramide and that inhibition of sphingolipid metabolism correlates with a reduction in PDGF-induced mitogenesis (9, 10). We have previously verified in MC and in A7r5 smooth muscle cells that treatment with either 10 ng/ml PDGF-AB or PDGF-BB for 1 h induces maximal elevations in [3H]sphingosine formation (9). To better understand the mechanism of growth factor-stimulated sphingosine formation, we have developed a cell-free ceramidase assay for MC. Ceramidase activity was assessed as TLC-separated [14C]oleate released from PDGF-treated or untreated membrane preparations and exogenous [14C]oleoylceramide substrate. In control experiments (data not shown), PDGF did not significantly stimulate ceramidase activity in heat (100 °C)-activated or calcium-free membrane preparations. Also, stimulated ceramidase activity was observed only when PDGF was added to intact cells and not when PDGF was incubated with the membrane preparation, suggesting that the PDGF responses require an intact receptor-linked signaling cascade. MC were treated with PDGF-BB (10 ng/ml, 1 h) or vehicle in the presence or absence of the tyrosine phosphatase inhibitor, vanadate (250 μM, 15 min preincubation) at pH 7.4, and then cell-free ceramidase activity was assessed at three distinct pH values (Fig. 1). PDGF-BB (and in data not shown, PDGF-AB) stimulates ceramidase activity at alkaline but not at acidic or neutral pH values, a finding consistent with activation of a plasma membrane-associated and not an endosomal/lysosomal ceramidase. PDGF-BB activation of alkaline ceramidase was maximal at 1 h and persisted for up to 6 h (data not shown). The mitogenic vasoconstrictor peptide, ET-1 (10^{-7} M, 1 h), did not induce an elevation in membrane-associated ceramidase activity in the absence or presence of vanadate. PDGF-stimulated membrane ceramidase activity was, however, further augmented in the presence of vanadate, suggesting that maximal activity is associated with a tyrosine phosphorylation mechanism. PDGF-stimulated ceramidase activity was observed at a neutral pH value in the presence but not the absence of vanadate, suggesting that, under optimal assay conditions, growth factors can stimulate ceramidase activity at physiological pH values.

A definitive role for tyrosine phosphorylation regulation of sphingosine formation was further suggested as PDGF-stimulated ceramidase activity was inhibited in the presence of the tyrosine kinase inhibitor, genistein (25 μM, 1-h preincubation). Genistein significantly inhibited PDGF-BB- but not vehicle-treated MC ceramidase activity (Fig. 2). These experiments were run in the presence of vanadate to ensure maximal phosphorylation and/or activity of ceramidase. Also, these MC data were confirmed in A7r5 vascular smooth muscle cells (data not shown) as to demonstrate that PDGF-stimulated ceramidase activity is not unique to a smooth muscle-like pericyte. We next assessed the relative specificity for ceramidase activation by growth factors (Fig. 3). Even though PDGF-BB was the most potent growth factor, basic fibroblast growth factor, insulin-like growth factor, and epidermal growth factor (all 10 ng/ml, 1 h) still significantly stimulated ceramidase activity. In contrast, the inflammatory cytokines, IL-1α and TNF-α, did not stimulate ceramidase activity even in the presence of vanadate. Thus, growth factors, but not cytokines or mitogenic vasoconstrictor proteins, stimulate ceramidase activity in vitro.

To assess if sufficient substrate could be generated in vivo for optimal ceramidase activity, we measured MC sphingomyelinase activity, in a cell-free system, as an indicator of ceramide formation (Fig. 4). PDGF-BB (10 ng/ml, 1 h) stimulates sphingomyelinase activity which generates sufficient substrate for subsequent ceramidase activity. Confirming reports by other investigators (5, 7), IL-1α and TNF-α (both at 10 ng/ml) were potent activators of sphingomyelinase activity in MC. However, these cytokine-receptor-induced signals were incapable of stimulating ceramidase activity. Thus, these data are consistent with the hypothesis that PDGF-stimulated sphingomyelinase activity correlates with ceramidase activation and mitogenesis.

![Graph showing ceramidase activity](image)

**Fig. 2.** PDGF stimulates MC ceramidase through a tyrosine kinase phosphorylation mechanism. PDGF-stimulated membrane-associated ceramidase activity was inhibited in the presence of the tyrosine kinase inhibitor, genistein. n = 3 separate experiments, each n replicated in duplicate or triplicate, mean ± S.E., p < 0.05.

**Fig. 3.** Growth factors but not cytokines stimulate MC ceramidase activity. MC were treated with all agonists (10 ng/ml) for 1 h and membrane ceramidase activity was assessed. n = 4 separate experiments, each n replicated in triplicate, mean ± S.E., p < 0.05.
with cytokines as inducers of ceramides and a resulting differentiated, nonproliferative, cellular phenotype. ET-1 (10^{-7} \text{ M}) neither stimulated sphingomyelinase nor ceramidase activities, suggesting that the mitogenic actions of this vasoconstrictor are not mediated by sphingolipid metabolites.

To further ascertain the role of sphingolipid metabolites, especially sphingosine, in mediating growth factor-induced proliferation, we have utilized an inhibitor of ceramidase, N-oleoylethanolamine (0.5 mM, 16 h dissolved in 5% Me2SO, 95% buffer containing 0.5% bovine serum albumin), in [3H]thymidine incorporation studies. N-Oleoylethanolamine significantly inhibited PDGF-BB (10 ng/ml)- but not ET-1 (10^{-7} \text{ M})-stimulated mitogenesis in MC (Fig. 5). N-Oleoylethanolamine had no effect by itself on proliferation. Exogenous sphingosine (10 \mu M) partially restored the mitogenic response to PDGF but not ET-1. These data imply that the mitogenic actions of PDGF, but not ET-1, were mediated through sphingosine or a sphingosine derivative. Moreover, in data not shown, IL-1\alpha or TNF-\alpha are not mitogens for MC under these assay conditions, supporting our observations that cytokines do not stimulate ceramidase activity. We conclude that, in mesenchymal cells, growth factors stimulate ceramidase activity resulting in the formation of the mitogenic lipid, sphingosine.

We have extended our earlier studies that implicate sphingosine as a component of PDGF-induced mitogenesis in mesenchymal cells (9). We now report that growth factors, but not mitogenic vasoconstrictor peptides or inflammatory cytokines, stimulate ceramidase activity in vitro. Both an increase in sphingosine and a decrease in ceramide content is consistent with a mitogenic phenotype. This is the first report to dissociate a role for distinct sphingolipid metabolites in a proliferative versus a differentiated phenotype. By making parallel evaluations of ceramidase and sphingomyelinase activities in vitro, we have demonstrated that even though both PDGF and cytokines induce sphingomyelinase activation, only growth factor receptors have the capacity to also augment ceramidase activity. These studies offer a novel explanation for the differentiating, antiproliferative, actions of cytokines and the promitogenic actions of growth factors. Consistent with our observations are studies that implicate ceramide in cell cycle arrest and apoptosis (2) and sphingosine metabolites in cellular proliferation (9, 10).

The role of sphingosine to mediate, in part, the mitogenic actions of growth factors but not of a mitogenic vasoconstrictor peptide is a particularly exciting finding. At the minimum, these observations suggest that the actions of N-oleoylethanolamine are specific to a sphingolipid-mediated signaling pathway. At the maximum, these studies suggest that mitogenic actions of sphingosine are distinct from proliferative signals induced by G protein-linked receptors. Yet, surprisingly, to date, all of the putative mechanisms postulated for the mitogenic actions of sphingosine can be induced by G protein-linked receptors. For example, sphingosine as well as ET-1 have been shown to induce elevations in intracellular free calcium concentrations (17, 18), activation of phospholipase D resulting in the formation of the mitogens, phosphatidic and lysophosphatidic acids (19, 20), and DNA binding activity of AP-1 transactivating elements (21, 22). Thus, sphingosine may regulate cellular proliferation through, as yet, unknown signaling mech-
In this regard, phospholipid-derived signaling pathways including phospholipase C, protein kinase C, or c-fos are not required for PDGF-induced proliferation (23, 24).

As PDGF-induced mitogenesis is, in part, sphingosine-dependent, it is surprising that sphingosine formation mediated by growth factor-regulated sphingolipid metabolism has not been investigated in pathophysologies that manifest a proliferative smooth muscle phenotype. To date, sphingolipid metabolism and resulting proliferation and/or hypertrophy has only been investigated in the streptozotocin-treated rat model of diabetic nephropathy (25). In this model, a glucosyl ceramide synthetase inhibitor has been used to demonstrate that an elevation in glucosyl ceramides with a concomitant reduction in ceramides is associated with renal growth (25). Thus, a decrease in ceramide content, either by an increase in ceramidase, sphingomyelin synthase, or glucosyl ceramide synthetase activities, correlates with a proliferative response. Even though alkaline ceramidase has not been isolated or cloned, a genetic disorder of acidic ceramidase (Farber’s lipogranulomatosis) has been identified and linked to accumulation of ceramide and resulting lethal neuronal abnormalities (26).

In conclusion, we suggest that growth factors, but not inflammatory cytokines or mitogenic vasoconstrictor peptides, mediate proliferation, in part, through ceramidase-regulated sphingosine formation. The dissociation of growth factor-induced mitogenesis from cytokine-mediated differentiation and apoptosis as a consequence of distinct sphingolipid-derived second messengers implicates the sphingomyelin cycle as an important homeostatic regulator of cellular division and differentiation.

Acknowledgment—We thank Norma Minear for preparing the manuscript.

REFERENCES

1. Jarvis, W. O., Kolesnick, R. N., Fornari, I. A., Taylor, R. S., Gewirtz, D. A., and Grant, S. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 73–77
2. Jayadev, S., Liu, B., Bielawska, A. E., Lee, J. Y., Nazaire, F., Pushkareva, M. Y., Obied, L. M., and Hannun, Y. A. (1995) J. Biol. Chem. 270, 2047–2052
3. Kim, M. Y., Linardic, C., Obied, L., and Hannun, Y. (1993) J. Biol. Chem. 268, 484–489
4. Mathias, S., Younes, A., Kan, C. C., Orlow, I., J. oseph, C., and Kolesnick, R. N. (1993) Science 259, 519–522
5. Okazaki, T., Bell, R. M., and Hannun, Y. A. (1989) J. Biol. Chem. 264, 19076–19080
6. Dressler, K. A., Mathias, S., and Kolesnick, R. N. (1992) Science 255, 1715–1717
7. Jayadev, S., Linardic, C. M., and Hannun, Y. A. (1994) J. Biol. Chem. 269, 5757–5763
8. Zhang, H., Buckley, N. E., Gibson, K., and Spiegel, S. (1990) J. Biol. Chem. 265, 76–81
9. Jacobs, L. S., and Kester, M. (1993) Am. J. Physiol. 265, C740–C747
10. Olivera, A., and Spiegel, S. (1993) Nature 365, 557–560
11. Sugita, M., Williams, M., Dulaney, J. T., and Moser, H. W. (1975) Biochim. Biophys. Acta 396, 125–131
12. Mene, P., Simonson, M. S., and Dunn, M. J. (1989) Physiol. Rev 69, 1347–1424
13. Sedor, J. R., Nakazato, Y., and Konieczkowski, M. (1992) Kidney Int. 41, 595–599
14. Kester, M. (1993) J. Cell. Physiol. 153, 244–255
15. Rao, B. G., and Spence, M. W. (1976) J. Lipid Res. 17, 506–515
16. Kester, M., Simonson, M. S., Mene, P., and Sedor, J. R. (1989) J. Clin. Invest. 83, 718–723
17. Ghosh, T. K., Bian, J., and Gill, D. L. (1990) Science 248, 1653–1656
18. Simonson, M. S., Wann, S., Mene, P., Dubyak, G. R., Kester, M., and Dunn, M. J. (1989) J. Clin. Invest. 83, 708–712
19. Desai, N. N., Zhang, H., Olivera, A., Mattie, M. E., and Spiegel, S. (1992) J. Biol. Chem. 267, 23122–23128
20. Kester, M., Simonson, M. S., McDermott, R. G., Baldi, E. D., and Dunn, M. J. (1992) J. Cell Physiol. 150, 578–585
21. Su, Y., Rosenthal, D., Smulson, M., and Spiegel, S. (1994) J. Biol. Chem. 269, 16512–16517
22. Simonson, M. S., Jones, J. M., and Dunn, M. J. (1992) J. Biol. Chem. 267, 8643–8649
23. Fukami, K., and Takenawa, T. (1992) J. Biol. Chem. 267, 10988–10993
24. Sharma, R. V., and Bhalla, R. C. (1993) Am. J. Physiol. 264, C71–C79
25. Shayman, J. A., Deshmukh, G. D., Zador, J., Kunkel, R., Johnson, K., and Radin, N. S. (1992) J. Am. Soc. Nephrol. 3, 766A
26. Prensky, A. L., Ferreira, G., Carr, S., and Moser, H. W. (1967) Proc. Soc Exp. Biol. Med. 126, 725–728