Tumor Necrosis Factor-α Autoregulates Interleukin-6 Synthesis via Activation of Protein Kinase C

FUNCTION OF SPHINGOSINE 1-PHOSPHATE AND PHOSPHATIDYLCHOLINE-SPECIFIC PHOSPHOLIPASE C*

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We investigated the mechanism of interleukin-6 (IL-6) synthesis induced by tumor necrosis factor-α (TNF) in osteoblast-like MC3T3-E1 cells. TNF stimulated the synthesis of IL-6 dose dependently in the range between 1 and 30 ng/ml. Stauroporin and calphostin C, inhibitors of protein kinase C (PKC), significantly enhanced the TNF-induced synthesis of IL-6. 1-Oleoyl-2-acetylglycerol, a specific activator of PKC, inhibited the TNF-induced IL-6 synthesis. The stimulatory effect of TNF was markedly increased in the PKC down-regulated cells. TNF produced diacylglycerol. TNF had little effect on the formation of inositol phosphates and choline. On the contrary, TNF significantly stimulated the formation of phosphocholine dose dependently. D-609, an inhibitor of phosphatidylcholine-specific phospholipase C, suppressed the TNF-induced diacylglycerol production. The TNF-induced IL-6 synthesis was significantly enhanced by D-609. TNF induced sphingomyelin hydrolysis. Neither C2-ceramide nor sphingosine but sphingosine 1-phosphate significantly stimulated the synthesis of IL-6. PKC down-regulation amplified the IL-6 synthesis by sphingosine 1-phosphate. These results strongly suggest that sphingosine 1-phosphate may act as a second messenger for TNF-induced IL-6 synthesis and that TNF autoregulates IL-6 synthesis due to PKC activation via phosphatidylcholine-specific phospholipase C in osteoblast-like cells.

Tumor necrosis factor-α (TNF)† is a multifunctional cytokine responsible for inflammation, infection, and cancer, and TNF induces numerous physiological effects on a wide variety of cells (1, 2). As for intracellular signaling of TNF, it has been reported that TNF stimulates breakdown of sphingomyelin through sphingomyelinase activation, which results in the formation of ceramide (2). It is subsequently metabolized to sphingosine and sphingosine 1-phosphate. Ceramide has been reported to induce apoptosis in several cells, whereas sphingosine and sphingosine 1-phosphate are mitogenic. Accumulating evidence suggests that these sphingomyelin metabolites mediate TNF-induced biological effects (3–5). In addition, TNF has been shown to catalyze phosphatidylcholine (PC) hydrolysis via activation of PC-specific phospholipase C, resulting in the production of phosphocholine and diacylglycerol (DAG), which is generally recognized to be a physiological activator of protein kinase C (PKC) (6, 7). However, the precise intracellular signaling system of TNF has not yet been fully clarified.

Bone metabolism is maintained by two types of functional bone cells, osteoblasts and osteoclasts, which are responsible for bone formation and bone resorption, respectively (8). Osteoclasts activity has been reported to be coupled through cytokines (such as TNF and interleukin-1), stimulation of osteoblasts, and the subsequent production of secondary peptide which activates osteoclasts (9, 10). It is well known that TNF is a potent bone resorptive agent (11). Bone resorptive agents such as TNF, parathyroid hormone, interleukin-1, and platelet-derived growth factor have been reported to stimulate interleukin-6 (IL-6) production and its secretion in cultured osteoclasts (11–14). IL-6 is a pleiotropic multifunctional cytokine which regulates diverse cell functions (15, 16), and it has been reported that IL-6 stimulates bone resorption and induces osteoclast formation (11, 17). Thus, accumulating evidence suggests that IL-6 secreted from osteoblasts plays an important role in bone resorption as a downstream effector of a variety of bone resorptive agents. However, the exact mechanism of IL-6 synthesis in osteoblasts has not yet been clarified.

In the present study, we investigated the mechanism of IL-6 synthesis induced by TNF in osteoblast-like MC3T3-E1 cells. We show here that sphingosine 1-phosphate may act as a second messenger for TNF-induced IL-6 synthesis and that TNF autoregulates IL-6 synthesis due to PKC activation via PC-hydrolyzing phospholipase C in osteoblast-like cells.

EXPERIMENTAL PROCEDURES

Materials—myo-[3H]Inositol (90 Ci/mmol), N-methyl-[3H]choline chloride (85 Ci/mmol), and mouse IL-6 enzyme immunoassay kit were purchased from Amersham Japan (Tokyo, Japan). TNF, potassium tricyclo-(5,2,1,0)-decayl-[9(8)-xanthogenate] (D-609), C2-ceramide, sphingosine, and sphingosine 1-phosphate were obtained from Funakoshi Pharmaceutical Co. (Tokyo, Japan). Calphostin C, stauroporin, and 12-O-tetradecanoylphorbol-13-acetate (TPA) were purchased from Sigma. 1-Oleoyl-2-acetylglycerol (OAG) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). rat-Propanolol hydrochloride (propranolol) was purchased from Wako Pure Chemical Co. (Osaka, Japan). Other materials and chemicals were obtained from commercial sources. Calphostin C, TPA, and propranolol were dissolved in dimethyl sulfoxide. The maximum concentration of dimethyl sulfoxide was 0.1%, which did not affect the assay for IL-6 nor the measurement of DAG production.

Cell Culture—Cloned osteoblast-like MC3T3-E1 cells derived from newborn mouse calvaria (18, 19) were generously provided by Dr. M. Kumezawa (Meikai University, Sakado, Japan) and maintained in α-minimum essential medium (α-MEM) containing 10% fetal calf serum (FCS) at 37 °C in a humidified atmosphere of 5% CO2, 95% air. The cells were cultured in minimum essential medium (MEM) containing 0.1% bovine serum albumin, 1 mM pyruvate, and 10 mM HEPES in a humidified atmosphere of 5% CO2, 95% air.
Fig. 1. Effect of TNF on IL-6 synthesis in MC3T3-E1 cells. A, the cultured cells were stimulated by 30 ng/ml TNF (●) or vehicle (○) for the indicated periods. B, the cultured cells were stimulated by various doses of TNF for 48 h. Each value represents the mean ± S.D. of triplicate determinations. Similar results were obtained with two additional and different cell preparations.

Fig. 2. Effect of calphostin C on the TNF-induced synthesis of IL-6 in MC3T3-E1 cells. The cultured cells were pretreated with various doses of calphostin C for 20 min, and then stimulated by 30 ng/ml TNF (●) or vehicle (○) for 48 h. Each value represents the mean ± S.D. of triplicate determinations. Similar results were obtained with two additional and different cell preparations.

Effect of calphostin C on the TNF-induced synthesis of IL-6 in MC3T3-E1 cells.

The cultured cells were pretreated with 0.1 mM OAG or vehicle for 20 min, and then stimulated by 30 ng/ml TNF or vehicle for 48 h. Each value represents the mean ± S.D. of triplicate determinations. Similar results were obtained with two additional and different cell preparations.

Table I

Effect of OAG on the TNF-induced synthesis of IL-6 in MC3T3-E1 cells

The cultured cells were pretreated with 0.1 mM OAG or vehicle for 20 min, and then stimulated by 30 ng/ml TNF or vehicle for 48 h. Each value represents the mean ± S.D. of triplicate determinations. Similar results were obtained with two additional and different cell preparations.

Measurement of the Formation of Water-soluble Choline Metabolites—To determine PC-hydrolyzing phospholipase C and phospholipase D activities, the cultured cells were labeled with [methyl-3H]choline chloride (2 μCi/dish) for 24 h. The labeled cells were stimulated by TNF in the assay buffer containing 0.01% bovine serum albumin for the indicated periods. The reaction was terminated by adding 0.75 ml of ice-cold methanol. The dishes were placed on ice for 10 min. The contents were transferred to tubes to which chloroform was added and placed on ice for a further 60 min. Chloroform and water were then added for a final chloroform:methanol:water ratio of 1:1:0.9. The tubes were centrifuged at 14,000 × g for 5 min and the upper aqueous methanolic phase was taken for analysis of the water-soluble choline-containing metabolites. The methanolic phase was separated on a 1-ml Dowex 50-WH column (200–400 mesh) as described by Cook and Wakeland (23) with a minor modification (24). In brief, the phase was diluted to 5 ml with water and applied to the column. Glycerocholines were removed by 4 ml of water. Phosphocholine was then eluted by 10 ml of water, and choline was eluted with 10 ml of 1 M HCl.

Measurement of DAG Production—The cultured cells were incubated in the assay buffer containing 0.01% bovine serum albumin at 37 °C for 20 min, and then stimulated by TNF for the indicated periods. The reaction was terminated by adding 0.75 ml of ice-cold methanol, and the lipids were extracted by the previously described method (25, 26). DAG was quantitated using sn-1,2-DAG assay reagents system (Amersham, Japan) essentially according to the procedure of Preiss et al. (27). The radioactive spot corresponding to phosphatidic acid was analyzed by BAS2000 (Fuji, Japan) equipped with imaging plates (28). When indicated, the cells were pretreated with D-609 or propranolol for 20 min.

Assay for sphingomyelin turnover—Sphingomyelin levels were measured as described by Okazaki et al. (29). In brief, the cultured cells were labeled with [methyl-3H]choline chloride (2 μCi/dish) for 24 h. The labeled cells were stimulated by TNF in the assay buffer containing 0.01% bovine serum albumin for the indicated periods. The lipids were extracted by the method of Bligh and Dyer (25). The samples were dried
down, dissolved in chloroform, and then applied on Gel 60A TLC plate. To identify sphingomyelin, TLC plate was developed in chloroform: methanol:acetic acid:water (50:30:8:5). The sphingomyelin spot was scraped.

**Determination**—The radioactivity of $^3$H-labeled samples was determined with a Beckman LS-6500IC liquid scintillation spectrometer.

**Statistical Analysis**—The data were analyzed by Student's $t$ test and a $p < 0.05$ was considered significant. All data are presented as the mean ± S.D. of triplicate determinations.

### RESULTS

**Effect of TNF on IL-6 Synthesis in MC3T3-E1 Cells**—TNF (30 ng/ml) significantly induced the synthesis of IL-6 in a time-dependent manner up to 48 h (Fig. 1A). The stimulative effect of TNF was dose-dependent in the range between 1 and 30 ng/ml (Fig. 1B). The maximum effect of TNF was observed at 30 ng/ml. Our findings are consistent with a previous report in osteoblasts (11).

**Effect of Calphostin C or Staurosporine on Synthesis of IL-6 Induced by TNF in MC3T3-E1 Cells**—PKC is well known to play a pivotal role in the regulation of various cellular functions (7). To investigate the role of PKC in the mechanism of TNF-induced IL-6 synthesis in MC3T3-E1 cells, we first examined the effect of calphostin C, a highly potent and specific inhibitor of PKC (30), on the TNF-induced IL-6 synthesis. Calphostin C, which alone had little effect on IL-6 synthesis, significantly enhanced the TNF-induced IL-6 synthesis (Fig. 2). The effect of calphostin C was dose-dependent in the range between 30 nM and 0.3 μM. Staurosporine (10 nM), an inhibitor of protein kinases (31), also enhanced the TNF-induced IL-6 synthesis (data not shown). It seems that PKC has an inhibitory effect on TNF-induced IL-6 synthesis.

**Effect of OAG on Synthesis of IL-6 Induced by TNF in MC3T3-E1 Cells**—We next examined whether the activation of PKC exogenously by addition of cell-permeant DAG would further inhibit the IL-6 synthesis in response to TNF. OAG (0.1 mM), a synthetic DAG known to be a specific activator of PKC (7), which alone had no effect on IL-6 synthesis, inhibited the TNF-induced IL-6 synthesis (Table I). Thus, it is probable that PKC activation suppresses the IL-6-synthesis by TNF.

**Effect of Down-regulation of PKC on Synthesis of IL-6 Induced by TNF in MC3T3-E1 Cells**—It has been shown that 24 h pretreatment of TPA (0.1 μM) down-regulates PKC in osteoblast-like MC3T3-E1 cells (32). We also found that the binding capacity of phorbol-12,13-dibutyrate, a PKC-activating phorbol ester (7), in PKC down-regulated MC3T3-E1 cells is reduced to approximately 30% of the capacity in intact cells (33). To further clarify the role of PKC in the TNF-induced IL-6 synthesis, we next examined the effect of TPA (0.1 μM) long-term pretreatment on the IL-6 synthesis stimulated by TNF. The effect of TNF on IL-6 synthesis was significantly enhanced in the PKC down-regulated cells compared with that in the cells without TPA pretreatment (Table II). These findings suggest that TNF activates PKC in MC3T3-E1 cells, and the PKC activation negatively regulates the TNF-induced IL-6 synthesis.

**Effect of TNF on Formation of Inositol Phosphates in MC3T3-E1 Cells**—We next investigated the intracellular signaling pathway responsible for TNF-activated PKC in MC3T3-E1 cells. It is well known that phosphatidylinositol hydrolysis by phospholipase C results in the formation of DAG. To test the effect of TNF on phosphatidylinositol-specific phospholipase C, we examined whether TNF affects the formation of inositol phosphates. TNF had no effect on the formation of inositol phosphates (control, 2,045 ± 99 cpm; 30 ng/ml TNF, 2,111 ± 106 cpm, as measured after 30 min stimulation). It is well recognized that heterotrimeric GTP-binding proteins are coupled to phosphatidylinositol-specific phospholipase C (34). So, we examined the effect of sodium fluoride, an activator of heterotrimeric GTP-binding proteins (34), on phosphatidylinositol hydrolysis, as a positive control. Sodium fluoride significantly stimulated the formation of inositol phosphates (control, 2,122 ± 101 cpm; 40 mM sodium fluoride, 30,300 ± 887 cpm, as measured after 30 min stimulation). It seems unlikely that TNF induces phosphatidylinositol hydrolysis by phospholipase C.

**Effect of TNF on Formation of Water-soluble Choline Metabolites in MC3T3-E1 Cells**—It is recognized that PC hydrolysis by PC-specific phospholipase C or D results in the formation of DAG and subsequent activation of PKC (35, 36). To clarify the effect of TNF on PC hydrolysis, we examined the effects of TNF on the formations of phosphocholine and choline, the products of PC hydrolysis by phospholipases C and D, respectively. TNF did not affect the formation of choline, but significantly stimulated the formation of phosphocholine (Fig. 3). The stimulative effect of TNF was dose-dependent in the range between 10 and 30 ng/ml, and the maximum effect of TNF was observed at 30 ng/ml (Fig. 3). Thus, these findings suggest that TNF activates not phospholipase D but PC-specific phospholipase C.

**Effect of TNF on Production of DAG in MC3T3-E1 Cells**—Based on our findings, it is probable that TNF activates PKC through PC hydrolysis by PC-specific phospholipase C in MC3T3-E1 cells. Thus, we examined the effect of TNF on DAG production. TNF (30 ng/ml) time dependently stimulated the production of DAG (Fig. 4A). The effect reached submaximum...
within 30 min, and decreased after 60 min. The stimulative effect of TNF was dose-dependent in the range between 1 and 30 ng/ml, and the maximum effect of TNF was observed at 30 ng/ml (Fig. 4B). The pattern of dose-response curve in TNF-induced DAG production was similar to that in TNF-induced IL-6 synthesis. D-609, a specific inhibitor of PC-specific phospholipase C (37), significantly inhibited the production of DAG induced by TNF (Table III). On the contrary, propranolol, an inhibitor of phosphatidic acid phosphohydrolase (38), had little effect on the TNF-induced DAG production (Table III). It is most likely that TNF induces DAG production through PC hydrolysis by phospholipase C, and then activates PKC.

**Effect of D-609 on TNF-induced Synthesis of IL-6 in MC3T3-E1 Cells**—To clarify the role of PC-specific phospholipase C in TNF-induced IL-6 synthesis, we examined the effect of D-609 on the TNF-induced IL-6 synthesis (Fig. 5). The stimulative effect of D-609 was dose-dependent in the range between 0.1 and 0.3 ng/ml. This finding suggests that TNF-induced IL-6 synthesis is negatively regulated by PC-specific phospholipase C activated by TNF itself.

**Effect of TNF on Sphingomyelin Turnover in MC3T3-E1 Cells**—It has been reported that sphingomyelin hydrolysis takes part in the signaling mechanism of TNF in several types of cells (2). Thus, we examined the effect of TNF on sphingomyelin levels in MC3T3-E1 cells. TNF (30 ng/ml) decreased sphingomyelin levels to 77% of control 30 min after the stim-ulation (Fig. 6). The levels then returned to control levels by 60 min. Our finding indicates that TNF truly induces sphingomyelin hydrolysis in MC3T3-E1 cells.

**Effects of C₂-Ceramide, Sphingosine, and Sphingosine 1-Phosphate on IL-6 Synthesis in MC3T3-E1 Cells**—To clarify whether sphingomyelin hydrolysis is involved in TNF-induced IL-6 synthesis, we next examined the effects of sphingomyelin metabolites on IL-6 synthesis. C₂-ceramide, a cell-permeable ceramide analogue, had no effect on the synthesis of IL-6 (Fig. 7). In addition, sphingosine did not stimulate IL-6 synthesis (Fig. 7). On the contrary, sphingosine 1-phosphate significantly induced the synthesis of IL-6 (Fig. 7). The stimulative effect was dose-dependent in the range between 0.1 and 30 μM. The long-term pretreatment with 0.1 μM TPA markedly enhanced the IL-6 synthesis by sphingosine 1-phosphate compared with that in intact cells (Table IV). Therefore, it is probable that sphingosine 1-phosphate mediates the stimulative effect of TNF on IL-6 synthesis.
We next investigated the exact mechanism behind the TNF-induced activation of PKC in osteoblast-like MC3T3-E1 cells. We first showed that TNF had no effect on the formation of inositol phosphates. It is well known that phosphatidylinositol is hydrolyzed by phospholipase C, resulting in the formation of inositol phosphates and DAG (39). Thus, it seems unlikely that TNF activates phosphatidylinositol-specific phospholipase C. Namely, our finding suggests that TNF does not activate PKC through phosphoinositide hydrolysis. In addition, we demonstrated that TNF did not affect the formation of choline, and that propranolol, did not affect the DAG production induced by TNF in MC3T3-E1 cells. It is recognized that phospholipase D hydrolyzes PC to yield phosphatidic acid and choline (35, 36). Phosphatidic acid is subsequently degraded into DAG by phosphatidic acid phosphohydrolase. Thus, it seems unlikely that TNF simulates PC-specific phospholipase D in MC3T3-E1 cells. On the contrary, TNF stimulated the formation of phosphocholine. PC is also hydrolyzed by phospholipase C, resulting in the formation of DAG and phosphocholine (35, 36). In addition, we found that D-609 reduced the DAG production by TNF. Therefore, these results suggest that TNF activates PKC via stimulating PC-specific phospholipase C in osteoblast-like MC3T3-E1 cells. Furthermore, we demonstrated here that D-609 enhanced the TNF-induced IL-6 synthesis due to PKC activation through PC-hydrolyzing phospholipase C in osteoblast-like MC3T3-E1 cells.

We showed here that TNF induced sphingomyelin turnover in osteoblast-like MC3T3-E1 cells and that neither C2-ceramide nor sphingosine but sphingosine 1-phosphate significantly stimulated the synthesis of IL-6. It is well known that sphingomyelin hydrolysis results in the production of ceramide. Ceramide can be subsequently metabolized to sphingosine and sphingosine 1-phosphate (1, 2). Recent evidence suggests that these sphingomyelin metabolites mediate TNF-induced biological effects (3–5). Thus, it seems that sphingosine 1-phosphate is involved in the TNF-induced IL-6 synthesis in MC3T3-E1 cells. In addition, we showed that PKC down-regulation significantly enhanced the sphingosine 1-phosphate-induced IL-6 synthesis as well as TNF-induced IL-6 synthesis. Therefore, our findings suggest that TNF exerts the stimulatory effect on IL-6 synthesis through sphingosine 1-phosphate in osteoblast-like MC3T3-E1 cells.

In conclusion, these results strongly suggest that TNF induces both PC hydrolysis by phospholipase C and sphingomyelin hydrolysis in osteoblast-like cells. Sphingosine 1-phosphate, a metabolite of the latter hydrolysis, may act as a second messenger for TNF-induced IL-6 synthesis, and that PKC ac-
activation due to the former hydrolysis autoregulates the TNF-induced IL-6 synthesis.

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REFERENCES
1. Hannun, Y. A. (1994) J. Biol. Chem. 269, 3125–3128
2. Divecha, N., and Irvine, R. F. (1995) Cell 80, 269–278
3. Schütze, S., Wiegmann, K., Machleidt, T., and Kronke, M. (1995) Immunobiol. 193, 193–203
4. Westwick, J. K., Biehlawska, A. E., Dhaibo, G., Hannun, Y. A., and Brenner, D. A. (1995) J. Biol. Chem. 270, 22689–22692
5. Kasnet, H., Hemi, R., Papa M. Z., and Karasik, A. (1996) J. Biol. Chem. 271, 9895–9897
6. Schütze, S., Berkovic, D., Tomsing, O., Unger, C., and Kronke, M. (1991) J. Exp. Med. 174, 975–988
7. Nishizuka, Y. (1986) Science 233, 305–312
8. Nijweide, P. J., Burger, E. H., and Feyen, J. H. M. (1986) Physiol. Rev. 66, 855–886
9. Thomson, B. M., Saklattuala, J., and Chambers, T. J. (1986) J. Exp. Med. 164, 104–112
10. Thomson, B. M., Mundy, G. R., and Chambers, T. J. (1987) J. Immunol. 138, 775–779
11. Ishimi, Y., Miyaura, C., Jin, C. H., Akatsu, T., Abe, E., Nakamura, Y., Yamaguchi, Y., Yoshi, S., Matsuda, T., Hirano, T., Kishimoto, T., and Suda, T. (1996) J. Immunol. 145, 3297–3303
12. Helle, M., Brakenhoff, J. P. J., DeGroot, E. R., and Aarden, L. A. (1988) Eur. J. Immunol. 18, 957–959
13. Feyen, J. H. M., Elford, P., Di Padovana, F. E., and Trechsel, U. (1989) J. Bone Miner. Res. 4, 633–638
14. Franchimont, N., and Canalis, E. (1995) Endocrinology 136, 5469–5475
15. Akira, S., Hirano, T., Taga, T., and Kishimoto, T. (1999) FASEB J. 4, 2860–2867
16. van Snick, J. V. (1990) Annu. Rev. Immunol. 8, 253–297
17. Goodman, G. D. (1992) J. Bone Miner. Res. 7, 475–478
18. Kodama, H., Amagai, Y., Sudo, H., Kasai, S., and Yamamoto, S. (1981) Jpn. J. Oral Biol. 23, 899–901
19. Sudo, H., Kodama, H., Amagai, Y., Yamamoto, S., and Kasai, S. (1983) J. Cell Biol. 96, 191–198
20. Berridge, M. J., Dawson, R. M. C., Downes, C., Heslop, J. P., and Irvine, R. F. (1985) Biochem. J. 212, 473–482
21. Berridge, M. J., Heslop, J. P., Irvine, R. F., and Brown, K. D. (1984) Biochem. J. 222, 195–201
22. Suzuki, A., Kozawa, O., Saito, H., and Oiso, Y. (1994) J. Cell. Biol. 126, 511–517
23. Cook, S. J., and Wakelam, M. J. O. (1989) Biochem. J. 263, 581–587
24. Shioda, J., Suzuki, A., Oiso, Y., and Kozawa, O. (1985) Am. J. Physiol. 269, E524–E529
25. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917
26. Suzuki, A., Kozawa, O., Shinoda, J., Watanabe, Y., Saito, H., and Oiso, Y. (1996) J. Exp. Med. 182, 209–216
27. Preiss, J., Loomis, C. R., Bishop, W. K., Niedel, J. E., and Bell, R. M. (1986) J. Biol. Chem. 261, 8597–8600
28. Amemiya, Y., and Miyahara, J. (1988) Nature 336, 89–90
29. Okazaki, T., Bell, R. M., and Hannun, Y. A. (1999) J. Biol. Chem. 264, 19076–19080
30. Kobayashi, E., Nakano, H., Morimoto, M., and Tamaoki, T. (1988) Biochem. Biophys. Res. Commun. 159, 548–553
31. Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M., and Tomita, F. (1986) Biochem. Biophys. Res. Commun. 135, 397–402
32. Sakai, T., Okano, Y., Nozawa, Y., and Oka, N. (1992) Cell Calcium 13, 329–340
33. Suzuki, A., Shinoda, J., Kanda, S., Oiso, Y., and Kozawa, O. (1996) J. Cell. Biol. 63, 491–499
34. Gilman, A. G. (1987) Annu. Rev. Biochem. 56, 615–649
35. Exton, J. H. (1990) J. Biol. Chem. 265, 1–4
36. Billah, M. M., and Anthes, J. C. (1990) Biochem. J. 269, 281–291
37. Schütze, S., Potthoff, K., Machleidt, T., Bergivic, D., Weigmann, K., and Kronke, M. (1992) Cell 71, 765–776
38. Pappu, A. S., and Hauser, G. (1983) Neurochem. Res. 8, 1565–1575
39. Berridge, M. J. (1993) Nature 361, 315–325

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