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ABSTRACT

Our previously result showed that PMA increased lipid droplet accumulation induced by oleic acid in RAW 264.7 macrophages. In this report, I further studied whether this effect is mediated via activation of mitogen-activated protein kinase (MAPK). Treatment of macrophages with 0.1 μM PMA induced rapid phosphorylation of ERKs (p44 and p42 MAPK) within 10 min, followed by returning to basal level at about 3 h. Results from immunoblot showed that PMA increased adipophilin expression at 4 h; however, cotreatment with 100 μM PD 98059, a MEK inhibitor, did not inhibit adipophilin protein level enhanced by PMA. In addition to have no effect on lipogenesis, PD 98059 did not stimulate lipolysis. Taken together, these results suggest that activity of ERK is not involved in lipid metabolism induced by oleic acid in RAW 264.7 macrophages

Keywords
macrophage; oleic acid; adipophilin; mitogen-activated protein kinase

INTRODUCTION

Adipophilin (also known as adipose differentiation-related protein, ADRP) is ubiquitously expressed in lipid droplet-containing cells in addition to adipocytes and steroidogenic cells. It can also be induced by oleic acid in RAW macrophages (Chen et al., 2001). Moreover, the expression of adipophilin protein level is enhanced by treatment with PMA (Chen et al., 2002). Nevertheless, the exact function of adipophilin in lipid metabolism and through what mechanism is it regulated remain uncertain. Moreover, nothing is known about its function during lipolysis. It should be clarified that adipophilin is necessary for lipid droplet formation or it simply acts by stabilization of lipid droplets.

Activation of mitogen-activated protein kinase (MAPK) in response to mitogen, cytokine, or various stresses plays a key role in transducing extracellular stimulus into intracellular signals which regulate a wide variety of cellular function. MAPK is composed of 3 distinct modules in vertebrates, i.e., extracellular-signal regulated kinase (ERK), c-Jun amino terminal kinase (JNK) and p38 (Hwang and Rhee, 1999). Recent studies indicate that oxidized LDL activate MAPK pathway via a PKC-dependent manner in smooth muscle cells and macrophages (Kusuhara et al., 1997; Yang et al., 2000), the two cell types involved in atherogenesis. Similar stimulation of MAPK by fatty acids has also been shown in rat liver epithelial WB cells (Hii et al., 1995) and U-937 cells (Barry et al., 1999). In adipocytes, activation of ERK has been shown to stimulate lipolysis via phosphorylation of hormone-sensitive lipase (Greenberg et al., 2001). However, these studies did not examine the correlation between MAPK activation and lipid droplet formation. Combined with our previous results (Chen et al., 2001) which demonstrated that lipid droplet accumulation and adipophilin expression are both up-regulated by PKC, it is thus necessary to investigate the putative involvement of PKC/MAPK in lipogenesis and adipophilin induction.

PURPOSES

Though adipophilin has been demonstrated to be located around lipid droplets, little is known about its functions and regulation in lipid metabolism. We utilize in vitro cultured RAW 264.7 macrophages to study the biochemical properties during lipogenesis and lipolysis. Since lipid-laden macrophage foam cells is one of the early characteristic in atherosclerosis, investigating the regulatory mechanisms of lipid metabolism in these cells may be helpful in understanding the process of atheroscerosis.

MATERIALS AND METHODS

Cell culture and drug treatment

RAW 264.7 macrophages (ATCC TIB-71) were purchased from the American Type Culture Collection (Rockville, MD) and cultured on coverglasses in DMEM (GibcoBRL, Long Island, NY) supplemented with 10% CPSR-1 (a low-lipid serum replacement) (Sigma), 100 units/ml of penicillin, and 100 μg/ml of streptomycin. To study lipid droplet accumulation, macrophages were loaded with 100 μg/ml of oleic acid (Sigma) (Jepson et al., 1996) in the presence of 0.1 μM PMA (Sigma) which activates ERK, or 100 μM PD 98059 (Calbiochem) which inhibits ERK.

Immunoblotting

RAW 264.7 macrophages were scraped off from the culture dishes, homogenized by sonication and determined for protein concentration (protein assay kit, BioRad). Cell lysates (50 μg/lane) were electrophoresed on 10 % SDS polyacrylamide gel (Fritz et al., 1989). Proteins were then transferred onto nitrocellulose membrane. After blocking with 5% non-fat milk for 30 min, the membranes were reacted with rabbit anti-adiophilin, mouse anti-phosphorylated ERK, followed by incubating with peroxidase-conjugated anti-rabbit and anti-mouse IgG. The blots were developed with the enhanced chemiluminence (ECL) system and exposed to Hyperfilm-ECL film (Amersham Pharmacia biotech, England).

Measurement of fatty acid release

After incubated with various agents, lipolysis was determined by measuring the extracellular free fatty acid using a colorimetric assay (Roche, Indianapolis; USA).

RESULTS

Fig. 1 shows that treatment of cells with oleic acid does not enhance obviously the phosphorylation of ERK 1 (p44 MAPK), though a slightly increase in ERK 2 (p42 MAPK) phosphorylation is observed. Incubation with 0.1 μM PMA significantly elevates phosphorylation levels of both p44 and p42 at 10 min in the presence or absence of oleic acid, followed by a decline to nearly basal level at about 3 h. As PMA potentiates lipid droplet accumulation & adipophilin expression, it is interesting to investigate whether PMA stimulates lipogenesis through activation of ERKs. Immunoblot results (Fig. 2)

shows that the level of adipophilin in PMA-treated cells is significantly elevated as compared with that in the absence of PMA. Unfortunately, PD 98059, a MEK inhibitor, at 100 μM does not inhibit the effect of PMA, nor does it affect adipophilin level induced by oleic acid alone. These results show that the PMA-enhanced adipophilin expression and lipid droplet accumulation is not mediated through activation of ERKs.

Though ERK is not involved in lipogenesis, it is worth to examine the effect of ERK on lipolysis. Measurement of extracellular fatty acid reveals that PD 98059 does not stimulate lipolysis in oleic acid-induced lipid-laden macrophages. All these findings indicate that ERK activity is irrelevant to lipid metabolism in RAW 264.7 macrophages incubated with oleic acid.

DISCUSSION

In this report, I present the data which demonstrate that both PMA-potentiated lipid droplet formation and adipophilin expression are not mediated via activation of ERK in RAW 264.7 macrophages incubated with oleic acid; moreover, inhibition of ERK activity does not stimulate lipolysis.

Previous results showed that PMA enhanced the oleic acid-induced PKC isozyme translocation in RAW 264.7 macrophages (Chen et al., 2002). It is possible that this increased translocation of PKC isozymes may explain the PMA-induced elevation of adipophilin expression and lipid droplet formation. Nevertheless, the downstream signaling mechanisms involved in this pathway remain unknown. Recent findings indicate that oxidized LDL-induced lipid-laden foam cell formation is mediated through activation of MAPK pathway via a PKC-dependent manner in smooth muscle cells and macrophages (Yang et al., 2000). Moreover, lipolysis in adipocytes is stimulated by activation of ERK (Greenberg et al., 2001). It is therefore my interest to explore the possibility that MAPK is also involved in lipid metabolism in macrophages incubated with oleic acid. My results show that, unlike adipocytes, neither lipogenesis nor lipolysis is regulated by activation or inhibition of ERK activity. These results indicate that lipolysis is regulated via different mechanisms between adipocytes and lipid-loaded RAW 264.7 macrophages, which may be due to the fact that adipocytes are “endowed” with lipid, whereas RAW 264.7 macrophages are “forced” to include exogenous lipid.

Though ERK does not participate in lipid metabolism in RAW 264.7 macrophages, the plausibility that this process is regulated by another modules of MAPK (JNK and p38) (Hwang and Rhee, 1999) can not be excluded out. Further studies are required to investigate the possible candidates involved in oleic acid- and PMA-induced lipid metabolism in RAW 264.7 macrophages.

REFERENCES

Barry, O.P., Kazanietz, M.G., Praticò, D., and FitzGerald, G.A. (1999) Arachidonic acid in platelet microparticles up-regulates cyclooxygenase-2 -dependent prostaglandin formation via a protein kinase C/mitogen- activated protein kinase-dependent pathway. *J. Biol. Chem.* **274**: 7545- 7556.

Chen, J.S., Greenberg, A.S., Tesng, Y.Z., and Wang, S.M. (2001) Possible involvement of protein kinase C in the induction of adipose differentiation-related protein by sterol ester in RAW 264.7 macrophages. *J. Cell. Biochem.* 83: 187-199.

Chen J.S., Greenberg, A.S., and Wang S.M. (2002) Oleic acid-induced PKC isozyme translocation in RAW 264.7 macrophages. *J. Cell. Biochem*. 86: 784-791.

Fritz, J.D., Swartz, D.R., and Greaser, M.L. (1989) Factors affecting polyacrylamide gel electrophoresis and electroblotting of high molecular-weight myofibrillar proteins. *Anal. Biochem.* **180**: 205-210.

Greenberg, A.S., Shen, W.J., Muliro, K., Patel, S., Souza, S.C., Roth, R.A., and Kraemer, F.B. (2001) Stimulation of lipolysis and hormone-sensitive lipase via the extracellular signal-regulated kinase pathway. *J. Biol. Chem.* 276: 45456-45461.

Hii, C.S.T., Ferrante, A., Edwards, Y.S., Huang, Z.H., Hartfield, P.J., Rathjen, D.A., Poulos, A., and Murry, A.W. (1995) Activation of mitogen-activated protein kinase by arachidonic acid in rat liver epithelial WB cells by a protein kinase C-dependent mechanism. *J. Biol. Chem.* **270**: 4201-4204.

Hwang, D., and Rhee, S.H. (1999) Receptor-mediated signaling pathways: potential targets of modulation by dietary fatty acids. *Am. J. Clin. Nutr.* **70**, 545-556.

Jepson, C.A., Harrison, J.A., Kraemer, F.B., and Yeaman, S.J. (1996) Down-regulation of hormone-sensitive lipase in sterol ester-laden J774.2 macrophages. *Biochem. J.* **318**: 173-177.

Kusuhara, M., Chait, A., Cader, A., and Berk, B.C. (1997) Oxidized LDL stimulated mitogen-activated protein kinases in smooth muscle cells and macrophages. *Arterioscler. Thromb. Vasc. Biol.* **17**: 141-148.

Yang, C.M., Xchiu, C.T., Wang, C.C., Chien, C.S., Hsiao, L.D., Lin, C.C., Tu, M.T., and Pan, S.L. (2000) Activation of mitogen-activated protein kinase by oxidized low-density lipoprotein in canine cultured vascular smooth muscle cells. *Cell. Signal.* **12**: 205-214.

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