Journal of Cellular Biochemistry 86:784–791 (2002)

Oleic Acid-Induced PKC Isozyme Translocation in RAW 264.7 Macrophages

Jin-Shan Chen,[1] Andrew S. Greenberg,[2] and Seu-Mei Wang[1]*

[1]Department of Anatomy and Cell Biology, College of Medicine, National Taiwan University, Taipei, Taiwan 100

[2]United States Department of Agriculture, Human Nutrition Research Center on Aging at Tufts University, Boston, Massachusetts 02111

Abstract Fatty acids are important second messengers that mediate various cellular functions, but their role in the formation of macrophage foam cells is not known. High plasma levels of oleic acid (OA) in obese patients are often associated with a high risk for atherosclerosis. In this study, we investigated the protein kinase C (PKC) isozymes involved in OA-induced lipid accumulation in RAW 264.7 macrophages. The results show that OA induces translocation of PKC α, β1, and δ from the cytosol to the cell membrane 5 min after the treatment. After 16 h incubation with OA, PKC δ was found to be colocalized with adipose differentiation-related protein (ADRP) on the surface of lipid droplets, but immunoprecipitation experiments showed that PKC δ was not biochemically associated with ADRP. After 16 h incubation with OA plus phorbol 12-myristate 13-acetate (PMA), PKC δ staining on the lipid droplet surface was not seen, whereas the accumulation of lipid droplets was unaffected. Furthermore, downregulation of PKC δ was confirmed by immunoblotting. These results demonstrate possible involvement of specific PKC isozymes in the early phase of lipid accumulation, possibly during the uptake of OA. J. Cell. Biochem. 86: 784–791, 2002. © 2002 Wiley-Liss, Inc.

Key words: oleic acid; PKC translocation; macrophage

In addition to acting as structural molecules in membrane lipids and as precursors of eicosanoids, fatty acids play important roles in intracellular signaling. Many studies have shown that they can activate PKC to modulate different cellular functions [Khan et al., 1992; Hwang and Rhee, 1999; Ron and Kazanietz, 1999]. At least three different effectors have been reported in oleic acid (OA)-induced PKC activation, namely OA itself, diacylglycerol synthesized from oleate, and the rise in intracellular calcium concentrations [Verkest et al., 1988; Díaz-Guerra et al., 1991; Lester et al., 1991; Khan et al., 1993; Yu et al., 2001]. PKC activation is involved in fatty acid-stimulated insulin secretion [Alcazar et al., 1997; Littman et al., 2000] and amylase secretion [Wooten and Wrenn, 1988] and, in neutrophils, in the formation of lipid droplets induced by *cis*-unsaturated fatty acids [Weller et al., 1991]. Moreover, the activation of phospholipase Cγ by unsaturated fatty acids (especially arachidonic acid) may trigger release of inositol triphosphate and diacylglycerol to activate PKC [Hwang et al., 1996]. Activation of PKC by fatty acids in various cell types is accompanied by translocation of various PKC isozymes from the cytosol to the cell membrane [Wooten and Wrenn, 1988; Díaz-Guerra et al., 1991; Shirai et al., 1998; Littman et al., 2000]. In addition to activating PKC, OA

Abbreviations used: ADRP, adipose differentiation-related protein; DMEM, Dulbecco's modified Eagle's medium; ERK, extracellular signal-related kinase; HSL, hormone-sensitive lipase; LDL, low density lipoprotein; MEK, mitogen-activated protein kinase kinase; PBS, phosphate-buffered saline; PKC, protein kinase C; PMA, phorbol 12-myristate-13 acetate.

Grant sponsor: National Science Council, Republic of China; Grant number: NSC 90-2314-B-002-205; Grant sponsor: US Department of Agriculture, Agriculture Research Service; Grant number: 3K06510; Grant sponsor: NIH; Grant number: P30DK34928; Grant sponsor: American Diabetes Association; Grant number: DK 50647.

Jin-Shan Chen's present address is Department of Anatomy, Taipei Medical University, Taipei, Taiwan 110

*Correspondence to: Seu-Mei Wang, Department of Anatomy and Cell Biology, College of Medicine, National Taiwan University, 1-1 Jen-Ai Road, Taipei, Taiwan 100. E-mail: smwang@ha.mc.ntu.edu.tw

Received 7 May 2002; Accepted 7 June 2002

DOI 10.1002/jcb.10266

© 2002 Wiley-Liss, Inc.

stimulates the expression of adipose differentiation-related protein (ADRP) in the mouse adipogenic cell line, 1,246 [Gao et al., 2000] and that of peroxisome proliferator-activated receptors, a marker in early adipocyte differentiation [Chawla et al., 1994], in the human osteosarcoma cell line, U2OS, transfected with a peroxisome proliferator-activated receptor expression vector [Yu et al., 1995]. Recently, expression of uncoupling protein-2, an uncoupler of oxidative phosphorylation in 3T3 preadipocytes, was shown to be upregulated by dietary fatty acids [Reilly and Thompson, 2000]. Taken together, these results support the idea that, in addition to being an energy fuel, fatty acids can also act as second messengers.

Increased plasma levels of non-esterified fatty acids are found in obese hypertensive patients [Lu et al., 1998a], and OA and angiotension II may interact to accelerate the progress of atherosclerosis [Lu et al., 1996; Lu et al., 1998b; Greene et al., 2001]. OA induces a mitogenic response in rat aortic smooth muscle cells through PKC- and extracellular signal-related kinase (ERK)-dependent pathways [Lu et al., 1996], contributing to the pathogenesis of atherosclerosis. The OA-induced activation of ERK is blocked by PKC inhibitors [Lu et al., 1996], suggesting possible crosstalk between ERK and PKC. Although sterol ester has been used to induce foam cells from macrophages [Jepson et al., 1996], the signaling pathway involved in the OA-mediated induction of foam cells remains to be explored. The formation of macrophage foam cells, an early characteristic of atherosclerosis, is initiated by uptake of oxidized low density lipoprotein (LDL) via scavenger receptors, followed by accumulation of lipid droplets within the cytoplasm [Wiklund et al., 1991; Juul et al., 1996]. Although induction of the scavenger receptor has been shown to be regulated by PKC in both smooth muscle cells [Mietus-Snyder et al., 1997] and the human monocyte cell line, THP-1 [Via et al., 1989], the role of the specific PKC isozymes involved remains to be elucidated. Our previous study showed that co-incubation of RAW 264.7 macrophages with 25-hydroxycholesterol, OA, and bovine serum albuminm results in the formation of lipid droplets and ADRP expression, both of which are enhanced by PMA treatment [Chen et al., 2001]. Although fatty acid stimulation of lipid droplet formation involving PKC has been well documented in neutrophils [Weller et al., 1991], the PKC isoforms involved have not been identified. In RAW 264.7 macrophages, PMA has been shown to induce the translocation of several PKC isozymes from the cytosol to the cell membrane [Lin and Chen, 1998]. In this study, we wished to determine the specific isoforms activated by OA.

MATERIALS AND METHODS

Cell Culture and Drug Treatments

RAW 264.7 macrophages (ATCC TIB-71) were purchased from the American Type Culture Collection (Rockville, MD) and cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO-BRL, Long Island, NY) supplemented with 10% CPSR-1 (a low-lipid serum replacement) (Sigma, St. Louis, MO), 100 U/ml of penicillin, and 100 μg/ml of streptomycin. To study the OA-induced translocation of PKC isoforms and lipid droplet accumulation, macrophages were loaded with 100 μg/ml of OA (Sigma) for 5 or 15 min in the presence or absence of 0.1 μM PMA.

Immunofluorescence Microscopy

RAW 264.7 macrophages cultured on coverslips were fixed for 1 min in cold methanol (−20°C), washed, and incubated with mouse anti-PKC α antibodies (Transduction Lab., San Diego, CA, USA) or rabbit anti-PKC β_1 antibodies (Santa Cruz Biotech., Santa Cruz, CA). In double-labeling experiments, cells were incubated with mouse anti-PKC δ antibody (Transduction Lab) and rabbit anti-ADRP antibody [a kind gift of Dr. Thomas Keenan; Heid et al., 1996]. After phosphate-buffered saline (PBS) washes, the cells were then reacted with FITC-conjugated anti-mouse IgG and Texas red-conjugated anti-rabbit IgG (Sigma), washed, mounted, and photographed using a Zeiss epifluorescence microscope (Zeiss, Inc., Thornwood, NY).

Uptake of Radiolabeled OA by RAW Macrophages

The following method was modified from Gao and Serrero [1999]. [9,10 (n)-^{3}H]OA (Amersham Biosciences) and BSA (Sigma) were added to the cells in each 35-mm dish at the final concentration of 3 μCi/ml and 0.3%, respectively. After incubation for 30 min, uptake was stopped by cold PBS containing 0.1% BSA. The cells were scraped off from the dishes, filtered

through GF/C filter pre-saturated with 0.1% BSA in PBS, and the filters washed three times with PBS–0.1% BSA. The filters were soaked in 5 ml of scintillation solution for 1 h before counting.

Separation of Cytosolic and Membrane Fractions for Immunoblotting

RAW 264.7 macrophages, incubated with OA in the presence or absence of PMA, were scraped off the culture dishes in buffer A (20 mM Tris, pH 7.5, 0.5 mM EGTA, 1 mM EDTA, 0.5 mM PMSF, 20 μg/ml of leupeptin, and 20 μg/ml of benzamidine). After sonication, the cell lysates were centrifuged at 20,000*g* at 4°C for 30 min, and the resulting supernatants and precipitates were used as cytosolic and membrane fractions, respectively. The protein concentration of each fraction was determined using a protein assay kit (Bio-Rad, Hercules, CA). Protein samples (50 μg per lane) were electrophoresed on a 10% SDS–polyacrylamide gel [Fritz et al., 1989], then transferred to nitrocellulose membranes. After blocking with 5% non-fat milk for 30 min at room temperature, the membranes were reacted for 1 h at 37°C with mouse or rabbit antibodies against various PKC isoforms, followed by peroxidase-conjugated anti-mouse or rabbit IgG (Amersham Pharmacia Biotech), then reactive bands were visualized using an enhanced chemiluminescence detection kit (Amersham Pharmacia Biotech). The relative density of each positive band on the X-ray film was quantified by densitometric scanning using an Image Master (Pharmacia Biotech, Hong Kong). When primary antibodies were omitted from negative controls, no bands were seen.

Immunoprecipitation

Immunoprecipitation of ADRP was performed at 4°C using anti-ADRP antibodies. Briefly, macrophages were scraped off the dishes in buffer B (10-mM Tris-HCl, pH 7.5, 1-mM EDTA, 1-mM PMSF, 0.5% NP-40), and centrifuged at 12,000*g* for 10 min. The supernatants were pretreated with protein G Sepharose, then incubated with rabbit polyclonal anti-ADRP antibody for 1 h, followed by incubation with protein G Sepharose for another 30 min, then centrifuged at 10,000*g* for 1 min. After several washes with buffer B, the beads were resuspended and boiled for 5 min in sample buffer containing 0.063 M Tris-HCl, pH 6.8, 5% β-mercaptoethanol, 3.3% SDS, and 10% glycerol, then the supernatants were electrophoresed on 10% polyacrylamide gels, transferred to membranes, and immunoblotted.

Nile Red Stain

To observe lipid droplets, macrophages were first fixed in 10% formalin in PBS for 10 min at room temperature, then stained with Nile red (Sigma) at a final concentration of 10 μg/ml [Greenspan et al., 1985]. Following brief washes with PBS, the cells were mounted and observed.

Data Analysis

To calculate the total surface area of the lipid droplets in each cell, lipid droplet containing macrophages were stained with Nile red and photographed, then the total surface area of the stained lipid droplets in each of 30 cells was measured using the BRS-2 version of the Microsoft Computer Image Device System (Brock University, St. Catharines, Ontario, Canada).

RESULTS

OA Induced Formation of Lipid Droplets

None of the lipid droplets were seen in untreated RAW macrophages in the presence or absence of PMA (data not shown). Radiolabeled OA was quickly uptaken by RAW macrophages within minutes. Cellular oleate uptake in cells incubated with radiolabeled OA for 30 min was 4.252 ± 0.06 pmole/mg and that in cells incubated with radiolabeled OA plus PMA was 6.29 ± 0.24 pmole/mg, which was a slight increase compared with radiolabeled OA group. Continuous incubation of RAW macrophages with OA for 2 h resulted in the accumulation of lipid droplets within the cytoplasm and this was accelerated by co-treatment with PMA (Fig. 1). The total surface area of lipid droplets within each cell increased from 11.3 ± 3.6 μm^2 in OA-treated cells to 26.1 ± 3.7 μm^2 in PMA plus OA-treated cells, while that in cells treated only with PMA was almost at control levels.

OA Stimulates Translocation of PKC α, β1, and δ

We next examined the effect of OA on PKC isozyme translocation in the presence or absence of PMA using immunoblotting of the cytosolic and plasma membrane fractions. Of the eight PKC isozymes (α, β_1, β_2, γ, δ, μ, λ, and ζ)

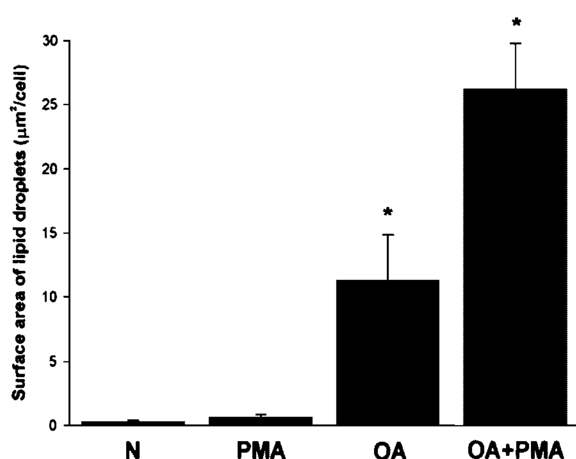

Fig. 1. Potentiation of OA-induced accumulation of lipid droplets by PMA. Macrophages were incubated with OA and/or PMA (0.1 μM) for 2 h and stained with Nile red. The total surface area of the Nile red-stained lipid droplets was measured and expressed as the mean ± SD (n=30). *, $P < 0.05$.

present in RAW 264.7 macrophages [Lin and Chen, 1998], only α, β1, and δ were found to show noticeable translocation to the cell membrane after OA and/or PMA treatment (data not shown). As shown in Figure 2, incubation with OA (100 μg/ml) for 5 min led to decreased levels of PKC α, β1, and δ in the cytosolic fractions compared to that in normal cells, and to their redistribution to the cell membrane; this effect was not further increased by 15 min incubation

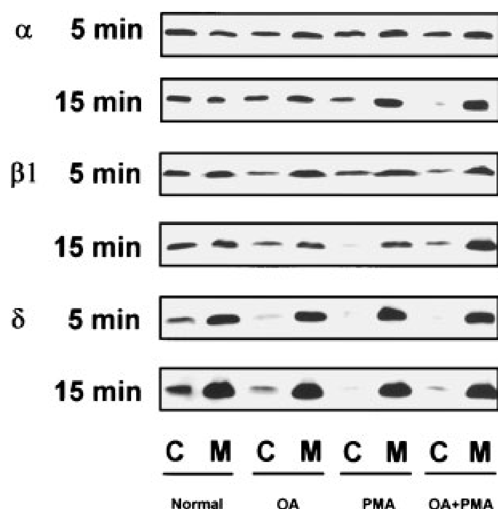

Fig. 2. Western blot analysis of PKC isoform translocation. Untreated macrophages (normal) or macrophages incubated with OA (100 μg/ml), PMA (0.1 μM), or OA plus PMA for 5 or 15 min, were fractionated into cytosolic (C) and membrane (M) fractions, which were then subjected to electrophoresis and immunoblotting with antibodies against PKC α, β1, or δ.

with OA. Treatment with 0.1 μM PMA alone or together with OA resulted in a much greater increase in the relative amounts of PKC α, β1, and δ translocated to the cell membrane. The densitometric scanning data (Table I) showed that, after 5 min treatment, cytosolic PKC α decreased from 67.6 ± 8.2% of the total cellular PKC α content in normal cells to 37.7 ± 0.1, 44.3 ± 0.4, and 35.0 ± 4.3% in cells treated with OA alone, PMA alone, or OA and PMA, respectively. Translocation was marked following PMA treatment for 15 min, as reflected in the ratios of cytosolic PKC α in PMA-treated cells in the absence or presence of OA being 23.2 ± 3.1 and 22.9 ± 0.5%, respectively, as compared with 58.7 ± 3.5% for normal cells. Similar trends in translocation to the cell membrane was seen for PKC β1 and δ, especially for β1 following 15 min treatment with PMA in the presence or absence OA. This translocation of PKC α and β1 from the cytosol to the cell membrane was confirmed by immunofluorescence microscopy. As shown in Figure 3, PKC α (Fig. 3A) and β1 (Fig. 3E) staining on the cell membrane was not so obvious in normal cells, but was increased in cells incubated with OA for 15 min (Fig. 3B,F), and even stronger in cells treated for 15 min with PMA alone (Fig. 3C,G) or with PMA plus OA (Fig. 3D,H).

Translocation of PKC δ to Lipid Droplet Surface by Long-Term OA Treatment

We then examined the distribution of different PKC isozymes after long-term OA treatment (16 h). Double labeling of untreated cells with anti-PKC δ antibody and anti-ADRP antibody showed a diffuse or punctate distribution of PKC δ (Fig. 4A) and virtually no ADRP staining (Fig. 4B). After incubation with OA for 16 h, of the eight isozymes examined by immunofluorescence, only PKC δ was found at high levels around lipid droplets in the form of a ring (arrows in Fig. 4C) or as small dots (arrowheads in Fig. 4C), which were colocalized with ADRP (Fig. 4D). Although PKC δ may have translocated to the lipid droplet surface after less than 16 h of OA treatment, the lipid droplets formed before this time were too small to be visualized for distinct PKC δ staining due to the limited resolution of immunofluorescence microscopy. When macrophages were treated with 0.1-μM PMA plus OA for 16 h to induce large amount of lipid droplets, PKC δ staining was lost from the surface of lipid droplets

TABLE I. Translocation of PKC Isozymes by OA, PMA, and OA + PMA

		α		β1		δ	
		5 min	15 min	5 min	15 min	5 min	15 min
Normal	Cytosol	67.6 ± 8.2	58.7 ± 3.5	44.5 ± 0.9	45.3 ± 3.6	20.4 ± 2.1	17.3 ± 4.4
	Membrane	32.9 ± 7.5	41.3 ± 3.5	55.6 ± 0.9	54.8 ± 3.6	79.7 ± 2.1	82.7 ± 4.4
OA	Cytosol	37.7 ± 0.1	44.6 ± 0.7	28.1 ± 0.3	35.6 ± 8.5	8.6 ± 3.5	12.6 ± 0.1
	Membrane	62.3 ± 0.1	55.4 ± 0.7	71.9 ± 2.3	64.4 ± 8.5	91.4 ± 3.5	87.5 ± 0.1
PMA	Cytosol	44.3 ± 0.4	23.2 ± 3.1	38.3 ± 7.5	17.0 ± 9.0	4.8 ± 1.3	13.7 ± 3.3
	Membrane	55.8 ± 0.4	76.8 ± 3.1	61.7 ± 8.0	83.1 ± 9.0	95.2 ± 1.3	90.0 ± 6.8
OA + PMA	Cytosol	35.0 ± 4.3	22.9 ± 0.5	31.8 ± 3.0	19.0 ± 0.5	7.9 ± 1.6	11.7 ± 4.0
	Membrane	65.1 ± 4.3	77.1 ± 0.5	68.3 ± 3.0	81.0 ± 0.5	91.7 ± 1.0	91.2 ± 5.9

Relative density of each reactive band in immunoblot was scanned with densitometer and the ratios shown are means ± SD from three experiments expressed as relative percentage.

(Fig. 4E), despite the continued presence of ADRP (Fig. 4F). Immunoblotting confirmed that long-term PMA treatment downregulated PKC δ, regardless of the presence or absence of OA (Fig. 5).

PKC δ is Not Physically Associated With ADRP

As PKC δ colocalized with ADRP on the surface of OA-induced-lipid droplets, it was important to determine whether they were physically associated. Immunoprecipitation studies showed that, although ADRP was precipitated from the lysates of OA-loaded cells by anti-ADRP antibody (Fig. 6A), it was not precipitated by anti-PKC δ antibody (Fig. 6B). These results clearly demonstrate that PKC δ is colocalized, but not biochemically associated, with ADRP on the lipid droplet surface.

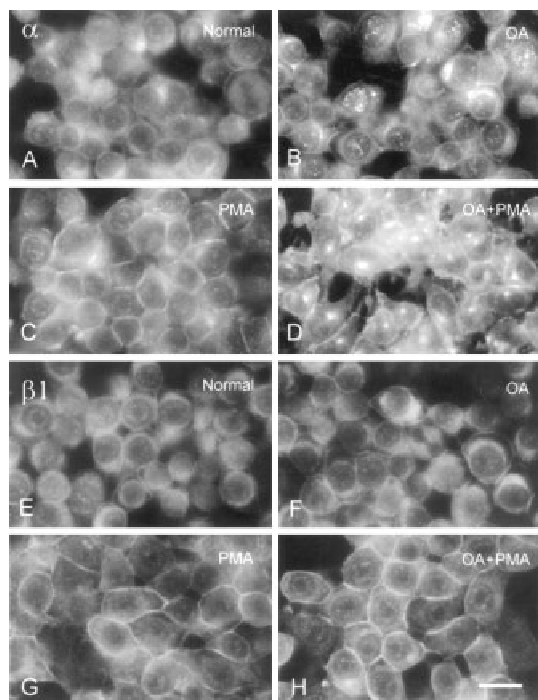

Fig. 3. Immunofluorescence demonstration of PKC isoform translocation to the cell membrane. Macrophages incubated with various agents for 15 min were immuno- stained with anti-PKC α (**A–D**) or anti-PKC β1 (**E–H**) antibodies. Normal untreated macrophages (A and E) display weak membrane staining, which becomes more prominent after incubation with OA (B and F). Treatment with 0.1 μM PMA in the absence (C and G) or presence (D and H) of OA results in strong membrane staining. Bar = 15 μm.

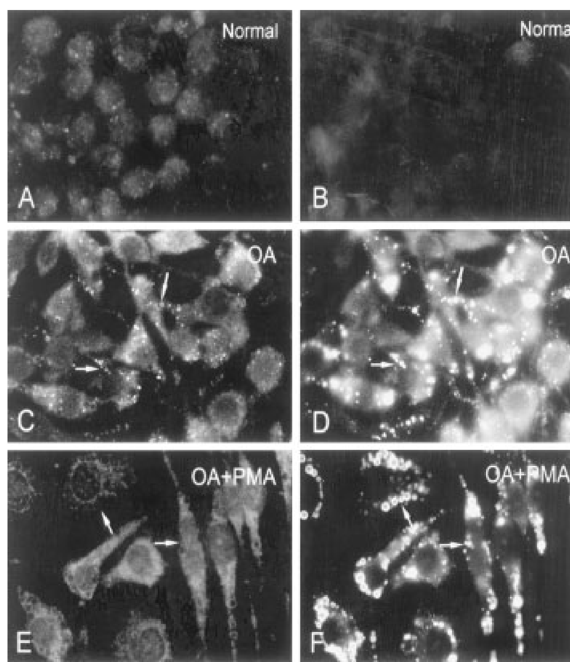

Fig. 4. Association of PKC δ with lipid droplets. Normal untreated cells (**A** and **B**) and cells incubated for 16 h with 100 μg/ml of OA (**C** and **D**) or OA plus 0.1 μM PMA (**E** and **F**) were double-stained with anti-PKC δ (A, C, and E) and anti-ADRP (B, D, and F) antibodies. The arrows in C and D indicate association of PKC δ (C) with ADRP-positive lipid droplets (D). Cells treated with OA plus PMA accumulate large amounts of ADRP-positive, but PKC δ-negative, lipid droplets (arrows in E and F). Bar = 15 μm.

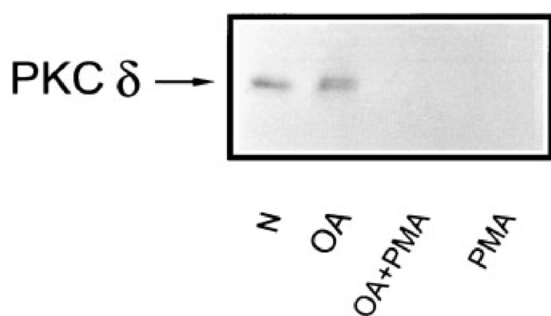

Fig. 5. Downregulation of PKC δ by long-term PMA treatment. Lysates of macrophages treated with various agents for 16 h were electrophoresed and transferred to membranes. PMA alone (PMA) or combined incubation with OA (OA + PMA) downregulates PKC δ.

DISCUSSION

The elucidation of the mechanism by which macrophages accumulate lipid droplets to form foam cells is important in understanding the pathogenesis of atherosclerosis. We previously demonstrated that OA induces ADRP expression, which correlates with lipid accumulation, and that PMA potentiates these effects [Chen et al., 2001]. In the present study, we identified PKC α, β1, and δ as the isozymes activated by OA or PMA, as judged by PKC isozyme translocation to the cell membrane. Furthermore, in both smooth muscle cells [Mietus-Snyder et al., 1997] and THP-1 human monocytes [Via et al., 1989], PKC activation is known to induce expression of the scavenger receptor, which is responsible for uptake of modified LDL. Taken together, these results suggest that PKC mediates lipid droplet accumulation by upregulating both scavenger receptor expression and translocation of PKC isozymes to the cell membrane, thus enhancing uptake of modified LDL or free fatty acid, and that the ADRP newly expressed as a result of OA or PMA treatment may be responsible for the packaging of these droplets.

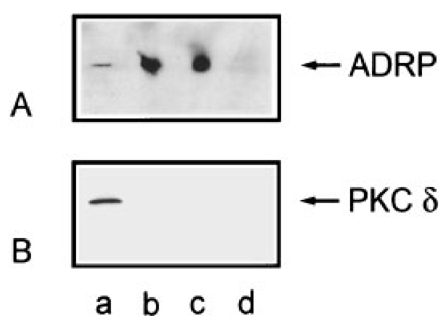

Fig. 6. Immunoprecipitation of ADRP from lysates of normal and OA-loaded macrophages. **Lane a**: untreated macrophage lysates (no immunoprecipitation with anti-ADRP antibody) (positive control). **Lanes b** and **c**: lysates of normal macrophages (b) and OA-loaded (c) macrophages immunoprecipitated with anti-ADRP antibody, followed by electrophoresis and immunoblotting of the immunoprecipitated material using anti-ADRP (**A**) or anti-PKC δ (**B**) antibodies. **Lane d**: OA-loaded macrophage lysates treated with immunoprecipitation buffer in the absence of anti-ADRP antibody. Lane a in (B) shows PKC δ in normal cell lysates, as a positive control for confirming the validity of this antibody.

Sodium oleate can activate soluble PKC [Murakami et al., 1986], and OA can activate PKC via diacylglycerol, the levels of which are increased 1.8-fold after OA treatment in cultured aortic smooth muscle cells [Yu et al., 2001]. Other investigators have reported that OA induces multifold increases in voltage-dependent calcium currents in ventricular myocytes [Huang et al., 1992]. Thus, OA can activate PKC directly or indirectly via calcium ion or diacylglycerol. Our results have shown that, as in other cell types [Boscá et al., 1989; Díaz-Guerra et al., 1991; Khan et al., 1993; Alcazar et al., 1997], OA induces PKC isozyme translocation in RAW macrophages. In a previous study [Lin and Chen, 1998], short-term treatment with 1 μM PMA induced translocation of PKC α, βI, βII, δ, ϵ, and μ from the cytosol to the cell membrane, whereas, in the present study, we only detected translocation of PKC α, βI, and β, probably due to the use of a low concentration of PMA (0.1 μM) in the present study. The finding that TPA (a specific PKC activator) reduces the lipolytic effect induced by norepinephrine in isolated rat adipocytes [Deaciuc and Spitzer, 1991] raises the possibility that PMA, in addition to enhancing OA uptake, may prevent lipolysis of accumulated lipid droplets. Moreover, in rat adipocytes, PMA stimulates glucose transport and activates lipogenesis from the glucose pathway, without any effect on lipid synthesis from acetate [Cherqui et al., 1986]. Thus, in addition to inducing ADRP expression and activating PKC isozymes, PMA also enhances lipogenesis from other pathways. Further studies are required to clarify whether phorbol ester has different effects on lipid metabolism in different cell types.

This is the first study to show an association of PKC δ with lipid droplets. Our results show that 15 min treatment of cells with OA in the absence of PMA induced PKC δ first to translocate to the cell membrane, then, after the formation of lipid droplets, to redistribute to the surface of lipid droplets. It seems unlikely that

PKC δ is involved in lipid droplet accumulation, since long term treatment with OA plus PMA induced the formation of enormous lipid droplets, but was accompanied by downregulation of PKC δ. Phosphotidylinositide 3-kinase (subunit p55, p85α, p85β) is shown to be present at isolated lipid bodies in RAW macrophages [Yu et al., 2000]. The phosphotidylinositide 3-kinase products, phopshotidylinositol (3,4) P2 and phosphotidylinositol (3,4,5) P3 are able to activate PKC δ [Toker et al., 1994]. Thus, an interaction between phosphotidylinositol 3-kinase and PKC [Hardy et al., 2000] might occur on the surface of lipid droplets. The application of OA to RAW macrophages may also directly activate PI-3 kinase or indirectly activate PKC isozymes via diacylglycerol derived from oleate, as shown in other cell types [Díaz-Guerra et al., 1991; Hardy et al., 2000]. Although PKC δ activation is involved in the induction of apoptosis in neutrophils [Pongracz et al., 1999] and in hyperglycemis-induced apoptosis of ventricular myocytes [Shizukuda et al., 2002], it is unlikely that PKC δ translocation to lipid droplets is related to apoptosis, since OA did not cause apoptosis in several types of cells [Hardy et al., 2000]. The significance of the PKC δ translocation and the density of the protein to which PKC δ binds on the lipid droplet surface remain to be elucidated. Similar downregulation of PKC isozymes (α, βI, βII, and δ) was seen after long-term PMA treatment of RAW 264.7 macrophages, but at a higher concentration (1 μM) of PMA and in the absence of OA [Lin and Chen, 1998]. It will now be of interest to determine whether PKC isozymes are associated with lipid droplets in other lipid-containing cells, such as adipocytes and adrenocortical cells.

ACKNOWLEDGMENTS

We thank Dr. Thomas Keenan for providing rabbit anti-ADRP antibody and Dr. Thomas Barkas for his critical reading and correction of this manuscript.

REFERENCES

Alcazar O, Zhu QY, Giné E, Tamarit-Rodriguez J. 1997. Stimulation of islet protein kinase C translocation by palmitate requires metabolism of the fatty acid. Diabetes 46:1153–1158.

Boscá L, Diaz-Guerra MJM, Mojena M. 1989. Oleate-induced translocation of protein kinase C to hepatic microsomal membranes. Biochem Biophys Res Commun 160:1243–1249.

Chawla A, Schwarz EJ, Dimaculangan DD, Lazar MA. 1994. Peroxisome proliferator-activated receptor (PPAR) γ: Adipose-predominant expression and induction early in adipocyte differentiation. Endocrinology 135:798–800.

Chen JS, Greenberg AS, Tseng YZ, Wang SM. 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.

Cherqui G, Caron M, Wicek D, lascols O, Capeau J, Picard J. 1986. Insulin stimulation of glucose metabolism in rat adipocytes: Possible implication of protein kinase C. Endocrinology 118:1759–1769.

Deaciuc IV, Spitzer JA. 1991. Insulin-like, antilipolytic effect of 12-*O*-tetradecanoyl phorbol 13-acetate in rat white adipocytes (43245). Proc Soc Exp Biol Med 197:193–196.

Díaz-Guerra MJM, Junco M, Boscá L. 1991. Oleic acid promotes changes in the subcellular distribution of protein kinase C in isolated hepatocytes. J Biol Chem 266:23568–23576.

Fritz JD, Swartz DR, Greaser ML. 1989. Factors affecting polyacrylamide gel electrophoresis and electroblotting of high molecular-weight myofibrillar proteins. Anal Biochem 180:205–210.

Gao J, Serrero G. 1999. Adipose differentiation related protein (ADRP) expressed in transfected COS-7 cells selectively by stimulates long chain fatty acid uptake. J Biol Chem 274:16825–16830.

Gao J, Ye H, Serrero G. 2000. Stimulation of adipose differentiation related protein (ADRP) expression in adipocyte precursors by long-chain fatty acids. J Cell Physiol 182:297–302.

Greene EL, Lu G, Zhang D, Egan BM. 2001. Signaling events mediating the additive effects of oleic acid and angiotensin II on vascular smooth muscle cell migration. Hypertension 37:308–312.

Greenspan P, Mayer EP, Fowler SD. 1985. Nile red: A selective fluorescent stain for intracellular lipid droplets. J Cell Biol 100:965–973.

Hardy S, Langelier Y, Prentki M. 2000. Oleate activates phosphotidylinositol 3-kinase and promotes proliferation and reduces apoptosis of MDA-MB-231 breast cancer cells, whereas palmitate has opposite effects. Cancer Res 60:6353–6358.

Heid HW, Schnölzer M, Keenan TW. 1996. Adipocyte differentiation-related protein is secreted into milk as a constitute of milk lipid globule membrane. Biochem J 320:1025–1030.

Huang JM, Xian H, Bacaner M. 1992. Long-chain fatty acids activate calcium channels in ventricular myocytes. Proc Natl Acad USA 89:6452–6456.

Hwang D, Rhee SH. 1999. Receptor-mediated signaling pathway: potential targets of modulation by dietary fatty acids. Am J Clin Nutr 70:545–556.

Hwang SC, Jhon DY, Bae YS, Kim JH, Rhee SG. 1996. Activation of phospholipase C-gamma by the concerted action of Tau proteins and arachidonic acid. J Biol Chem 271:18342–18349.

Jepson CA, Harrison JA, Kraemer FB, Yeaman SJ. 1996. Down-regulation of hormone-sensitive lipase in sterol ester-laden J774.2 macrophages. Biochem J 318:173–177.

Juul K, Nielsen LB, Munkholm K, Stender S, Nordestgaard BG. 1996. Oxidation of plasma low-density lipoprotein

accelerates its accumulation and degradation in the arterial wall in vivo. Circulation 94:1698–1704.

Khan WA, Blobe G, Hannun YA. 1992. Activation of protein kinase C by oleic acid. Determination and analysis of inhibition by detergent micelles and physiologic membranes: Requirement for free oleate. J Biol Chem 267: 3605–3612.

Khan WA, Blobe G, Halpern A, Taylor W, Wetsel WC, Burns D, Loomis C, Hannun YA. 1993. Selective regulation of protein kinase C isoenzymes by oleic acid in human platelets. J Biol Chem 268:5063–5068.

Lester DS, Collin C, Etcheberrigaray R, Alkon DL. 1991. Arachidonic acid and diacylglycerol act synergistically to activate protein kinase C in vitro and in vivo. Biochem Biophys Res Commun 179:1522–1528.

Lin WW, Chen BC. 1998. Distinct PKC isoforms mediate the activation of cPLA2 and adenylyl cyclase by phorbol ester in RAW 264.7 macrophages. Br J Pharmacol 125: 1601–1609.

Littman ED, Pitchumoni S, Garfinkel MR, Opara EC. 2000. Role of protein kinase C isoenzymes in fatty acid stimulation of insulin secretion. Pancreas 20:256–263.

Lu G, Morinelli TA, Meier KE, Rosenzweig SA, Egan BM. 1996. Oleic-induced mitogenic signaling in vascular smooth muscle cells. A role for protein kinase C. Circ Res 79:611–618.

Lu G, Greene EL, Nagai T, Egan BM. 1998a. Reactive oxygen species are critical in the oleic acid-mediated mitogenic signaling pathway in vascular smooth muscle. Hypertension 32:1003–1010.

Lu G, Meier KE, Jaffa AA, Rosenweig SA, Egan BM. 1998b. Oleic acid and angiotensin II induce a synergistic mitogenic response in vascular smooth muscle cells. Hypertension 31:978–985.

Murakami K, Chan SY, Routtenberg A. 1986. Protein kinase C activation by cis-fatty acid in the absence of Ca^{2+} and phospholipids. J Biol Chem 261:15424–15429.

Mietus-Snyder M, Friera A, Glass CK, Pitas RE. 1997. Regulation of scavenger receptor expression in smooth muscle cells by protein kinase C. A role for oxidative stress. Arterioscler Thromb Vasc Biol 17:969–978.

Pongracz J, Webb P, Wang K, Deacon E, Lunn OJ, Lord JM. 1999. Spontaneous neutrophil apoptosis involved caspase 3-mediated activation of protein kinase C-δ. J Biol Chem 274:37329–37334.

Reilly JM, Thompson MP. 2000. Dietary fatty acids upregulate the expression of UCP2 in 3T3-L1 preadipocytes. Biochem Biophys Res Commun 277:541–545.

Ron D, Kazanietz MG. 1999. New insights into the regulation of protein kinase C and novel phorbol ester receptors. FASEB J 13:1658–1676.

Shirai Y, Kashiwagi K, Yagi K, Sakai N, Saito N. 1998. Distinct effects of fatty acids on translocation of γ- and ε-subspecies of protein kinase C. J Cell Biol 143:511–521.

Shizukuda Y, Reyland ME, Buttrick PM. 2002. Protein kinase C-δ modulates apoptosis induced by hyperglycemia in adult ventricular myocytes. Am J Physiol Heart Circ Physiol 282H:1625–1634.

Toker A, Meyer M, Reddy KK, Falck JR, Aneja S, Parra A, Burns DJ, Ballas LM, Cantley LC. 1994. Activation of protein kinase C family members by the novel polyphosphoinositides PtdIns-3,4-P2 and PtdIns-3, 4, 5-P3. J Biol Chem 269:32358–32367.

Verkest V, McArthur M, Hamilton S. 1988. Fatty acid activation of protein kinase C: Dependence on diacylglycerol. Biochem Biophy Res Commun 152:825–829.

Via DP, Pons L, Dennison DK, Fanslow AE, Bernini F. 1989. Induction of acetyl-LDL receptor activity by phorbol ester in human monocyte cell line THP-1. J Lipid Res 30:1515–1524.

Weller PF, Ryeom SW, Picard ST, Ackerman SJ, Dvorak AM. 1991. Cytoplasmic lipid bodies of neutrophils: Formation induced by cis-unsaturated fatty acids and mediated by protein kinase C. J Cell Biol 113:137–146.

Wiklund O, Mattsson L, Björnheden T, Camejo G, Bondjers G. 1991. Uptake and degradation of low density lipoproteins in atherosclerotic rabbit aorta. J Lipid Res 32:55–62.

Wooten MW, Wrenn RW. 1988. Linoleic acid is a potent activator of protein kinase C type III-a isoform in pancreatic acinar cells; its role in amylase secretion. Biochem Biophys Res Commun 153:67–73.

Yu K, Bayona W, Kallen CB, Harding HP, Ravera CP, McMahon G, Brown M, Lazar MA. 1995. Differential activation of peroxisome proliferator-activated receptors by eicosanoids. J Biol Chem 270:23975–23983.

Yu W, Cassara J, Weller PF. 2000. Phosphotidylinositide 3-kinase localizes to cytoplasmic lipid bodies in human polymorphonuclear leucocytes and other myeloid-derived cells. Blood 95:1078–1085.

Yu HY, Inoguchi T, Kakimoto M, Nakashima N, Imamura M, Hashimoto T, Umeda F, Nawata H. 2001. Saturated non-esterified fatty acids stimulate de no diacylglycerol synthesis and protein kinase c activity in cultured aortic smooth muscle cells. Diabetologia 44:614–620.