Effects of phorbol 12-myristate 13-acetate on triglyceride and cholesteryl ester synthesis in cultured coronary smooth muscle cells and macrophages

Madelaine Moinat, Jean-Michel Chevey, Patrick Muzzin, Jean-Paul Giacobino, and Michel Kossovsky

Département de Biochimie Médicale, Centre Médical Universitaire, 9 Avenue de Champel, 1211 Genève 4, Switzerland

Abstract In cultured pig coronary smooth muscle cells phorbol 12-myristate 13-acetate (PMA) stimulated the conversion of [4-^{14}C]cholesterol into cholesteryl esters and the incorporation of [2-^{3}H]glycerol into triglycerides 6.4- and 4.5-fold, respectively. The maximal effects occurred after 3 h of treatment and there was a return to basal values after 72 h. In the presence of 400 μM oleic acid, PMA stimulated the converstion of [4-^{14}C]cholesterol into cholesteryl esters and that of [2-^{3}H]glycerol into triglycerides 5.3- and 2.3-fold, respectively. The stimulatory effects were more sustained (still significant after 72 h) and their maxima were delayed (peaks after 24 h). PMA was also found to increase 2-fold the amount of triglyceride that accumulated in the cells in the presence of oleic acid after 24 h. In macrophages IC-21, the effects of PMA were observed only in the presence of oleic acid. They consisted of a 1.9-fold stimulation in the conversion of [4-^{14}C]cholesterol into cholesteryl esters after 72 h and of a 1.7-fold stimulation in the incorporation of [2-^{3}H]glycerol into triglycerides after 24 h. PMA also increased the amount of triglyceride that accumulated in the cells 1.9-fold after 72 h. It is concluded that PMA, and possibly growth factors, may promote lipid storage in smooth muscle cells and that fatty acids favor long lasting effects of PMA in smooth muscle cells and are necessary for any effect of PMA in macrophages. — **Moinat, M., J-M. Chevey, P. Muzzin, J-P. Giacobino, and M. Kossovsky.** Effects of phorbol 12-myristate 13-acetate on triglyceride and cholesteryl ester synthesis in cultured coronary smooth muscle cells and macrophages. *J. Lipid Res.* 1990. **31:** 329–334.

Supplementary key words phorbol ester • oleic acid

The atherosclerotic lesion is due to an accumulation in the intima of the artery of lipid-laden foam cells that are derived from both macrophages and vascular smooth muscle cells (1).

The lipids of the atherosclerotic lesion consist mostly of cholesteryl esters in the aorta (2, 3) and of triglycerides associated with cholesteryl esters in the coronary arteries (4). The circulating low density lipoproteins (LDL) probably contribute most of the lipid deposited in the lesion. Incubation of cultured aorta smooth muscle cells with LDL has been shown to result in a marked intracellular accumulation of cholesterol and cholesteryl esters (5, 6). Exposure of macrophages to various modified lipoproteins also caused a marked intracellular accumulation of cholesteryl esters (7). An increase in circulating free fatty acid (FFA) is frequently associated with conditions predisposing to atherosclerosis. Exposure of cultured smooth muscle cells and macrophages J774 to FFA complexed to albumin has been found to cause a marked intracellular accumulation of triglycerides and to increase the esterification of cholesterol with fatty acids (8–12).

Possible factors that might modulate lipid storage pathways in smooth muscle cells and in macrophages have received little study. Since various growth factors are synthesized and released in large amounts in the developing atherosclerotic lesion (1), it would be of interest to know whether they can modulate the lipid accumulation in smooth muscle cells and in macrophages. Platelet derived growth factor (PDGF), in fact, has been shown to increase cholesterol synthesis (13, 14) and the uptake of LDL in smooth muscle cells (15) and in macrophages (16). Protein kinase C activators have also been shown to increase LDL binding and LDL receptor mRNA levels (17) as well as lipoprotein lipase mRNA levels (18) in the human monocytic leukemic cell line THP-1. Nothing is known, however, about a possible effect of growth factors on cholesteryl ester and triglyceride synthesis in smooth muscle cells and in macrophages. Growth factors are supposed to act via activation of the protein kinase C (19).

Abbreviations: LDL, low density lipoproteins; FFA, free fatty acids; PDGF, platelet-derived growth factor; PMA, phorbol 12-myristate 13-acetate.

Downloaded from www.jlr.org by guest, on June 18, 2012

Phorbol 12-myristate 13-acetate (PMA), known to stimulate Ca^{2+}- and phospholipid-dependent protein kinase C (20), can be used to study directly protein kinase C-mediated effects of growth factors. The first aim of this work was to study the effects of PMA on lipid accumulation in cultured coronary smooth muscle cells and in a well-characterized macrophage cell line IC-21 (21). The parameters tested were the rate of synthesis of cholesteryl esters and triglycerides as well as the amount of cholesterol, cholesteryl esters and triglycerides in the cells.

It is also of interest to ascertain whether exposure of cultured cells to lipids and/or the subsequent transformation into foam cells would interfere with the effects of factors modulating intracellular lipid storage. The second aim of this work was thus to study the effects of PMA on lipid accumulation in cells loaded with lipids by the addition of oleic acid to the culture medium.

MATERIALS AND METHODS

Materials

All organic and inorganic chemicals were of analytical grade and were purchased from Merck (Darmstadt, FRG), Sigma (St. Louis, MO), and Fluka (Buchs, Switzerland). Products for tissue culture were purchased from Gibco (New York, NY) and [4-^{14}C]cholesterol and [2-^{3}H]-glycerol were from Amersham (Amersham, UK). 12-Myristate 13-acetate (PMA) was purchased from Sigma and stored at −20°C in absolute ethanol. Silica gel G high performance thin-layer chromatography glass plates (HPTLC 60) were obtained from Merck.

Cell culture

Artery smooth muscle cells from medial explants of pig heart coronaries were subcultured, propagated, and harvested as described previously (22). They were grown at 37°C in Dulbecco's modified Eagle's medium supplemented with nonessential amino acids (MEM), 1 mM sodium pyruvate, 100 IU per ml penicillin G, 100 μg per ml streptomycin, 2.5 μg per ml fungizone, 0.38% sodium bicarbonate, and 5% fetal calf serum in a humidified atmosphere of 95% air–5% CO_2. The cells were used after the fourth passage. Macrophages IC-21 were a gift from Dr J. Mauël, Institut de Biochimie (Lausanne, Switzerland). They were grown as the smooth muscle cells except that the medium was supplemented with arginine (0.2%), glutamine (0.8%), asparagine (0.36%), and folate (0.006%); the concentration of fetal calf serum was 10%.

Preparation of oleic acid

Oleic acid (800 mM) was prepared in absolute ethanol, diluted to 8 mM under sonication (Branson B-30 Sonifier, microtip No 2, 3 × 1 min) in a solution of fatty acid-free bovine serum albumin (14%) at pH 8.0, and filtered (0.22 μm). Free fatty acid concentrations were measured after filtration with the NEFAC kit from Wako Chemicals (Dallas, TX). Protein concentrations were determined by the method of Lowry et al. (23). The oleic acid/albumin ratio was 4. The solution was diluted to a final concentration of 400 μM oleic acid in the culture medium.

Measurement of the incorporation of labeled precursors into cholesteryl esters and triglycerides

[4-^{14}C]Cholesterol and [2-^{3}H]glycerol in absolute ethanol were added to the cell culture medium to a final concentration of 0.1 μCi per ml and 0.5 μCi per ml, respectively (final concentration of ethanol was 0.05%). The cells were harvested by trypsinization, washed in 10 ml of Dulbecco's phosphate-buffered saline, and counted with an Industrial D Coulter Counter. The total lipids of the cells were extracted according to the method described by Folch, Lees, and Sloane Stanley (24), applied with a Camag Linomat III on Silica gel G thin-layer chromatography plates with the appropriate standards and developed (2 times, 10 min) in hexane–ether–acetic acid 90:10:1 (v/v/v). The plates were dried, charred according to the technique of Schmitz and Assman (25) by dipping into a solution containing 0.6 g of $MnCl_2$ in 50 ml methanol–water–sulfuric acid 150:30:6 (v/v/v), dried, and heated at 150°C for 1.5 min on a Desaga HP-thermoplate. The spots corresponding to cholesterol, cholesteryl esters, and triglycerides were scraped off and the radioactivity was measured in a liquid scintillation counter (Beckman LS 6800 with automatic compensation).

Measurement of the cell lipid content

The lipid classes were separated on silica gel G thin-layer chromatography plates as described above except that, before the lipid was applied, the plates were washed in absolute methanol, dried, and washed in the developing solvent system. After charring, the cholesterol, cholesteryl ester, and triglyceride spots were quantified by fluorescence scanning at 366 nm using a Camag TLC scanner (25). The fluorescence was found to increase linearly with the amount of lipids applied on the plates in ranges varying between 5 and 50 ng, 10 and 100 ng, and 50 and 350 ng for cholesterol, cholesteryl esters, and triglycerides, respectively.

RESULTS

Effect of oleic acid on smooth muscle cells

Incubation of cells with oleic acid for 72 h was found to increase the amount of intracellular free [4-^{14}C]cholesterol from 69 ± 3 to 107 ± 6 dpm per 10^6 cells

Downloaded from www.jlr.org by guest, on June 18, 2012

TABLE 1. Cholesterol, cholesteryl ester, and triglyceride content of smooth muscle cells and IC-21 macrophages: effect of oleic acid (400 μM) in the culture medium

	Basal Medium	Basal Medium + Oleic Acid
	$\mu g/10^6$ cells	
Smooth muscle cells		
Cholesterol	20.6 ± 2.2 (17)[a]	22.3 ± 2.1 (11)
Cholesteryl ester	0.920 ± 0.160 (9)	1.00 ± 0.13 (10)
Triglyceride	1.76 ± 0.16 (12)	110 ± 18 (12)[b]
Macrophages		
Cholesterol	5.28 ± 0.49 (10)	6.29 ± 0.61 (20)
Cholesteryl ester	0.028 ± 0.002 (6)	0.252 ± 0.062 (9)[c]
Triglyceride	0.151 ± 0.016 (4)	31.3 ± 4.7 (7)[b]

[a]Values given as mean ± SEM of the number of experiments in parentheses.
[b]$P < 0.001$ vs basal values.
[c]$P < 0.005$ vs basal values.

($\times 10^3$) (1.6-fold) and to stimulate the conversion of [4-^{14}C]cholesterol into cholesteryl esters from 402 ± 73 to 1028 ± 102 dpm per 10^6 cells (2.6-fold) and that of [2-^{3}H]glycerol into triglycerides from 4.6 ± 0.4 to 180 ± 16 dpm 10^3 cells ($\times 10^3$) (29-fold) (see Figs. 1-3).

As shown in **Table 1**, oleic acid for 72 h did not change the free cholesterol and cholesteryl ester content of smooth muscle cells but it increased the triglyceride content 63-fold.

Effect of oleic acid on macrophages

Oleic acid for 72 h had no effect on the amount of intracellular free [4-^{14}C]cholesterol but stimulated the conversion of [4-^{14}C]cholesterol into cholesteryl esters from 116 ± 16 to 373 ± 58 dpm per 10^6 cells and that of [2-^{3}H]glycerol into triglycerides from 7.7 ± 1.8 to 38 ± 2 dpm per 10^6 cells ($\times 10^3$) (3.2-fold) ($P < 0.005$) and 7.3-fold ($P < 0.001$), respectively (results not shown).

As shown in Table 1, oleic acid for 72 h did not change the cellular free cholesterol content of macrophages but increased the cholesteryl ester and triglyceride content 9-fold and 207-fold, respectively.

Effects of PMA on smooth muscle cells

In preliminary experiments, different concentrations of PMA were tested for their effects on the conversion of [4-^{14}C]cholesterol into cholesteryl esters and on that of [2-^{3}H]glycerol into triglycerides in smooth muscle cells and in macrophages. It was found that 100 ng/ml of PMA elicited a maximal stimulatory effect. This concentration was thus chosen for all subsequent studies.

As shown in **Fig. 1**, in smooth muscle cells under basal conditions, PMA increased the amount of free [4-^{14}C]-cholesterol recovered in the cells with a maximal effect of 1.6-fold after 3 h. In the presence of oleic acid, PMA increased the amount of intracellular free [4-^{14}C]cholesterol with a maximal effect of 1.8-fold after 72 h. As shown in **Fig. 2**, under basal conditions, PMA stimulated the conversion of [4-^{14}C]cholesterol into cholesteryl esters 6.4- and 2.1-fold, after 3 h and 24 h, respectively, but it had no detectable effect after 72 h. In the presence of oleic acid, PMA stimulated the conversion of [4-^{14}C]cholesterol into cholesteryl esters 1.5-, 5.3-, and 2.6-fold after 3 h, 24 h, and 72 h, respectively. As shown in **Fig. 3**, under basal conditons, PMA stimulated the conversion of [2-^{3}H]glycerol into triglycerides 4.5-fold after 3 h, but it had no detectable effect after 24 h and 72 h. In the presence of oleic acid, PMA stimulated the conversion of [2-^{3}H]glycerol into triglycerides 1.7-, 2.3-, and 1.7-fold, after 3 h, 24 h, and

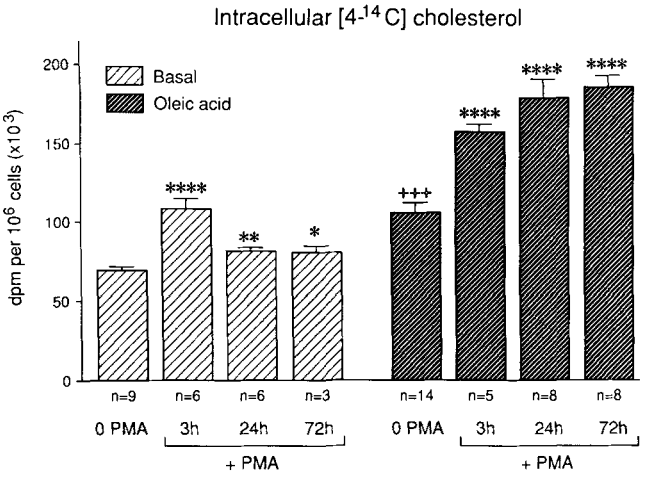

Fig. 1. Effect of PMA (100 ng/ml) on the amount of [4-^{14}C]cholesterol recovered in smooth muscle cells under basal conditions or in the presence of 400 μM oleic acid. The results represents the mean ± SEM of the number (n) of experiments. The cells were exposed to [4-^{14}C]cholesterol and [2-^{3}H]glycerol and, when specified, to oleic acid 3 days before they were harvested. PMA was either present throughout the 3 days or added 24 h or 3 h before harvesting. $^{+++}P < 0.001$ vs control without oleic acid; $^{*}P < 0.05$; $^{**}P < 0.01$ $^{***}P < 0.005$; $^{****}P < 0.01$ vs respective control without PMA.

Downloaded from www.jlr.org by guest, on June 18, 2012

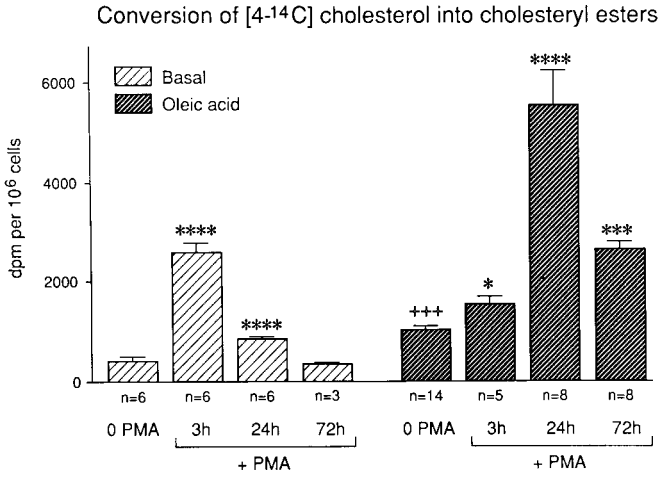

Fig. 2. Effect of PMA (100 ng/ml) on the incorporation of [4-^{14}C]cholesterol into cholesteryl esters by smooth muscle cells under basal conditons or in the presence of 400 μM oleic acid. The results represent the mean ± SEM of the number (n) of experiments. The cells were exposed to [4-^{14}C]cholesterol and [2-^{3}H]glycerol and, when specified, to oleic acid 3 days before they were harvested. PMA was either present throughout the 3 days or added 24 h or 3 h before harvesting. $^{+++}P < 0.001$ vs control without oleic acid; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$; $^{****}P < 0.001$ vs respective control without PMA.

72 h, respectively. It is noteworthy that the effects of PMA on all the above mentioned parameters were maximal after 3 h under basal conditons and only after 24 h in the presence of oleic acid. These effects were generally abolished after 72 h under basal conditions whereas they were still present after 72 h in the presence of oleic acid.

The intracellular content of cholesteryl esters and triglycerides was measured after 24 h and 72 h of PMA treatment under basal conditions or in the presence of oleic acid. The only effects of PMA observed were 2.0-fold increases after 24 h ($P < 0.05$) and after 72 h ($P < 0.005$) in the accumulation of triglycerides in the presence of oleic acid.

Effects of PMA on macrophages

Contrary to what was observed in smooth muscle cells, in macrophages there was no effect of PMA on the conversion of [4-^{14}C]cholesterol into cholesteryl esters and on that of [2-^{3}H]glycerol into triglycerides under basal conditions (results not shown). As illustrated in **Fig. 4**, in the presence of oleic acid, PMA stimulated the conversion of [4-^{14}C]cholesterol into cholesteryl esters 1.9-fold after 72 h and that of [2-^{3}H]glycerol into triglycerides 1.7-fold after 24 h and 72 h.

When the intracellular content of cholesteryl esters and triglycerides was measured after 72 h of PMA treatment, the only effect observed was a 1.9-fold increase ($P < 0.01$) in the accumulation of triglycerides in the presence of oleic acid.

DISCUSSION

The first aim of this work was to study the effect of PMA on the lipid storage pathways of cultured smooth muscle cells and macrophages IC-21. The results show that, in smooth muscle cells, PMA causes in 3 h a marked increase in the conversion of [4-^{14}C]cholesterol into cholesteryl esters and of [2-^{3}H]glycerol into triglycerides under basal conditions. A stimulatory effect of PMA on cholesteryl ester synthesis was still observed after 24 h but was less marked than after 3 h. In macrophages, contrary to what was observed in smooth muscle cells, PMA did not affect the conversion of [4-^{14}C]cholesterol into cholesteryl esters and that of [2-^{3}H]glycerol into triglycerides. These results show that PMA, and possibly growth factors, might control the lipid storage pathways in smooth muscle cells.

The second aim was to check the hypothesis that intracellular lipid accumulation might interfere with effects of PMA. The condition chosen to load the cells with lipids was the addition to the culture medium of an excess (400 μM) of oleic acid complexed to albumin. The observed effects, of exposure of the cells to fatty acids, agreed with those in the literature, i.e., a marked intracellular accumulation of triglycerides in cultured smooth muscle cells (8, 9) and in macrophages (9, 10) and an increase in the incorporation of [4-^{14}C]cholesterol into cholesteryl esters in these two cell types (11, 12). The study furthermore showed that the oleic acid-induced increase in cholesteryl ester synthesis

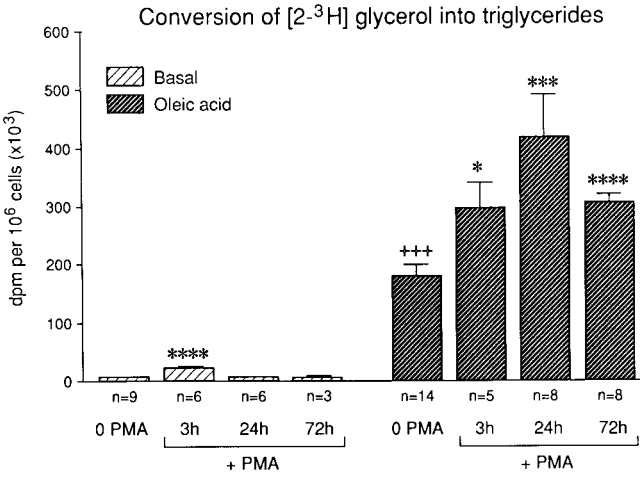

Fig. 3. Effect of PMA (100 ng/ml) on the incorporation of [2-^{3}H]glycerol into triglycerides by smooth muscle cells under basal conditions or in the presence of 400 μM oleic acid. The results represent the mean ± SEM of the number (n) of experiments. The cells were exposed to [4-^{14}C]cholesterol and [2-^{3}H]glycerol and, when specified, to oleic acid 3 days before they were harvested. PMA was either present throughout the 3 days or added 24 h or 3 h before harvesting. $^{+++}P < 0.001$ vs control without oleic acid; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$; $^{****}P < 0.001$ vs respective control without PMA.

Downloaded from www.jlr.org by guest, on June 18, 2012

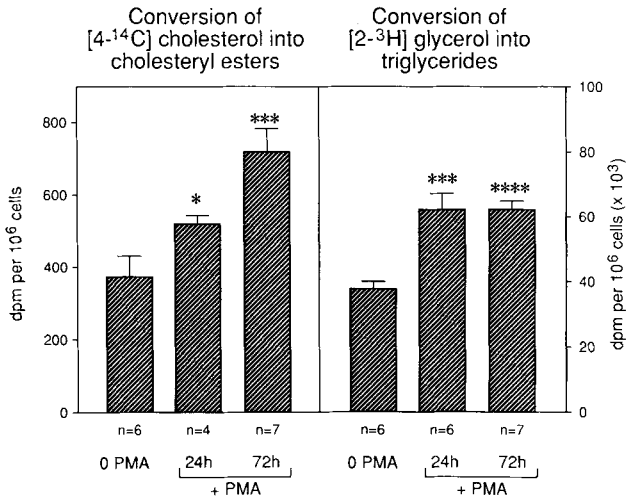

Fig. 4. Effect of PMA (100 ng/ml) on the incorporation of [4-^{14}C]cholesterol into cholesteryl esters and on the incorporation of [2-^{3}H]glycerol into triglycerides by macrophages IC-21 in the presence of 400 μM oleic acid. The results represent the mean ± SEM of the number (n) of experiments. The cells were exposed to [4-^{14}C]cholesterol, [2-^{3}H]glycerol, and oleic acid 3 days before they were harvested. PMA was either present throughout the 3 days or added 24 h before harvesting. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$; $^{****}P < 0.001$ vs respective control without PMA.

in 72 h resulted in an accumulation of cholesteryl esters in macrophages. The concentration of oleic acid used in this study was in the physiological range of circulating FFA in humans. This concentration is probably rather high compared to that in the extracellular fluid of the arterial intima in vivo. These results, nevertheless, suggest that an increase not only in circulating LDL but also in FFA might play a role in foam cell formation.

The response to PMA of the cells grown in the presence of oleic acid differed in two respects from that of the cells under basal conditions. First, the stimulatory effects of PMA on the conversion of [4-^{14}C]cholesterol into cholesteryl esters and of [2-^{3}H]glycerol into triglycerides in smooth muscle cells were more sustained in the presence of oleic acid than under basal conditions (still significant after 72 h) and their maxima were delayed (peaks after 24 h). Second, stimulatory effects of PMA on the synthesis of cholesteryl esters and triglycerides were observed in macrophages only in the presence of oleic acid. Thus, an excess of fatty acids and/or the intracellular accumulation of lipids seem to favor long lasting effects of PMA in smooth muscle cells and are necessary for any effect of PMA in macrophages.

The stimulatory effect of oleic acid or of PMA on the incorporation of [2-^{3}H]glycerol into triglycerides was correlated with an increase in the triglyceride content of the cell in smooth muscle cells and in macrophages. The stimulatory effect of oleic acid or of PMA on the incorporation of [4-^{14}C]cholesterol into cholesteryl esters, however, was not correlated with an increase in the cholesteryl ester content of the cell in smooth muscle cells. This suggests that the amount of cholesteryl ester synthesized in 3 days represents only a small portion of the cholesteryl ester present in the cell. Thus, the increase in cholesteryl ester synthesis is not reflected by a detectable increase above basal level of the cellular cholesteryl ester content. In macrophages, probably because the basal level of cholesteryl esters is small compared to that of smooth muscle cells (see Table 1), oleic acid induced an increase in the cholesteryl ester content of the cell. In these same cells however, PMA, whose effect was seen only after 72 h, did not promote any increase in the cholesteryl ester content. The induction by PMA of an increase in the cholesteryl ester content of smooth muscle cells and of macrophages might necessitate repeated exposure to the drug.

This is the first time that an effect of PMA on the lipid storage pathways of smooth muscle cells and macrophages has been described. The only report in the literature on similar effects is that of Leslie, Antonidades, and Geyer (26) which showed that PDGF stimulates [^{3}H]oleic acid incorporation into cholesteryl esters and phospholipids in human skin fibroblasts.

The mechanism of the small but significant stimulatory effects of oleic acid and of PMA on the uptake of [4-^{14}C]-cholesterol by smooth muscle cells is not clear and deserves further study. The observed effects indicate some specificity in the process of cholesterol adsorption and/or uptake. This suggests that part of the [4-^{14}C]cholesterol added to smooth muscle cells is incorporated in the lipoproteins of the fetal calf serum and penetrates into the cells via the LDL receptor. Since PMA has been shown to induce LDL receptor synthesis in a human monocytic cell line (17), the stimulatory effect of PMA on [4-^{14}C]cholesterol uptake by smooth muscle cells could be due to an increase by PMA of the LDL receptor number in these cells. The effect of PMA on [4-^{14}C]cholesterol uptake is too small to explain completely the large effects of PMA on the incorporation of [4-^{14}C]cholesterol into cholesteryl esters. This latter effect, as well as that on the conversion of [2-^{3}H]glycerol into triglycerides, might involve either the activation of acyl-CoA: cholesterol acyltransferase and acyl-CoA:glycerophosphate acyltransferase activities, or the inhibition of cholesteryl ester esterase and triglyceride lipase activities, or both. Further studies would be necessary to define precisely the mechanism of action of PMA. It would be interesting to try to determine whether the changes in the responsiveness to PMA observed in the presence of oleic acid are due to a modification in the properties of the protein kinase C and/or in the effect of protein kinase C on target enzymes of lipid metabolism.

Other messenger systems could be involved in the regulation of lipid metabolism in smooth muscle cells and in macrophages. It has recently been shown, in fact, that in

Downloaded from www.jlr.org by guest, on June 18, 2012

J774 monocytes dibutyryl-cyclic AMP decreases oleic acid incorporation into cholesteryl esters and increases oleic acid incorporation into triglycerides (27).

Despite the fact that cholesteryl esters are found mainly in the atherosclerotic lesion, at least in the aorta (2, 3), triglyceride accumulation may be involved in the initial stage of the development of the lesion. In the coronary arteries, triglyceride accumulation is part of the atherosclerotic lesion (4). The capacity of smooth muscle cells and macrophages to accumulate triglycerides is striking, and it is likely that if such an accumulation occurs in vivo it would lead to metabolic changes in the cells.

Taken together, the results suggest that growth factors might play a stimulatory role in cholesteryl ester and triglyceride synthesis in smooth muscle cells and in macrophages during the development of atherosclerosis and that elevated circulating FFA might exert a permissive effect.

We greatly appreciate the excellent technical help of Miss C. Colomb. This work was supported in part by grants from Carlos et Elsie de Reuter Foundation, Sandoz Stiftung zur Förderung des Medizinisch-Biologischen Wissenschaften, Fondation du Centenaire de la Société Suisse d' Assurances Générales sur la Vie Humaine pour la Santé Publique et les Recherches Médicales, and Fondation Suisse de Cardiologie.

Manuscript received 11 July 1989 and in revised form 29 September 1989.

REFERENCES

1. Ross, R. 1986. The pathogenesis of atherosclerosis: an update. *N. Engl. J. Med.* **314:** 488–500.
2. Katz, S. S., and D. M. Small. 1980. Isolation and partial characterization of the lipid phases of human atherosclerotic plaques. *J. Biol. Chem.* **255:** 9753–9759.
3. Lundberg, B. 1985. Chemical composition and physical state of lipid deposits in atherosclerosis. *Atherosclerosis.* **56:** 93–110.
4. Reinilä, A., H. K. Akerblom, and R. O. Scow. 1980. Accumulation of lipid in muscular arteries of short-term diabetic rats. An electron microscopic study. *Diabetologia.* **19:** 529–534.
5. Stein, O., J. Vanderhoek, and Y. Stein. 1977. Cholesterol ester accumulation in cultured aortic smooth muscle cells. *Atherosclerosis.* **26:** 465–482.
6. Leake, D. S., and T. J. Peters. 1982. Lipid accumulation in arterial smooth muscle cells in culture. Morphological and biochemical changes caused by low density lipoproteins and chloroquine. *Atherosclerosis.* **44:** 275–291.
7. Klimov, A. N. 1988. In vivo formation of modified lipoproteins with increased affinity for macrophages. *Atheroscler. Rev.* **17:** 75–86.
8. Laughton, C. W., D. L. Ruddle, C. J. Bedord, and E. L. Alderman. 1988. Sera containing elevated nonesterified fatty acids from patients with angiographically documented coronary atherosclerosis cause marked lipid accumulation in cultured human arterial smooth muscle-derived cells. *Atherosclerosis.* **70:** 233–246.
9. Bergstraesser, L. M., and S. R. Bates. 1988. Comparison of fatty acid and triacylglycerol metabolism of macrophages and smooth muscle cells. *Lipids.* **23:** 641–646.
10. Von Hodenberg, E., J. C. Khoo, D. Jensen, J. L. Witztum, and D. Steinberg. 1984. Mobilization of stored triglycerides from macrophages as free fatty acids. *Arteriosclerosis.* **4:** 630–635.
11. Stein, O., G. A. Coetzee, and Y. Stein. 1980. Modulation of cytoplasmic cholesteryl ester of smooth muscle cells in culture derived from rat, rabbit and bovine aorta. *Biochim. Biophys. Acta.* **620:** 538–549.
12. McCloskey, H. M., J. N. Glick, A. C. Ross, and G. H. Rothblat. 1988. Effect of fatty acid supplementation on cholesterol and retinol esterification in J774 macrophages. *Biochim. Biophys. Acta.* **963:** 456–467.
13. Habenicht, A. J. R., J. A. Glomset, and R. Ross. 1980. Relation of cholesterol and mevalonic acid to the cell cycle in smooth muscle and swiss 3T3 cells stimulated to divide by platelet-derived growth factor. *J. Biol. Chem.* **255:** 5134–5140.
14. Fairbanks, K. P., L. D. Witte, and D. S. Goodman. 1986. Effects of platelet-derived growth factor on 3-hydroxy-3-methylglutaryl Coenzyme A reductase activity in cultured human fibroblasts. *Arteriosclerosis.* **6:** 34–41.
15. Chait, A., R. Ross, and E. L. Bierman. 1988. Stimulation of receptor-dependent and receptor-independent pathways of low-density lipoprotein degradation in arterial smooth muscle cells by platelet-derived growth factor. *Biochim. Biophys. Acta.* **960:** 183–189.
16. Aviram, M. 1989. Platelet secretory products enhance LDL receptor activity and inhibit scavenger receptor activity in human monocyte-derived macrophages. *Metabolism.* **38:** 425–430.
17. Auwerx, J. H., A. Chait, G. Wolfbauer, and S. S. Deeb. 1989. Involvement of second messengers in regulation of the low-density lipoprotein receptor gene. *Mol. Cell. Biol.* **9:** 2298–2302.
18. Auwerx, J. H., S. Deeb, J. D. Brunzell, G. Wolfbauer, and A. Chait. 1989. Lipoprotein lipase gene expression in THP-1 cells. *Biochemistry.* **28:** 4563–4567.
19. Nishizuka, Y. 1984. The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature.* **308:** 693–698.
20. Nishizuka, Y. 1986. Studies and perspectives of protein kinase C. *Science.* **23;** 305–309.
21. Mauel, J., and V. Defendi. 1971. Infection and transformation of mouse peritoneal macrophages by simian virus 40. *J. Exp. Med.* **134:** 335–350.
22. Stout, R. W., E. L. Bierman, and R. Ross. 1975. Effect of insulin on the proliferation of cultured primate arterial smooth muscle cells. *Circ. Res.* **36:** 319–326.
23. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurment with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
24. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
25. Schmitz, G., and G. A. Assmann. 1984. A quantitative densitometric method for the rapid separation and quantification of the major tissue and lipoprotein lipids by high performance thin-layer chromatography. I. Sample preparation, chromatography, densitometry. *J. Chromatogr.* **307:** 65–79.
26. Leslie, C. C., H. N. Antoniades, and R. P. Geyer. 1982. Stimulation of phospolipid and cholesterol ester synthesis by platelet-derived growth factor in normal and homozygous familial hypercholesterolemia human skin fibroblasts. *Biochim. Biophys. Acta.* **711:** 290–304.
27. Houtia, N. E., J. C. Mazière, C. Mazière, M. Auclair, L. Mora, J. Gardette, and J. Polonovski. 1987. Dibutyryl-cyclic AMP inhibits cholesterol esterification in J774 monocyte-like cells. *Biochem. Biophys. Res. Commun.* **142:** 120–127.

Downloaded from www.jlr.org by guest, on June 18, 2012