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DISSIMILAR EFFECTS OF 1-OLEOYL-2-ACETYLGLYCEROL AND PHORBOL 12-MYRISTATE 13-ACETATE ON FATTY ACID SYNTHESIS IN ISOLATED RAT-LIVER CELLS

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SUMMARY: Exogenous 1-oleoyl-2-acetylglycerol (OAG) is known to mimic the action of tumour-promoting phorbol esters in various cell types. However, in isolated rat hepatocytes OAG depressed the rate of *de novo* fatty acid synthesis and the activity of the key enzyme acetyl-CoA carboxylase (EC 6.4.1.2), in contrast to the pronounced stimulation of both parameters by phorbol 12-myristate 13-acetate (PMA). The inhibition by OAG appeared to be dose- and time-dependent. On the other hand, medium-chain 1,2-diacylglycerols like 1,2-dioctanoyl-*sn*-glycerol did mimic the stimulatory action of PMA. The anomalous effect of OAG may well be explained by its metabolic breakdown leading to liberation of oleate and subsequent inhibition of acetyl-CoA carboxylase activity by endogenously formed oleoyl-CoA. The stimulatory effects of both PMA and medium-chain diacylglycerols are likely to be mediated by protein kinase C.

Calcium-activated, phospholipid-dependent protein kinase (protein kinase C, PK-C) is widely distributed in mammalian tissues (1) and represents an integral component of the phosphoinositide signal transduction pathway used by various hormones and biologically active compounds (2,3). Small amounts of diacylglycerols dramatically increase the affinity of PK-C for Ca^{2+} as well as for phospholipids, thereby causing activation of PK-C at physiological concentrations of Ca^{2+} (4). As first shown by Castagna *et al.* (5), tumour-promoting phorbol esters such as phorbol 12-myristate 13-acetate (PMA) are able to substitute for endogenous diacylglycerols in activating PK-C in intact cells.

Exogenous diacylglycerols with two long-chain acyl moieties, though being excellent activators of isolated PK-C (6), fail to mimic PMA because they poorly penetrate cell membranes. Therefore, Nishizuka and co-workers (6,7) introduced 1-oleoyl-2-acetyl-*sn*-glycerol (OAG), a synthetic diacylglycerol which is able to intercalate into the plasma membrane and directly activates PK-C without involvement of receptor-generated diacylglycerol (7). As might be anticipated, OAG and PMA exert similar

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Abbreviations: ACC, acetyl-CoA carboxylase; DMSO, dimethylsulfoxide; OAG, 1-oleoyl-2-acetyl-*sn*-glycerol; PDD, 4α-phorbol 12,13-didecanoate; PK-C, protein kinase C; PMA, 4β-phorbol 12β-myristate 13α-acetate.

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effects in various cell types, including release of histamine (8) and arachidonic acid (9), increase in cytoplasmic pH (10), induction of ornithine decarboxylase activity (11), stimulation of DNA synthesis and cell division (12), and inhibition of EGF-receptor interaction (13) and of phenylephrine-induced increase in cytoplasmic free Ca^{2+} (14).

Previously we had already observed a substantial stimulation by PMA of *de novo* fatty acid synthesis (15) and of acetyl-CoA carboxylase (ACC) activity (16) in freshly isolated rat-liver cells. In both cases the dose-response relationship for PMA closely coincided with that found for PMA-induced translocation - and concomitant activation - of PK-C from soluble to particulate fractions of rat hepatocytes (17). To corroborate the involvement of PK-C in these metabolic actions of PMA, the above-mentioned similarity between phorbol esters and OAG led us to use OAG in our experimental system as well.

This communication shows that both the rate of fatty acid synthesis and ACC activity were decreased rather than promoted by OAG. However, medium-chain diacylglycerols like 1,2-dioctanoyl-*sn*-glycerol which were recently reported to be as effective as OAG in activating PK-C *in situ* and to elicit PMA-like effects in A431 cells (18), HL-60 cells (19) and platelets (20), did indeed mimic PMA in our experiments. The results of this study suggest (i) that the adverse effect of OAG is due to its metabolism resulting in inhibition of ACC by oleoyl-CoA; and (ii) that, as far as hepatic fatty acid synthesis is concerned, there is no need to invoke an additional mechanism for the acute metabolic action of PMA independent of PK-C.

MATERIALS AND METHODS

OAG was obtained from Sigma and R 59022 from Janssen. 1,2-Diacyl-*sn*-glycerols (dihexanoyl-, dioctanoyl-, didecanoyl-) were prepared prior to use, by phospholipase C treatment of the corresponding phosphatidylcholines (Sigma) according to (21). In addition, commercially available 1,2-dioctanoyl-*sn*-glycerol (Sigma D5156) was used. Sources of other materials, isolation and incubation of hepatocytes, and measurement of fatty acid synthesis and of cell protein were exactly as described previously (16). Full details concerning the assay of ACC activity in digitonin-permeabilized hepatocytes are given in (22). All incubations were performed in triplicate. The 100%-contro values for fatty acid synthesis and ACC activity were in the range of 0.5 - 2.3 nmol ^3H_2O incorporated/mg cell protein per min and 0.3 - 0.8 nmol malonyl-CoA formed/mg cell protein per min, respectively. Results are presented as means ± S.D. of the number of experiments indicated. Significance was determined using paired *t*-testing.

RESULTS AND DISCUSSION

In line with our previous observations (15) PMA increased the rate of fatty acid synthesis in a dose-dependent fashion, whereas the biologically inactive analogue 4α-phorbol 12,13-didecanoate (PDD) had no significant effect (Fig. 1). In the same hepatocyte preparations the presence of 100 μM OAG caused approx. 25% inhibition, relative to control (DMSO alone), of *de novo* fatty acid synthesis over a 30-min incubation period (Fig. 1). A similar picture emerged when the activity of ACC, the key regulatory enzyme of this pathway, was determined in digitonin-permeabilized hepatocytes. The inhibitory effect of OAG was dose-dependent (Fig. 1) as well as

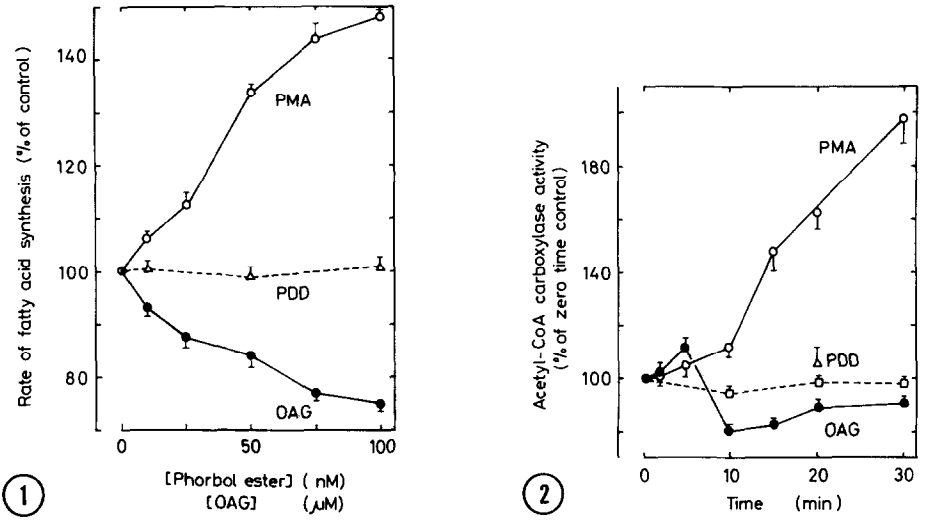

Fig. 1. Influence of agonist concentration on the rate of fatty acid synthesis in isolated rat hepatocytes. Incubation time, 30 min. Open circles, PMA; closed circles, OAG; triangles, PDD. For further information, see Materials and Methods. Values are means ± S.D. of four experiments.

Fig. 2. Time-course of agonist-induced effects on acetyl-CoA carboxylase activity in digitonin-permeabilized isolated rat hepatocytes. Open circles, 100 nM PMA; closed circles, 100 μM OAG; triangle, 100 nM PDD; squares, vehicle (DMSO at 0.1% (v/v) final concentration). After incubation for the indicated time-periods the intact cells were permeabilized prior to determination of ACC activity. For further details, see Materials and Methods. Values are means ± S.D. of three experiments and are expressed as percentages of zero-time control.

time-dependent (Fig. 2). Inhibition of cellular ACC activity by 100 μM OAG was optimal after about 10 min under the conditions specified (Fig. 2).

These data may be interpreted in several ways. Firstly, having in common with OAG the capacity to activate PK-C, tumour-promoting phorbol esters could cause additional effects. In fact, several reports have documented the inability of OAG in various cell types to reproduce effects of PMA on cell differentiation and maturation (23-25), on the phosphoinositide cycle (26,27) and on intracellular redistribution of PK-C (25,28). These additional effects of PMA might involve the activation of protein kinases other than PK-C (29-33) and of phospholipase A_2 (34; cf. 35), or even protein alkylation (36). However, this interpretation would imply that inhibition of ACC activity by OAG is due to PK-C activation, and that the supposed side-effect of PMA is able to overrule this inhibitory action of PK-C. Such an implication - a negative effect on ACC activity of its phosphorylation by PK-C - is not supported by other studies using isolated enzymes (16).

Secondly, one might assume that OAG and PMA have a single mechanism of action, *i.e.* activation of PK-C, and yet yield different results because phorbol esters are metabolized less readily than OAG. Hence activation of PK-C by PMA is bound to be more persistent and, as ACC is associated with microsomes (37) and mitochondria (38), the transient activation of PK-C at the plasma membrane by OAG may be too short-lived and too remote to affect ACC activity. In addition, one might postulate more subtle

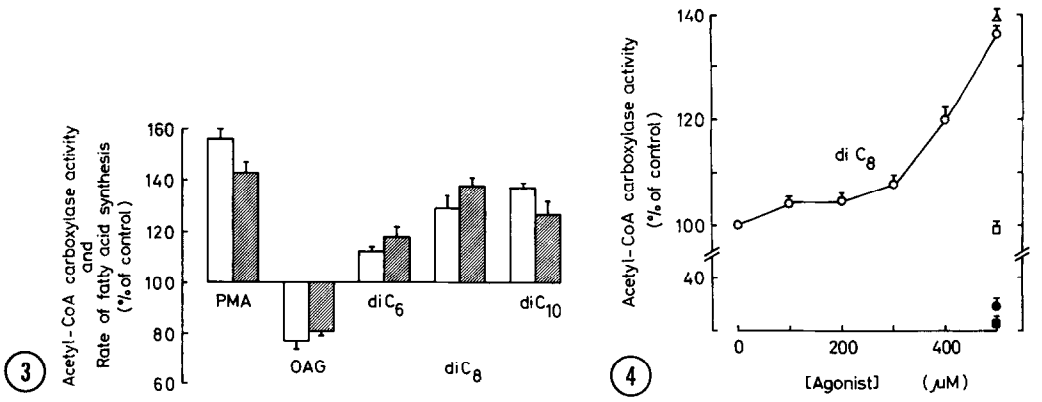

Fig. 3. Comparison of the effects of PMA and 1,2-diacylglycerols. Isolated hepatocytes were incubated for 15 min (ACC activity, open columns) or 30 min (rate of fatty acid synthesis, hatched columns) with 100 nM PMA, 100 μM OAG or 100 μM 1,2-diacyl-*sn*-glycerol (diC_6, 1,2-dihexanoylglycerol; diC_8, 1,2-dioctanoylglycerol; diC_{10}, 1,2-didecanoylglycerol) present. Preparation of diacylglycerols from phosphatidylcholines, see Materials and Methods. In case of ACC activity the hepatocytes were permeabilized at t=15 min followed by the ACC assay. Values are means ± S.D. of two experiments.

Fig. 4. Effect of 1,2-dioctanoylglycerol concentration on acetyl-CoA carboxylase activity in digitonin-permeabilized isolated rat hepatocytes. Incubation time, 20 min (followed by permeabilization and assay procedure). Open circles, 1,2-dioctanoyl-*sn*-glycerol (diC_8, commercial preparation); closed circle, 500 μM OAG; open square, 10 μM R 59022; closed square, OAG + R 59022; triangle, 500 nM PMA. Stock solutions of R 59022 were prepared as in (47). All incubations contained DMSO at 0.5%(v/v) final concentration. Shown are means ± S.D. of triplicate incubations from a representative experiment.

differences between OAG and PMA in that (i) both agents activate different species of PK-C (39), or that PK-C molecules in the presence of OAG or PMA (ii) bind to different sites in the plasma membrane, (iii) have different mobilities, (iv) have a slightly different substrate specificity (cf. 40), or have a different catalytic activity due to dissimilar rates of (v) autophosphorylation (41) and/or (vi) proteolytic cleavage (42). However, in any of these circumstances one would expect to find only quantitative differences between effects of OAG and PMA rather than the opposite effects shown in Figs. 1 and 2.

Thirdly, if activation of PK-C by PMA and OAG leads to stimulation of ACC activity OAG may exert an additional effect overriding this stimulation. The question then arises whether other diacylglycerols mimic OAG. 1,2-Diacylglycerols with two medium-chain acyl moieties were recently reported to be capable of PK-C activation in intact cells (18-20). Using these compounds it turned out (see Fig. 3) that they stimulate, like PMA, the rate of fatty acid synthesis as well as the ACC activity in isolated rat hepatocytes. The degree of stimulation varied from one cell preparation to another the smallest effects being observed in preparations with the highest control values and *vice versa*. The data shown in Fig. 3 and the dose-response plot of 1,2-dioctanoyl-*sn*-glycerol (Fig. 4) represent typical examples of hepatocyte preparations with low and high basal rates of fatty acid synthesis, respectively. In fact, the increment of ACC activity induced by 500 μM 1,2-dioctanoylglycerol ranged from 30 to 110% of

basal ACC activity in our experiments. Nevertheless, despite variations in control values and in the percentage of effect, a stimulatory action of these diacylglycerols was consistently observed, and their relative potency (Fig. 3) agreed well with data from the literature (19,43).

Taken together our data indicate that, at least in case of fatty acid synthesis by isolated rat-liver cells, (*i*) addition of medium-chain 1,2-diacylglycerols mimicks the effects of PMA (cf. 44); (*ii*) inhibitory effects are only observed with OAG; and (*iii*) activation of PK-C is probably instrumental in the acute metabolic effects of both medium-chain diacylglycerols and phorbol esters.

Based on phorbol-ester binding studies with HL-60 cells, Ebeling *et al.* (19) had stated already that medium-chain diacylglycerols are presumably better tools to assess biological effects of diacylglycerols than OAG. Our own results serve to illustrate another drawback of OAG, that is, its metabolic conversion yields products interfering with parameters under investigation. Diacylglycerols will either be rapidly deacylated or be converted into the corresponding phosphatidic acids by diacylglycerol lipase (45) and kinase (46), respectively. As addition of 10 μM R 59022, a diacylglycerol kinase inhibitor (47), did not prevent inhibition of ACC activity by OAG (Fig. 4), the deacylation pathway is likely to be responsible for the anomalous effects of OAG. Endogenous liberation of oleate from the C-1 position of OAG will be followed by formation of oleoyl-CoA which, unlike medium-chain acyl-CoA esters, is a strong inhibitor of ACC activity (48). The delayed inhibition observed with OAG (Fig. 2) and the data in Table 1 provide suggestive evidence in favour of this explanation. Whereas inhibition of fatty acid synthesis and of ACC activity by OAG can be reproduced by an equimolar concentration of oleate, the distinct stimulation of ACC activity by 1,2-dioctanoylglycerol is not found with octanoate (Table 1). The small octanoate-induced increase in fatty acid synthesis may be attributed to an increased supply of carbon precursor via octanoate oxidation (49).

In conclusion, both PMA and 1,2-diacylglycerols are capable to induce short-term activation of ACC in isolated hepatocytes and in neither case there is reason to doubt the involvement of PK-C in signal transduction. However, our results do not necessarily imply a direct phosphorylation of ACC by PK-C, because we cannot exclude the possibility of a PK-C-initiated cascade of reactions eventually leading to ACC activation. The deviating effects of OAG may well be explained by its metabolic degradation and subsequent formation of oleoyl-CoA. OAG metabolism, be it the interference by OAG metabolites or other secondary effects like ATP depletion (50) or the rapid disappearance of OAG itself, may also account for some differences between PMA and OAG reported by others. Yet, at the present state of knowledge it is hard to understand why, *e.g.*, diacylglycerols (OAG as well as 1,2-dioctanoylglycerol) affect phosphatidylcholine metabolism in GH_3 cells (51) and maturation of HL-60 cells (52) differently from phorbol esters. Such discrepancies, as well as the implications of

Table 1. Effects of exogenous 1,2-diacylglycerol and free fatty acid on fatty acid synthesis and acetyl-CoA carboxylase activity in rat hepatocytes

Addition	Percentage of control	
	Rate of fatty acid synthesis	Acetyl-CoA carboxylase activity
(A) OAG	81 ± 5 [a] (n=6)	73 ± 10 [a] (n=7)
oleate	69 ± 6 [a] (n=4)	49 ± 6 [a†] (n=4)
diC_8	126 ± 14 [b] (n=5)	131 ± 15 [a] (n=7)
octanoate	113 ± 8 [c] (n=3)	98 ± 7 (n=4)
(B) OAG	47 (n=1)	37 (n=1)
oleate	48 (n=1)	19 [†] (n=1)
diC_8	118 (n=2)	163 (n=2)
octanoate	113 (n=1)	96 (n=2)

Isolated hepatocytes were incubated for 30 min (fatty acid synthesis) or 15 min (ACC activity) in the basic medium (16) supplemented as indicated with 100 μM (A) or 500 μM (B) of: OAG, oleate, 1,2-dioctanoylglycerol (diC_8) and octanoate. All incubations contained DMSO at (A) 0.1% or (B) 0.5% (v/v) final concentration. Fatty acid synthesis was measured as ^3H_2O-incorporation into non-saponifiable lipids over the 30-min period. In case of ACC activity the hepatocytes were permeabilized at t=15 min prior to a 6-min assay (22) of the carboxylase activity. Data shown in (A) and (B) are means ± S.D. and means, respectively, of triplicate incubations. The number of cell preparations is given in parentheses; 100%-control values, see Materials and Methods.

[a] $P < 0.001$ vs control; [b] $P < 0.01$ vs control; [c] $P < 0.05$ vs control.

[†] As residual oleate is carried over with the permeabilized cells into the ACC-assay medium, some oleoyl-CoA may still be formed during the assay (cf. 22). Its maximal contribution to the indicated % inhibition of ACC activity can be judged from zero-time control assays with added oleate: 82 (A) and 65 (B) % of the ACC activity in zero-time control assays without added oleate.

PMA-induced accumulation of diacylglycerols in hepatocytes (53,54), MDCK kidney cells (55) and Swiss 3T3 cells (56), remain an intriguing challenge for future research.

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