Diradylglycerols Alter Fatty Acid Inhibition of Monoacylglycerol Acyltransferase Activity in Triton X-100 Mixed Micelles†

Rosalind A. Coleman,* Ping Wang, and B. Ganesh Bhat‡

Departments of Nutrition and Pediatrics, The University of North Carolina, Chapel Hill, North Carolina 27599-7400

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ABSTRACT: The activity of hepatic monoacylglycerol acyltransferase (MGAT) (EC 2.3.1.22), a developmentally expressed microsomal enzyme, is inhibited by long-chain fatty acids, and stimulated by its product 1,2-diacyl-*sn*-glycerol. Because the quantities of fatty acids and diacylglycerols are likely to vary in membranes during different physiological conditions and could thereby alter MGAT activity, we examined their combined effects on MGAT in Triton X-100/phospholipid mixed micelles. MGAT's product, 1,2-diC18:1-*sn*-glycerol, which is also normally a cooperative activator of the activity, reversed the 50% inhibition caused by 10 mol % oleic acid. The presence of oleic acid also allowed low concentrations (<10 mol %) of 1,2-diC18:1-*sn*-glycerol to stimulate MGAT activity without the lag that is observed in the absence of fatty acid. At 12.6 mol %, 1,2-monoC18:1-*sn*-glycerol ether, which alone has no effect on MGAT activity, became an activator in the presence of 10 mol % oleic acid. Kinetic studies revealed that in the presence of 15 mol % oleic acid, 1,2-diC18:1-*sn*-glycerol ether increased the apparent V_{max} by 3.8-fold while minimally altering the apparent K_m for palmitoyl-CoA. Other neutral lipids including tri-C18:1-glycerol, ceramide, and cholesterol oleate did not stimulate MGAT in either the presence or the absence of fatty acid. Assay conditions altered MGAT's apparent relative preferences for potential monoradylglycerol substrates. The presence of phospholipids and of MGAT's 1,2-diacyl-*sn*-glycerol product increased the enzyme's apparent preference for its 2-monoacyl-*sn*-glycerol substrate by selectively increasing the apparent V_{max} 2.7-fold only when 2-monoC18:1-*sn*-glycerol was the substrate. Thus, in addition to previously reported regulation of MGAT by phospholipids and intracellular lipid second messengers, these studies lend additional support to the hypothesis that changes in other membrane-associated lipids, such as long-chain fatty acids and diradylglycerols, may also profoundly alter the activity of MGAT.

Monoacylglycerol acyltransferase (MGAT)[1] (EC 2.3.1.22) from rat liver catalyzes the synthesis of 1,2-diacyl-*sn*-glycerol from long-chain acyl-CoAs and 2-monoacyl-*sn*-glycerol (*1*). The partially purified enzyme has been studied extensively in Triton X-100 mixed micelles. These studies suggest that MGAT may be regulated by a variety of specific membrane lipids (*2*−*4*). MGAT has been shown to be activated by anionic phospholipids and lysophospholipids and inhibited by sphingolipids such as sphingosine and sphinganine that contain a free amino group and a long-chain hydrocarbon (*2*, *3*). MGAT's product, 1,2-diacyl-*sn*-glycerol, activates MGAT activity in a highly cooperative fashion, and long-chain fatty acids stimulate MGAT activity at low concentrations and inhibit at concentrations greater than 7.5 mol % (*2*, *4*).

MGAT may play a critical role in lipid metabolism during physiological periods when lipolysis and β-oxidation are stimulated (*5*, *6*), and several of the lipids that activate or inhibit MGAT are known second messengers that are released during hormone-mediated signal transduction. Using Triton X-100/phospholipid mixed micelles, we showed that such potential regulatory lipids include sphingolipids, which are inhibitors, and 1,2-diacyl-*sn*-glycerol, anionic phospholipids, phosphatidic acid, and lysophosphatidic acid, which are activators (*2*−*4*). Phospholipid activators require at least one long-chain fatty acyl group, and sphingolipid inhibitors require a long-chain hydrocarbon, suggesting that the anionic phospholipid headgroup or sphingolipid free amino group must be anchored close to the membrane or micelle surface in order to interact with the enzyme. Further, we have calculated that MGAT is likely to be present in endoplasmic reticulum that contains very low amounts of its monoacylglycerol and acyl-CoA substrates (*4*). Michaelis−Menten kinetics suggest that MGAT's dependence on its acyl-CoA substrate is highly cooperative, and that the presence of long-chain fatty acids significantly decreases MGAT's apparent K_m for palmitoyl-CoA. Thus, relatively small changes in membrane fatty acid content might have important physiological effects on MGAT activity. To better define the conditions under which fatty acids can inhibit

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* Correspondence should be addressed to this author at the Department of Nutrition, CB# 7400, University of North Carolina, Chapel Hill, NC 27599-7400. Phone: (919) 966-7213. Fax: (919) 966-7216.

‡ Current address: MailZone 44B, Monsanto Co., Nutrition and Consumer Products Sector, 800 N. Lindbergh Ave., St. Louis, MO 63167. Telephone: (314) 694-3589.

[1] Abbreviations: BSA, bovine serum albumin; 2-MO, 2-monoC18:1-, *sn*-glycerol; 1,2-DO, 1,2-diC18:1-*sn*-glycerol; 1,2-DOE, 1,2-diC18:1-, *sn*-glycerol ether; DTT, dithiothreitol; PA, phosphatidic acid; PC, phosphatidylcholine; PS, phosphatidylserine; EDTA, (ethylenedinitrilo)-tetraacetic acid; MGAT, monoacylglycerol acyltransferase.

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MGAT activity and to determine whether other lipids might counteract this inhibition, in this study we have examined the interrelationship of long-chain fatty acid and other possible regulatory lipids on MGAT activity in Triton X-100 mixed micelles.

EXPERIMENTAL PROCEDURES

Materials

Oleic acid and all lipids were from Serdary Research Laboratories, Inc., Canada. BSA, DTT, EDTA, and Triton X-100 were from Sigma Chemical Co. Thin layer plates were from Analtech. [^{3}H]Palmitate was from NEN Life Science Products.

Methods

MGAT Assay. Animal use was approved by the Animal Care Committee of the University of North Carolina. MGAT was partially purified from livers of 8-day-old Sprague-Dawley rats obtained from pregnant dams (Zivic-Miller) (*7*). After the hydroxylapatite chromatography step, the solubilized and highly purified enzyme preparation is free of phospholipids (*2*). Aliquots were stored at −70 °C. MGAT was assayed in mixed micelles as described (*4*). Briefly, dried lipids were solubilized in 0.2% Triton X-100 and added to the reaction mixture. Concentrations of each lipid and of the hydrophobic MGAT substrate 2-monoC18:1-*sn*-glycerol are expressed as mole percent, calculated by the equation: 100 × {[added lipid]/([total lipid] + [Triton X-100])} (*8*). MGAT activity was assayed in a 200 μL volume containing 100 mM Tris-HCl (pH 7.0), 0.5 mg/mL BSA, 0.22% Triton X-100 (3 mM micelle concentration), 150 μM 2-MO, 25 μM [^{3}H]palmitoyl-CoA (115 Ci/mol), 0.25−0.5 μg of hydroxylapatite-purified protein, and the indicated concentrations of specific lipids. Concentrations of palmitoyl-CoA, which is water-soluble, are reported as molar concentrations, because we cannot be certain about the amount that partitions into the micelles, particularly in the presence of BSA. Concentrations of palmitoyl-CoA higher than 60 μM were not used because inhibition was observed. After a 5 min incubation at 23 °C, the products were extracted and analyzed (*7*). When necessary, a portion of the heptane extract was chromatographed with carrier lipids on 10 cm silica gel G plates in heptane/isopropyl ether/acetic acid (60:40:4; v/v), and the triacylglycerol and diradylglycerol areas were scraped and counted. All assays contained optimal amounts of substrates and measured initial rates.

Other Methods. To purify 2-MO, 1,2-DO, and tri-C18:1-glycerol, lipids were spotted on a 3% sodium borate TLC plate and chromatographed in $CHCl_3$/acetone/methanol/acetic acid (90:50:2:0.5; v/v). Lipid bands were scraped and extracted twice with 2 mL of $CHCl_3$. After evaporation, the lipid was resuspended in 1.0 mL of hexane and quantified (*9*). Where needed, diC18:1-glycerol ethers were quantified by weight. Protein was measured using BSA as the standard (*10*). [^{3}H]Palmitoyl-CoA was synthesized enzymatically (*11*).

RESULTS

1,2-DiC18:1-sn-glycerol Ether and 1,2-DiC18:1-sn-glycerol Reverse MGAT Inhibition by Oleic Acid. At concentrations from 0.5 to 5.0 mol %, long-chain fatty acid stimulates MGAT activity in mixed micelles, but at higher concentrations, fatty acid inhibits MGAT activity (Figure 1 and (*4*)). Inhibition is about 50% at 10 mol % oleic acid and is virtually complete in the presence of 18 mol % oleic acid.

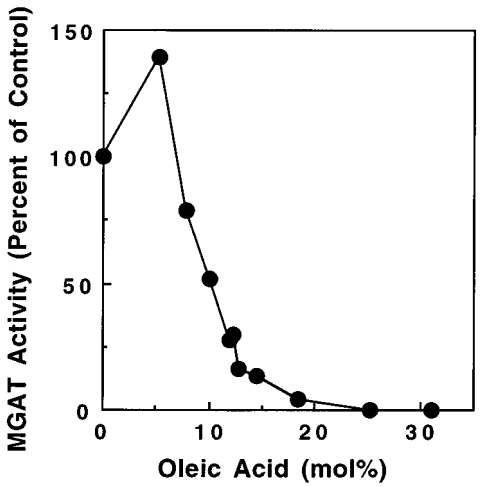

FIGURE 1: Effect of oleic acid on MGAT activity in Triton X-100 mixed micelles. Each point is the average of duplicate determinations. Control activity without oleic acid was 65.7 nmol min^{-1} (mg of protein)$^{-1}$.

In an extensive study of activators and inhibitors of MGAT in the absence of activating phospholipids, we found that the ether analogue of 1,2-DO, 1,2-diC18:1-*sn*-glycerol ether (1,2-DOE), had no effect on MGAT activity (Figure 2A and (*2*)). However, when 10 mol % oleic acid was present, 1,2-DOE at 5 mol % reversed oleic acid's inhibitory effect. Higher concentrations of 1,2-DOE (between 10 and 28 mol %) in the presence of 10% oleic acid stimulated MGAT activity. Thus, 1,2-DOE, which is not normally an activator of MGAT activity, stimulated MGAT almost 4-fold in the presence of a concentration of oleic acid that would otherwise inhibit MGAT activity about 50% (Figures 1 and 2A). Synergy was also observed with 1,2-DO in the presence of 10 mol % oleic acid. In the mixed micelles, 1,2-DO, a cooperative activator of MGAT that stimulates MGAT activity only at concentrations above 10 mol %, was able to stimulate MGAT even at very low concentrations (Figure 2B).

A concentration of 12.6 mol % of 1,2-DOE was required to fully counteract the inhibitory effect of 10 mol % oleic acid (Figure 3A). In contrast, 1,2-DO synergized with the stimulatory effects of low concentrations of oleic acid (2.5−5 mol %), and attenuated, but failed to prevent, the inhibition caused by higher concentrations of oleic acid (Figure 3B).

In contrast to the synergistic effect of 1,2-DO at low concentrations of oleic acid (1−5 mol %) that normally stimulate MGAT activity in mixed micelles, adding 1,2-DOE had little effect (Figure 4A,B). However, 1,2-DOE reversed the potent inhibitory effect of higher concentrations of oleic acid (Figure 5A,B). This combined effect was not due to physical aggregation of the micelles, since no turbidity change was observed at OD_{600nm} (data not shown). Other neutral lipids that do not alter MGAT activity in mixed micelles (ceramide, triC18:1-glycerol, cholesterol oleate) neither stimulated nor inhibited MGAT activity in the presence of oleic acid.

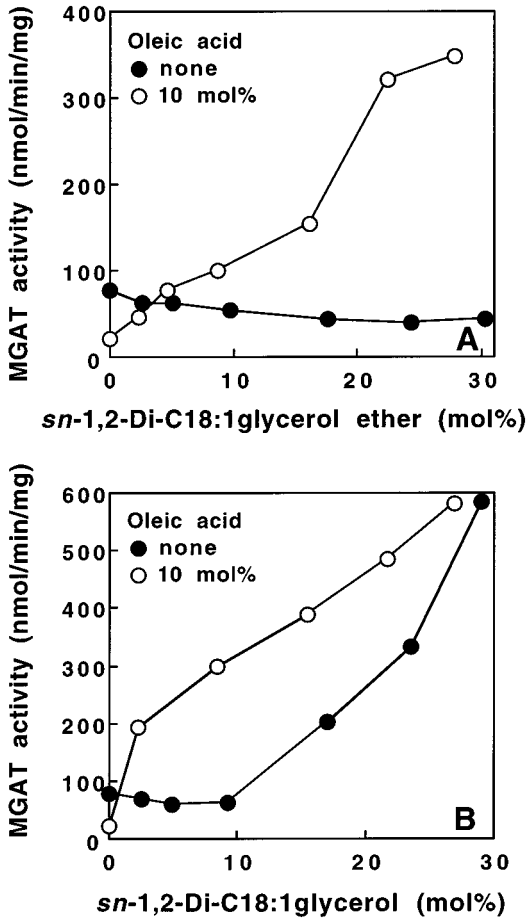

FIGURE 2: Effect of (A) *sn*-1,2-diC18:1-glycerol ether and (B) *sn*-1,2-diC18:1-glycerol on MGAT activity in the presence or absence of oleic acid in Triton X-100 mixed micelles. Assay conditions are described under Experimental Procedures. Each point is the average of duplicate determinations.

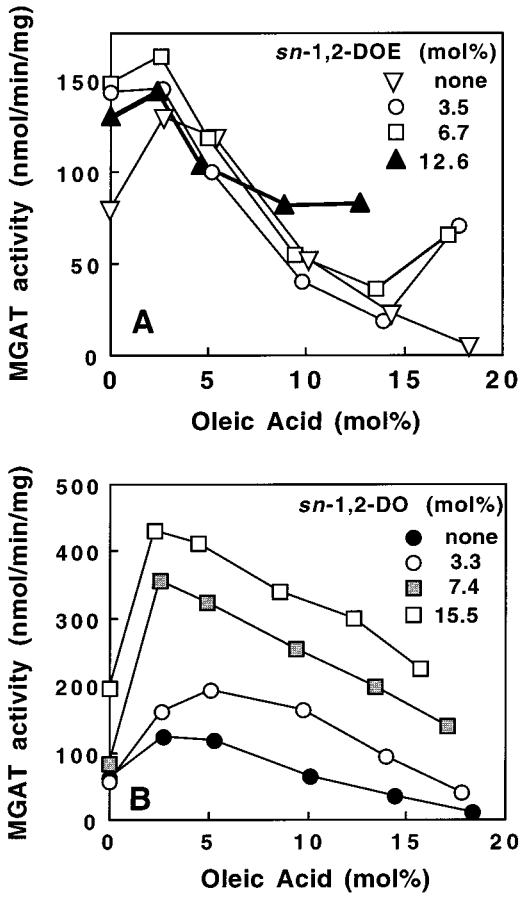

FIGURE 3: Effect of oleic acid on MGAT activity in mixed micelles in the presence of varying amounts of (A) 1,2-DOE and (B) 1,2-DO. Assay conditions are described under Experimental Procedures.

Assay Conditions Alter MGAT's Apparent Relative Substrate Preferences. In previous studies, we showed that hepatic MGAT is highly specific for its 2-monoacyl-*sn*-glycerol substrate and uses 1(3)-monoacylglycerol and the *sn*-1- and *sn*-2-monoalkylglycerol analogues very poorly (*12*). Thus, we were surprised to find that assay conditions appeared to alter MGAT's relative preference for its substrates (Table 1). In a nonmicellar assay in which the lipid substrate was added dispersed in acetone, compared to acylation observed with 2-MO (100%), acylation of 1(3)-MO was poor both with partially purified MGAT (7.3%) and with microsomes (7.5%). In the mixed micellar assay, however, acylation of 1(3)-MO increased to 7.9 and 13.1% for partially purified MGAT and microsomes, respectively. For the ether analogues, the change in preference was even more striking; acylation of 1-MOE and 2-MOE in the mixed micelle assay more than doubled compared with nonmicellar assay conditions. Examination of MGAT specific activities, however, indicated that the changes in relative preference for each substrate were almost entirely due to the effect of the assay conditions on MGAT's ability to use its primary substrate, 2-MO. Thus, the specific activity with 2-MO increased 3.8-fold and 2.6-fold, respectively, when acetone was the dispersing agent (assays 1 and 3) compared with the mixed micellar assay (assays 2 and 4). When detailed dependences on 2-MO were performed under each condition, the apparent K_m values changed minimally, whereas the apparent V_{max} values increased more than 2.7-fold in the nonmicellar assays (data not shown).

To determine which of the assay components was altering MGAT's relative substrate utilization, MGAT in Triton X-100 mixed micelles was assayed using either 2-MO or 1-MOE, together with the various components of the alternative assay (Figure 6). Adding phosphatidylcholine (PC) and phosphatidylserine (PS) doubled the activity observed with the ether analogue, but even when PC and PS were present, acetone severely inhibited acylation of 1-MOE. In contrast, the addition of PC and PS increased MGAT activity with 2-MO 3.9-fold, and the further addition of 1,2-DO increased MGAT activity an additional 60%. Microsomal lipids decreased acylation of 2-MO but not 1-MOE, 30%, and other additions (DTT, EDTA, and acetone) had no specific effect with either substrate. Thus, the apparent change in relative preference for substrates occurred because PC and PS and 1,2-DO increased MGAT activity primarily with 2-MO, and because acetone inhibited MGAT activity with the ether analogue, 1-MOE.

To determine whether known activators of MGAT in mixed micelles would have equivalent effects with both the primary 2-MO substrate and the less preferred 1-MOE substrate, assays were performed using each of these substrates plus other lipids that had previously been shown either to activate MGAT in Triton X-100 mixed micelles or to have little effect (*2*) (Table 2). Anionic phospholipids are the best activators of MGAT in Triton X-100 mixed micelles, PC and PE are very weak activators, TAG has no

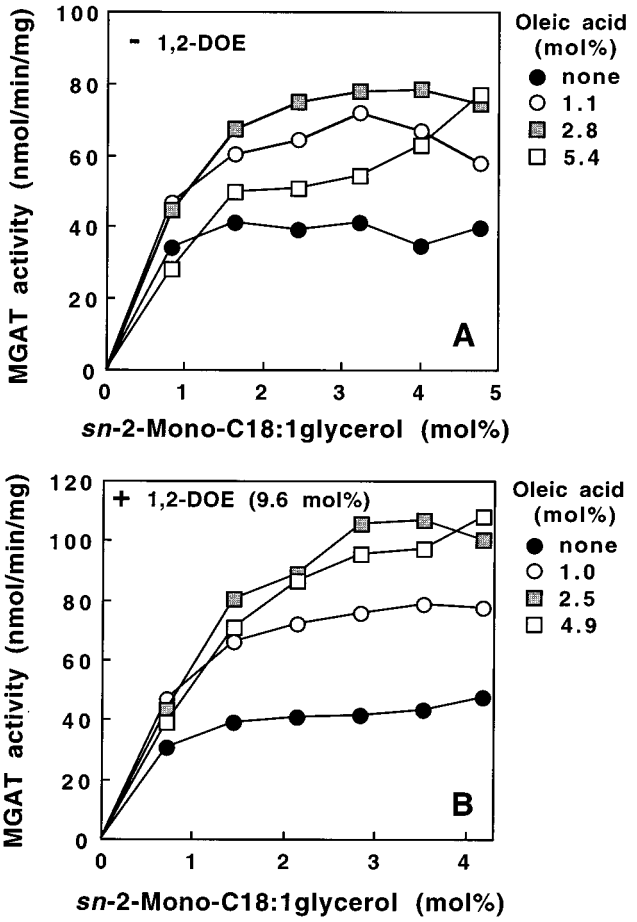

FIGURE 4: Effect of *sn*-1,2-diC18:1-glycerol ether on stimulatory concentrations of oleic acid on the dependence of 2-monoC18:1-*sn*-glycerol. The concentrations of oleic acid were varied as indicated. Determination in the absence (A) or presence (B) of *sn*-1,2-diC18:1-glycerol ether. Assay conditions are described under Experimental Procedures.

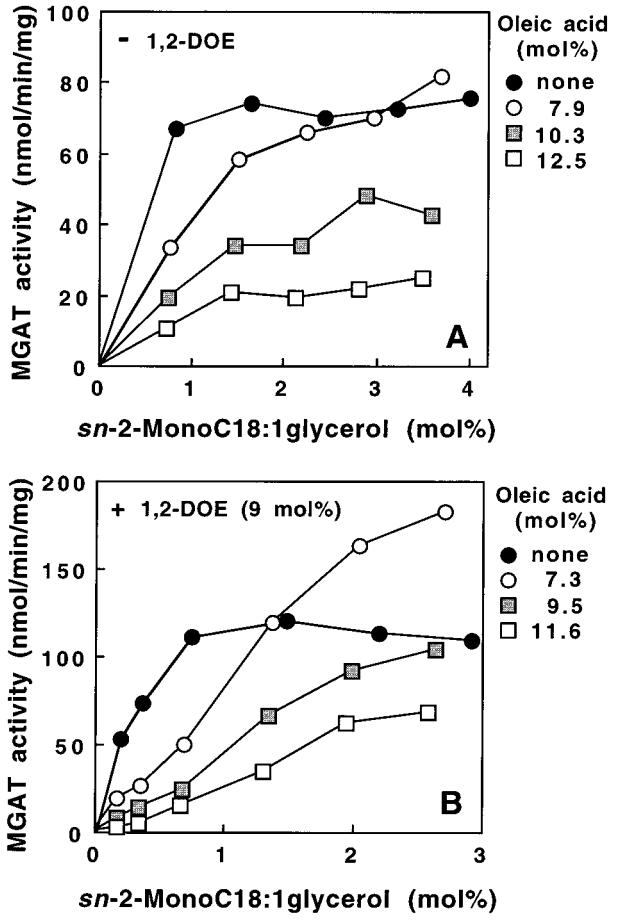

FIGURE 5: Effect of *sn*-1,2-diC18:1-glycerol ether on inhibitory concentrations of oleic acid on the dependence of 2-monoC18:1-*sn*-glycerol. The concentrations of oleic acid were varied as indicated. MGAT activity was measured in the (A) absence or (B) presence of *sn*-1,2-diC18:1-glycerol ether. Each point is the average of duplicate determinations.

effect, 1,2-DO is a good activator only at high mole percent (>10 mol %), and oleic acid at 10−18 mol % is a potent inhibitor (*2*, *4*). Table 2 compares the response of MGAT to each of these lipids when either 2-MO or 1-MOE was used. The potent activators PA and PS were 2.8- and 2.5-fold more effective, respectively, with 2-MO than with 1-MOE as the substrate. PC and PE increased activity about 60% with 2-MO, but had no effect with 1-MOE, and triC18:1-glycerol and 1,2-DO (at low mole percent) were equally ineffective with both substrates. Oleic acid at 10−18 mol % inhibited acylation of both substrates. Like the data shown in Figure 6, these studies also suggested that lipid activators of MGAT were more effective when 2-MO, than when 1-MOE, was the substrate.

Palmitoyl-CoA Dependence Is Affected by Oleic Acid. The dependence of MGAT activity on palmitoyl-CoA concentration showed a sigmoidal curve in the absence of oleic acid, a hyperbolic curve when a concentration of oleic acid that stimulates MGAT (5 mol %) was present, and an inhibited dependence when 15 mol % oleic acid was present (Figure 7A). When these studies were repeated in the presence of 12.6 mol % *sn*-1,2-DOE, there was no change in the palmitoyl-CoA dependence at 0 and 5 mol % oleic acid. However, at 15 mol % oleic acid, the profound inhibition of MGAT activity (compared with 5 mol % oleic acid) was reversed when *sn*-1,2-DOE was present (Figure 7B). Kinetic analysis of the dependences obtained when 15 mol % oleic acid was present showed that the addition of 1,2-DOE altered the apparent K_m very little (14.5−21.5 μM palmitoyl-CoA), whereas it increased the apparent V_{max} 3.8-fold [83−312 nmol min^{-1} (mg of protein)$^{-1}$].

DISCUSSION

The activities of most enzymes that are intrinsic membrane proteins are highly dependent on the lipid milieu of the membrane (*13*), which, in addition to its content of bulk phospholipid and cholesterol, also contains a variety of lipid second messengers. In the case of enzymes that synthesize glycerolipids, the lipid milieu also contains the enzymes' hydrophobic and amphipathic substrates and products, several of which may act as activators or inhibitors of specific enzyme function. Because most of the enzymes in the pathway of triacylglycerol and phospholipid synthesis have resisted purification to homogeneity, many questions remain about their dependences on lipid cofactors and mediators. With highly purified proteins, some of these difficulties can be overcome by examining kinetic functions in mixed micelles. Mixed micelles, comprised of purified enzyme and detergent, provide a surface of known composition that functionally mimics the endoplasmic reticulum bilayer and in which putative activators, inhibitors, and cofactors can

Table 1: Relative Preference for Monoradylglycerol Substrates Depends on Assay Conditions

condition: phospholipids:	assay 1 acetone microsomal		assay 2 mixed micelle microsomal		assay 3 acetone PC/PC		assay 4 mixed micelle PC/PC	
	percent[b]	SA	percent[b]	SA	percent[b]	SA	percent[b]	SA
sn-2-MO	100	1395	100	365	100	993	100	375
1(3)-MO	7.3	102	7.9	29	7.5	75	13.1	49
sn-2-MOE	4.5	62	9.1	33	6.6	66	20.3	76
1(3)-MOE	5.7	79	14.1	52	11.7	116	27.3	103
app K_m (μM) (with 2-MO		187		118		55		74
app V_{max} (nmol min^{-1} mg^{-1}) (with 2-MO)		4380		1610		1065		368

[a] MO, monoC18:1-glycerol; MOE, monoC18:1-glycerol ether; SA, specific activity [nmol min^{-1} mg of protein)$^{-1}$]. Each substrate was used at 100 μM. Assays 1 and 2 contained 150 μg/mL microsomal lipids, 5 mM DTT, 2.5 mM EDTA, 1 mg/mL BSA, 300 μM 1,2-diC18:1-*sn*-glycerol, 25 μM [^{3}H]palmitoyl-CoA, and 0.55 μg of partially purified MGAT. Assays 3 and 4 contained 225 μg/mL PC/PS (1:1; wt/wt), 1 mg/mL BSA, and 25 μM [^{3}H]palmitoyl-CoA. In assays 1 and 3, the monoradylglycerol substrates were added in 5 μL of acetone (5% of final volume), and in assays 2 and 4, the substrates were added in 0.2% Triton X-100 (final concentration was 0.22%). After incubation for 5 min at 23 °C, the reaction was stopped as described under Experimental Procedures, and the products were separated by TLC, scraped, and counted. Each value is the average of duplicate values. K_m and V_{max} values were obtained from dependences on 2-MO in assays using different lots of purified protein preparations. Regression coefficients for these studies were greater than 0.99 for assays 1−3 and greater than 0.967 for assay 4. [b] Percent of activity with *sn*-2-MO.

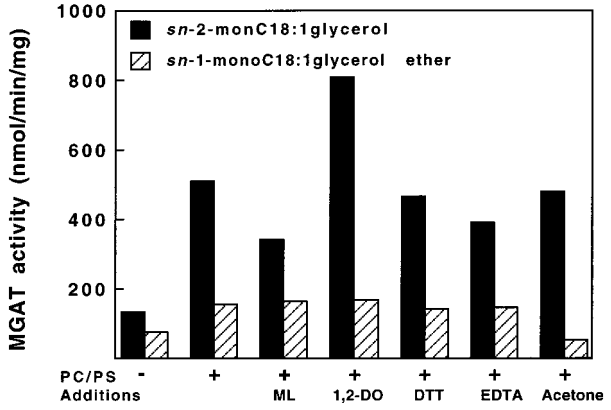

FIGURE 6: Effect of assay components on MGAT activity with the substrates 2-monoC18:1-*sn*-glycerol and 2-monoC18:1-*sn*-glycerol ether. Phosphatidylcholine and phosphatidylserine (225 μg/mL) (PC/PS), microsomal lipids (450 μg/mL) (ML), 1,2-diC18:1-glycerol (8.6 mol %) (1,2-DO), EDTA (5 mM), and acetone (5%) were added as indicated.

be added to study the enzyme's kinetic properties. Since only 1 enzyme molecule and 140 molecules of Triton X-100 are present in each micelle, one can calculate and report the number of lipid activators or inhibitors present as mole percent.

We have used the Triton X-100 mixed micellar system to study highly purified MGAT from neonatal rat liver and have reported that MGAT is activated by 1,2-DO and by anionic phospholipids which increase both the apparent V_{max} and K_m with respect to 2-monoacyl-*sn*-glycerol (*2*). MGAT is inhibited by sphingolipids that contain a free amino group and long-chain hydrocarbon (*3*). This inhibition is competitive with respect to activation by anionic phospholipids, suggesting that the same site may be involved in activation by anionic phospholipids and in inhibition by sphingolipids. Studies of inhibition of MGAT by fatty acids suggest that long-chain fatty acids may induce a conformational change that alters the enzyme's interaction with its acyl-CoA substrate.

The present study carries the kinetic studies of MGAT forward by examining the ability of 1,2-DO and 1,2-DOE to reverse oleic acid's inhibition of MGAT activity. 1,2-DO, a cooperative activator of MGAT with a Hill coefficient of 3.6, cannot be acylated by MGAT but, in the absence of activating phospholipids, stimulates MGAT activity after an initial lag, requiring about 18 mol % 1,2-DO for full activation (*2*).[2] If MGAT has already been activated by the addition of PC and PS, the lag diminishes, and 1,2-DO at only 6 mol % is required for maximal activation (*2*). An even more striking difference was observed in the current study in which the 1,2-DO activation lag disappeared entirely when an inhibitory concentration of oleic acid (10 mol %) was present (Figure 2B). Even more surprising was the effect of 1,2-DOE. Although normally 1,2-DOE neither inhibits nor stimulates, it became an activator when 10 mol % oleic acid was present in the mixed micelle (Figure 2A). These data suggest that regulation of MGAT may be critically altered by small changes in the concentrations of many of the individual lipids that make up the endoplasmic reticulum. MGAT specific activity is highest in liver from 7- to 9-day-old rat pups (*1*). We have calculated that the concentration of nonesterified fatty acid in the hepatic endoplasmic reticulum of 9-day-old rat pups is around 3 mol % (*4*). Although this concentration is well below the amount that inhibits MGAT activity, membrane microdomains that concentrate specific lipid species have been well described (*14*), and it is possible that the endoplasmic reticulum contains regions that are enriched in fatty acids, acyl-CoAs, and/or diradylglycerols.

Several membrane-associated enzymes are activated by fatty acid or phospholipid plus diacylglycerol in a synergistic manner. NADPH oxidase activity increases about 2-fold with phosphatidic acid or with diacylglycerol separately and about 12-fold with the two lipids together (*15*), and anionic phospholipids together with diacylglycerol activate CTP: phosphocholine cytidylyltransferase in small unilamellar vesicles (*16*). Most extensively studied are the various isoforms of protein kinase C, in which synergistic activation has been observed by arachidonic acid plus diacylglycerol (*17*), saturated fatty acids plus diacylglycerol (*18*), phorbol

[2] Because ^{3}H-labeled products are not produced when 1,2-DO, but not 2-MO, is present in the assay mixture, MGAT does not hydrolyze (and reacylate) 1,2-DO.

Table 2: Ability of Different Lipids To Stimulate MGAT Activity When 2-MonoC18:1-*sn*-glycerol or 1(3)-MonoC18:1-glycerol Ether Is the Substrate[a]

substrate	mol % tested	% of control at each mol % tested		
		sn-2-MO (4.76 mol %)	1(3)-MOE (4.76 mol %)	*sn*-2-MO/1(3)-MOE
no addition (control)		100	100	1.0
phosphatidic acid	2.1, 4.2	856, 823	303, 298	2.8
phosphatidylserine	3.9, 7.5	736, 772	306, 302	2.5
phosphatidylcholine	3.9, 7.5	163, 202	111, 114	1.6
phosphatidylethanolamine	4.1, 7.9	158, 235	106, 160	1.5
trioleoylglycerol	3.5, 6.7	96, 87	76, 66	–
dioleoylglycerol	4.9, 9.3	98, 100	82, 66	–
oleic acid	10.1, 18.4	69, 4	0, 0	–

[a] MO, monooleoylglycerol; MOE, monooleylglycerol ether. Effect of phospholipids and neutral lipids on MGAT activity with 2-monoC18:1-*sn*-glycerol and 1(3)-monoC18:1-glycerol ether substrates in Triton X-100 mixed micelles. Control activities (100%) for 2-MO and 1(3)-MOE measured in the absence of lipids were 38.6 and 25.0 nmol min^{-1} mg^{-1}, respectively. Percents of control values are given for each lipid mediator at each of the two concentrations listed in the mol % column.

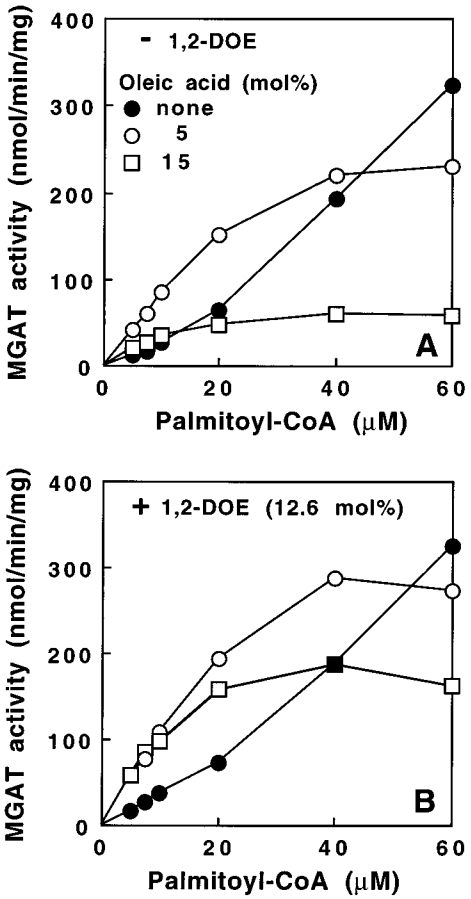

FIGURE 7: Effect of oleic acid on MGAT activity's dependence on palmitoyl-CoA in the (A) absence or (B) presence of 12.6 mol % 1,2-DOE. Assays were performed in Triton X-100 mixed micelles as described under Experimental Procedures.

esters and retinoic acid (*19*), and *cis*-unsaturated fatty acids plus diacylglycerol (*20*). Perhaps most closely related to our observations is the report of a nonactivating phospholipid sparing the requirement for phosphatidylserine and reducing its cooperativity in its ability to activate protein kinase C (*21*).

The second new finding reported here is the ability of the assay condition itself to alter the apparent preference of MGAT for its monoradylglycerol substrates. Hepatic MGAT is highly specific for 2-monoacyl-*sn*-glycerols and is relatively poor at acylating 1(3)-monoacylglycerols and 1,2- and 1(3)-monoalkyl-*sn*-glycerols (less than 11% of the activity observed with 2-monoacyl-*sn*-glycerol) (*1*, *12*). Our present study clearly indicates that the components of the MGAT assay can induce distinct changes in MGAT's relative ability to acylate these different monoradylglycerol substrates. The observed changes in relative preference, however, result from a change in the enzyme's ability to acylate its primary substrate, 2-MO, while having little effect with the alternative substrates. It is as yet unclear how this selective change in preference is effected, that is, how the presence of specific phospholipids or of 1,2-DO might increase MGAT's activity with only one of its four possible monoradylglycerol substrates. However, this observation suggests the possibility that the less restrictive intestinal MGAT isoform (*12*) may only appear to be able to readily acylate the ether analogues of 2-MO because of intrinsic differences in membrane phospholipid composition or the presence of high amounts of diacylglycerol in the endoplasmic reticulum, rather than due to intrinsic differences in substrate affinity. Functional changes in MGAT activity may occur via the ability of different lipid species to interact with specific sites on the enzyme's surface.

Although firm answers to these questions will require structural studies of MGAT, the data reported here clearly suggest that MGAT, and possibly other enzymes of glycerolipid biosynthesis, is exquisitely sensitive to small changes in its membrane composition, and that a variety of putative second messengers and phospholipids could play critical roles in regulating the synthesis of 1,2-diacyl-*sn*-glycerol by MGAT.

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