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Potential role for triglycerides in signal transduction

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Abstract We previously reported that endothelin-1 or platelet-derived growth factor promoted in aortic smooth muscle cells a rapid hydrolysis of 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine (alkyl-PE) which was immediately converted into 1-*O*-alkyl-2,3-diacyl-*sn*-glycerol (alkyl-TG) within 5 s or 60 s respectively [C. Comminges et al. (1996) Biochem. Biophys. Res. Commun. 220, 1008–1013 and C. Comminges et al. (1997) Biochim. Biophys. Acta 1355, 69–80]. In this study, we show that this alkyl-PE hydrolysis is triggered by a transient activation of a specific phospholipase C (PLC) regulated by pertussis toxin-sensitive heterotrimeric G-proteins. Moreover, this PLC can be triggered through a Ca^{2+} influx depending on L-type Ca^{2+} channel activation, as suggested by the use of a specific 'activator' *S*(−)-BayK 8644 and of selective inhibitors such as nimodipine. Interestingly, low concentrations (10^{-8}–10^{-7}M) of alkyl-TG block the opening of L-type Ca^{2+} channels, whereas identical concentrations of DG do not alter L-type Ca^{2+} channels. This study thus unravels a hitherto unrecognized signaling pathway generating alkyl-TG as a novel lipid second messenger, potentially acting as a negative feedback regulator of L-type Ca^{2+} channels.

Key words: Ether triglyceride; Calcium channel; Phospholipase C; Ether phosphatidylethanolamine

1. Introduction

Since the pioneering study of Nishizuka's group [1] describing diacylglycerol as a specific activator of protein kinase C, the last 20 years have seen the emergence of a number of intracellular lipid messengers able to modulate the activity of different targets involved in cell signaling. Generation of these second messengers involves a complex set of intracellular enzymes.

In previous studies, we observed that endothelin-1 (ET-1) or platelet-derived growth factor (PDGF-BB) induced a very rapid conversion of 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine (alkyl-PE) into 1-*O*-alkyl-2,3-diacyl-*sn*-glycerol (alkyl-TG) in pig aortic smooth muscle cells (SMC) [2,3]. Although TG are essentially known for their role in fatty acid storage, their very transient accumulation suggested that we were dealing with a novel signaling pathway, possibly involving alkyl-TG as a new intracellular lipid messenger. This effect did not appear to be restricted to SMC signaling because a similar response was also obtained with RAW 264.7 macrophage-like cells stimulated by silica (unpublished results). The simplest pathway to explain the conversion of alkyl-PE into alkyl-TG would involve the removal of the phosphoethanolamine group by a specific phospholipase C (PLC) and simultaneous acylation of the intermediate alkyl-DG. In this study, PLC activation was followed by [^{3}H]ethanolamine labeling instead of [^{3}H]myristic or [^{3}H]octadecyl [2,3]. Thus, we demonstrate that ET-1 activates a PLC specific for alkyl-PE and suggest that alkyl-TG generated downstream of this novel PLC act as potent inhibitors of L-type Ca^{2+} channels.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), fetal calf serum, glutamine and penicillin/streptomycin were purchased from Gibco BRL (Paisley, UK). [1-^{3}H]Ethan-1-ol-2-amine (25 Ci/mmol) and 1-*O*-[^{3}H]octadecyl-*sn*-glycero-3-phosphocholine (163 Ci/mmol) were from Amersham Pharmacia Biotech (Les Ulis, France). Fura Red™/AM and Pluronic F-127 were from Molecular Probes (Eugene, OR, USA). Nimodipine was from Interchim (Montluçon, France). *S*(−)-BayK 8644, ET-1, ATP (magnesium salt), tricaprin (1,2,3-tridecanoylglycerol), dicaprin (1,2-didecanoyl-*rac*-glycerol), 1-*O*-hexadecyl-*sn*-glycerol and other chemicals were from Sigma (St. Louis, MO, USA).

2.2. Synthesis of 1-O-hexadecyl-2,3-didecanoyl-sn-glycerol

This was classically obtained by coupling decanoic anhydride to 1-O-hexadecyl-*sn*-glycerol in the presence of *N*,*N*′-dicyclohexylcarbodiimide and 4-dimethylaminopyridine (62% yield). The purity of the synthesized product was checked [4].

2.3. Lipid metabolism in stimulated SMC

SMC were obtained from the thoracic aorta of 6-week-old pigs and cultured as described [2]. For experiments, cells between the first and fourth passage were seeded in 60 mm diameter dishes. After 2 days, they were labeled for 18 h in calf serum-free DMEM supplemented with 1 μCi/ml of [^{3}H]ethanolamine or 1-*O*-[^{3}H]hexadecyl-*sn*-glycero-3-phosphocholine as previously described [2]. Cells were then incubated with different pharmacological compounds as indicated, with or without addition of ET-1 (100 nM) or various concentrations of *S*(−)-BayK 8644 or A23187. Alternatively, cell stimulation was achieved by changing the medium to DMEM containing 50 mM KCl. Reactions were blocked by the addition of 1 ml of ice-cold methanol. Cells were scraped and lipids extracted with the Bligh and Dyer method [5] as already mentioned [2]. Major phospholipids and neutral lipids in the organic phase were separated by thin-layer chromatography using two successive runs with chloroform/methanol/acetic acid (90/10/10, v/v) and hexane/diethyl ether/formic acid (55/45/1, v/v). Ethanolamine,

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Abbreviations: ET-1, endothelin-1; PLC, phospholipase C; $[Ca^{2+}]_i$, cytoplasmic free calcium concentration; SMC, smooth muscle cells; alkyl-PE, 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine; alkyl-TG, 1-*O*-alkyl-2,3-diacyl-*sn*-glycerol; PDGF, platelet-derived growth factor; alkyl-DG, 1-*O*-alkyl-2-acyl-*sn*-glycerol; $InsP_3$, inositol 1,4,5-trisphosphate; diC10-alkyl-TG, 1-*O*-hexadecyl-2,3-didecanoyl-*sn*-glycerol; diC10-DG, didecanoyl-*rac*-glycerol

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phosphoethanolamine, and *sn*-glycero-3-phosphoethanolamine were separated using methanol/2 M ammonia/0.6% NaCl (60/40/1, v/v) as a solvent. All the spots were identified, scraped off and quantified [2].

2.4. Inositol 1,4,5-trisphosphate ($InsP_3$) determination

Unlabeled cells were incubated as above in the presence of ET-1 and the reaction was stopped by adding 5% (w/v) trichloroacetic acid. The $InsP_3$ level was then determined using a kit from Amersham, the assay being based on the competition between $InsP_3$ generated by the reaction and radiolabeled $InsP_3$ from the kit according to the manufacturer's instructions.

2.5. Determination of cytoplasmic free calcium concentration ($[Ca^{2+}]_i$)

SMC seeded on glass cover slides were incubated in a buffer containing 137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 8.4 mM HEPES and 5.6 mM glucose, pH 7.4. Cells were then incubated for 45 min at 37°C with 10 μM Fura Red acetoxymethyl ester and 0.2% (v/v) Pluronic F-127 (Molecular Probes, Eugene, OR, USA). Drugs were pressure-injected from an extracellular micropipette, the tip of which was positioned in the vicinity of the recorded cells. The concentrations reported are those in the pressure pipette. $[Ca^{2+}]_i$ changes were routinely measured by a real-time (30 frames/s) confocal laser scanning microscope (Odyssey XL with InterVision 1.5.1 software, Noran Instruments Inc.). Because Fura Red is a single-wavelength dye, its emission is a function of both intracellular Ca^{2+} and dye concentrations. Since fluorescence of Fura Red decreased once the indicator bound Ca^{2+}, $[Ca^{2+}]_i$ changes were first expressed as F/F_{max} ratio. To illustrate $[Ca^{2+}]_i$ rises, the data were then plotted as F/F_{min} ratio after application of the following equation: $F/F_{min} = 2-(F/F_{max})$.

3. Results

3.1. ET-1 induces the rapid activation of a PLC specific for PE closely followed by alkyl-TG production

When cells were challenged with ET-1, we observed a rapid drop in the radioactivity of alkyl-PE with a corresponding increase of [^{3}H]phosphoethanolamine after 5 s (Fig. 1A). No change was detected in the radioactivity of ethanolamine and glycerophosphoethanolamine whose level was 100-fold lower (not shown). Moreover (Fig. 1B), when alkyl-PE were labeled on the alkyl backbone using 1-*O*-[^{3}H]hexadecyl-*sn*-glycero-3-phosphocholine as a precursor [2], the decrease in alkyl-PE was reproduced, and a concomitant increase in alkyl-TG was measured. Both events, release of phosphoethanolamine and increase of alkyl-TG, were closely related (compare Fig. 1A,B). The return to baseline occurring within 30 s was the same for the two labeling conditions. In addition, accumulation of DG and phosphatidic acid occurred later and was 50-fold lower (Fig. 1C).

Because we never observed any DG and phosphatidic acid increase correlated with alkyl-PE decrease, it is likely that the simplest pathway to explain the conversion of alkyl-PE into alkyl-TG requires the removal of the phosphoethanolamine group by a PLC and a simultaneous acylation of the intermediate alkyl-DG.

Other data indicated that alkyl-PE were hydrolyzed by a specific PLC: (i) in the presence of ethanol, there was no formation of [^{3}H]alkyl-phosphatidylethanol, which excluded the involvement of a phospholipase D; (ii) the transient accumulation of phosphoethanolamine was dissociated from the generation of $InsP_3$ by phosphoinositide-specific PLC, which peaked at 60–90 s (Fig. 1D) when compared with 5 s for phosphoethanolamine and alkyl-TG.

Altogether these data provided strong evidence that ET-1 activates a PLC selective for alkyl-PE, the resulting alkyl-DG being immediately acylated into alkyl-TG because they never accumulated.

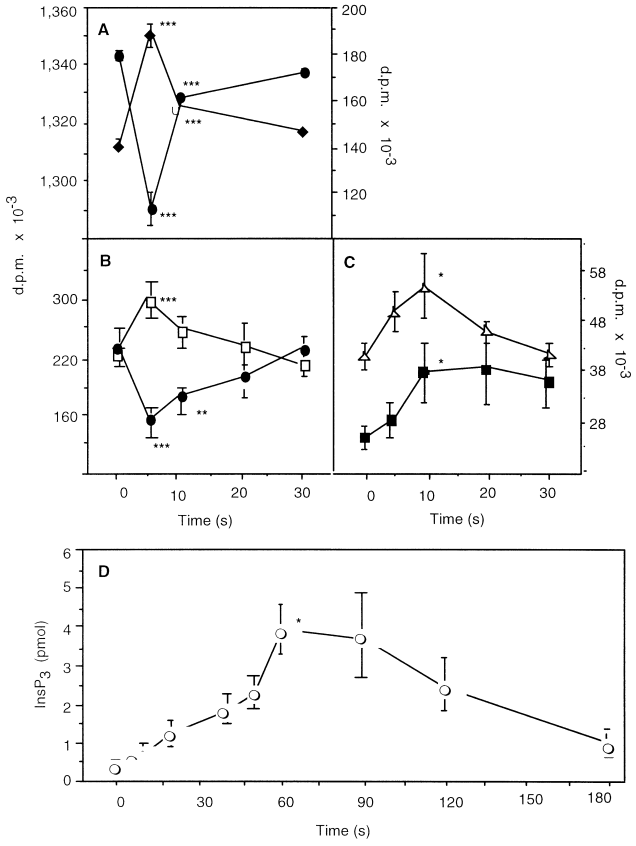

Fig. 1. Time course of phosphoethanolamine (A) and alkyl-TG (B) synthesis as evidence for alkyl-PE hydrolysis by an alkyl-PE-specific PLC after ET-1 (100 nM) activation, compared to time course of DG and phosphatidic acid (C) and $InsP_3$ (D) synthesis. A: Cells were labeled with [^{3}H]ethanolamine, stimulated with 100 nM ET-1 and the radioactivity of alkyl-PE (●) and phosphoethanolamine (♦) was determined. The right scale indicates labeling level of alkyl-PE, while the left is for phosphoethanolamine labeling. B: Cells were labeled with 1-[^{3}H]*O*-alkyl-*sn*-glycero-3-phosphocholine. The radioactivity of alkyl-PE (●) and alkyl-TG (□) was determined [2]. C: Cells were labeled with 1-[^{3}H]*O*-alkyl-*sn*-glycero-3-phosphocholine. The radioactivity of DG (△) and phosphatidic acid (■) was determined [2]. D: $InsP_3$ (○, pmol/assay) was determined as reported in Section 2. Data are means ± S.E.M. of three separate experiments each performed in quadruplicate. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (Student's *t*-test).

3.2. Alkyl-PE-specific PLC is activated by calcium entering SMC through L-type Ca^{2+} channels

We previously reported that extracellular Ca^{2+} was absolutely required for the generation of alkyl-TG induced by ET-1 [2]. Removal of extracellular calcium also abolished ET-1-dependent production of phosphoethanolamine, and neither Mg^{2+} nor Ba^{2+} were able to restore PLC activity (data not shown). In addition, the Ca^{2+} ionophore A23187 (1 μM) mimicked the effects of ET-1 on phosphoethanolamine accumulation, whereas membrane depolarization induced by 50 mM KCl generated phosphoethanolamine at a level 1.6-fold higher than ET-1 (Fig. 2A). This observation suggested the involvement of a voltage-dependent Ca^{2+} channel. Nimodipine, a specific blocker of L-type Ca^{2+} channels [6], inhibited ET-1-induced formation of phosphoethanolamine in SMC

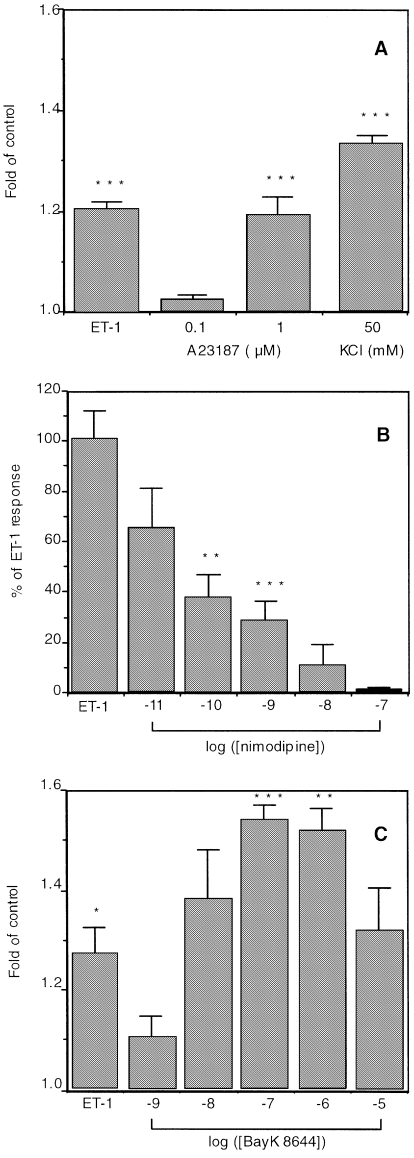

Fig. 2. Alkyl-PE-specific PLC is activated via L-type Ca^{2+} channels. [^{3}H]Ethanolamine-labeled cells were stimulated for 5 s with various agents, then the reaction was blocked and the radioactivity of phosphoethanolamine was determined. A: Stimulation with ET-1 (100 nM), A23187 (0.1 and 1 μM), or KCl (50 mM). B: Cells were preincubated in the absence or in the presence of various concentrations of nimodipine before addition of 100 nM ET-1. C: Stimulation with 100 nM ET-1 or various concentrations of BayK 8644. Data are expressed as fold stimulation compared to non-stimulated cells (A,C) or as percentages of the response obtained with ET-1 alone (B). They are means ± S.E.M. from three separate experiments performed in quadruplicate. $*P<0.05$; $**P<0.01$; $***P<0.001$ (Student's *t*-test).

with an apparent IC_{50} of 3.4×10^{-11} M (Fig. 2B). Moreover, *S*(−)-BayK 8644, which was described as a specific 'activator' of L-type Ca^{2+} channels [7], was twice as effective as ET-1 in activating alkyl-PE-specific PLC from SMC (Fig. 2C). This strongly suggested that alkyl-PE-specific PLC was activated by Ca^{2+} entering the cells through L-type Ca^{2+} channels.

3.3. Activation of alkyl-PE-specific PLC requires a pertussis toxin-sensitive heterotrimeric G-protein

Because it was demonstrated that G-proteins can regulate

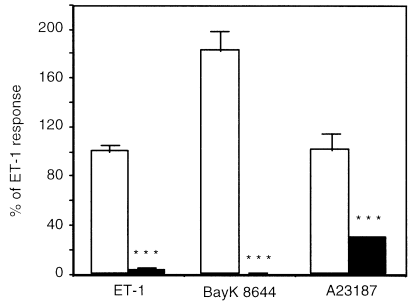

Fig. 3. Effect of pertussis toxin on the activation of alkyl-PE-specific PLC. [^{3}H]Ethanolamine-labeled cells were treated for 6 h in the absence (open bars) or in the presence (full bars) of pertussis toxin (10 ng/ml), followed by stimulation for 5 s with ET-1 (100 nM), BayK 8644 (100 nM) or A23187 (1 μM). The reaction was blocked and the radioactivity of phosphoethanolamine was determined. Data (means ± S.E.M. from three separate experiments performed in quadruplicate) are percentages of the response obtained with ET-1 alone. $***P<0.001$ (Student's *t*-test).

voltage-dependent Ca^{2+} channels [8], we investigated the possible involvement of G_i or G_0 heterotrimeric proteins in alkyl-PE-specific PLC regulation. As shown in Fig. 3, pretreatment of cells with pertussis toxin abolished the accumulation of phosphoethanolamine induced by ET-1 or BayK 8644, the effect of A23187 (1 μM) being reduced by 70%. Although

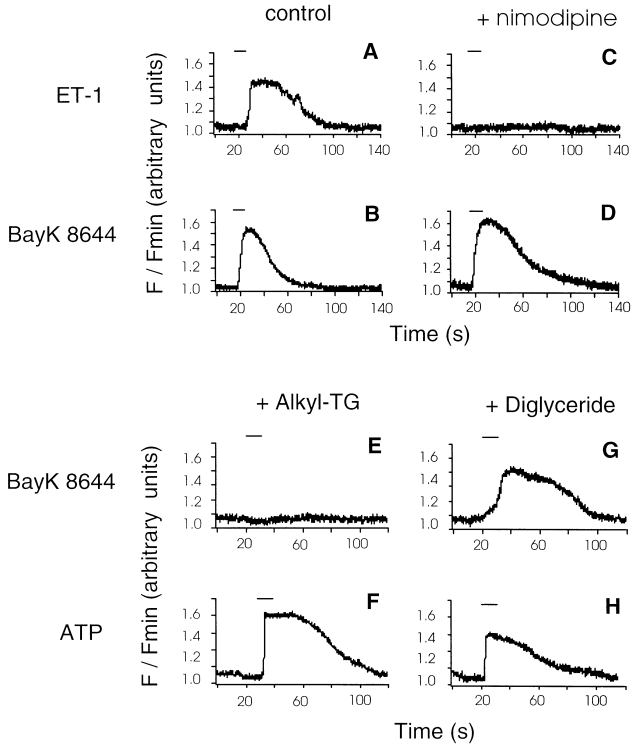

Fig. 4. Closure of L-type Ca^{2+} channels by alkyl-TG. Variations of $[Ca^{2+}]_i$ were determined by measuring the fluorescence of SMC loaded with Fura Red. A: ET-1, 100 nM; B: BayK 8644, 100 nM; C: nimodipine (1 μM, 15 min), then ET-1 (100 nM); D: nimodipine (1 μM, 15 min), then BayK 8644 (100 nM); E: DiC10-alkyl-TG (100 nM, 15 min), then BayK 8644 (100 nM); F: DiC10-alkyl-TG (100 nM, 15 min), then ATP (1 mM); G: DiC10-DG (100 nM, 15 min), then BayK 8644 (100 nM); H: DiC10-DG (100 nM, 15 min), then ATP (1 mM). Each trace is representative of 14–63 determinations.

we cannot distinguish between a direct and an indirect regulation of alkyl-PE-specific PLC by heterotrimeric G-proteins, these data clearly demonstrated the involvement of a G_i or G_0 protein in the activation of the PE-specific PLC pathway.

3.4. Alkyl-TG inhibits both alkyl-PE-specific PLC and L-type Ca^{2+} channels

We synthesized diC10-alkyl-TG containing a long alkyl chain (16 carbons) at the *sn*-1 position and two short acyl chains (10 carbons) at positions 2 and 3 of glycerol, in order to allow intercalation of the lipid in the membrane and permeation to the internal leaflet, as currently achieved with DG or ceramides [9]. Preincubation of cells with diC10-alkyl-TG for 15 min inhibited BayK 8644-induced accumulation of phosphoethanolamine with a narrow dose–response curve. There was no effect at 1 nM, a half maximal effect at 10 nM and total inhibition at 100 nM. The effects of diC10-alkyl-TG were then tested on single cells loaded with the fluorescent Ca^{2+} indicator Fura Red. ET-1 promoted an increase in $[Ca^{2+}]_i$ which was abolished by nimodipine (Fig. 4A,C). *S*(−)-BayK 8644 induced a similar Ca^{2+} influx and reversed the effect of nimodipine, confirming the presence of functional L-type Ca^{2+} channels in SMC (Fig. 4B,D). The effects of *S*(−)-BayK 8644 were abolished in cells preincubated with 100 nM diC10-alkyl-TG, whereas didecanoyl-*rac*-glycerol (diC10-DG) had a marginal effect or even prolonged to some extent the Ca^{2+} signal (Fig. 4E,G). Eventually, the increase in $[Ca^{2+}]_i$ evoked by ATP, which involves a purinergic receptor acting via intracellular store Ca^{2+} mobilization [10,11], was insensitive to diC10-alkyl-TG (Fig. 4F), whereas di-C10-DG produced some slight inhibition (Fig. 4H). These data indicated that diC10-alkyl-TG was not toxic for the cells and that its inhibitory effect was probably directed against L-type Ca^{2+} channels.

4. Discussion

Altogether, our present data indicate that we are dealing with a novel signaling pathway leading to the generation of a hitherto unrecognized lipid second messenger, i.e. alkyl-TG. In response to various agents able to elevate $[Ca^{2+}]$ in SMC, PE, especially alkyl species, are rapidly degraded, generating phosphoethanolamine and alkyl-TG [2,3]. It has been shown that platelet-activating factor was able to induce simultaneous production of phosphocholine and phosphoethanolamine, indicating that both phosphatidylcholine and PE were degraded probably by the same enzyme [12]. Although two previous studies suggested that some signaling pathways might involve phosphatidylethanolamine-specific phospholipases C or D [13,14], this is the first report of an alkyl-PE-specific PLC activated very early upon cell challenge with ET-1 as an agonist.

The PLC described in the present study differs from previously identified enzymes by several points. First, it appears to be selective for alkyl-PE, as shown by the lack of phosphatidylcholine, diacyl and alkenyl-acyl PE decrease [2]. Secondly, this enzyme is activated very early following ET-1 stimulation, before phosphoinositide-specific phospholipase C, by a mechanism involving Ca^{2+} influx through L-type Ca^{2+} channels and a pertussis toxin-sensitive heterotrimeric G-protein. Such a dual regulation is reminiscent of the behavior of cytosolic phospholipase A_2 [15,16]. Third, alkyl-PE-specific PLC does not lead to the accumulation of DG because of their immediate acylation into TG. So far, we do not know which is the fatty acyl donor involved in this secondary transacylation, i.e. acyl-CoA or a similar phospholipid.

This peculiar metabolism evoked by ET-1 in SMC led us to consider that alkyl-TG might represent a novel lipid second messenger. Alkyl-DG was previously reported to exert biological effects similar or opposite to those of diacylglycerol [17–20], however a specific biological activity has never been reported for triglycerides with or without a 1-*O*-alkyl chain.

Using short chain analogues able to cross the membrane, we found that alkyl-TG exerted potent inhibitory effects on L-type Ca^{2+} channels, which explains why alkyl-PE-specific PLC was also inhibited. Such an unexpected finding cannot be explained by a putative degradation of TG into DG, which were previously shown to depress L-type Ca^{2+} channels independently of protein kinase C activation [21], for two reasons: (i) effective DG concentrations in previous studies were significantly higher (1–10 μM) than the concentrations of diC10-alkyl-TG used herein (10–100 nM) which are very low compared to usual agonists used; (ii) diC10-DG, under our experimental conditions, was unable to reproduce the effects of diC10-alkyl-TG (compare Fig. 4E,G). The possibility that TG could be active via fatty acids release by a lipase is excluded as well because fatty acids at the same concentration do not block L-type Ca^{2+} channels [22]. We cannot distinguish whether alkyl-TG act directly on L-type Ca^{2+} channels or involve protein intermediates such as a kinase or a phosphatase. Protein kinase C is known to regulate the activity of L-type Ca^{2+} channels, but inhibition [23], activation [24] or dual effects [25] have been reported, suggesting additional mechanisms of regulation by classical activators of this enzyme [21]. Moreover, acyl-TG have been shown to be inactive on protein kinase C in vitro [20]. Further studies will be undertaken to elucidate the mechanism of action of this novel second messenger and to investigate other possible targets.

A critical question concerns the biological relevance of our observations. Total blockade of L-type Ca^{2+} channels was obtained with 100 nM of di-C10-alkyl-TG which is equivalent to 100 pmol di-C10-alkyl-TG (1 ml medium) for 12.5 nmol of total cell phospholipids. We previously found that alkyl-PE represent 6.75% of total phospholipids from pig aortic SMC [2]. In other words, a 10% hydrolysis of alkyl-PE would generate 85 pmol alkyl-TG in the cell samples used in our various experiments, basically, an amount in the same range of magnitude as the di-C10-alkyl-TG added to the cells. This should reinforce our conclusion that alkyl-TG could be considered as a potential novel lipid second messenger. Moreover, it has to be noted that the inhibitory effect of di-C10-alkyl-TG on L-type Ca^{2+} channels is reversible: after 45 min incubation, L-type Ca^{2+} channels were no longer blocked, cells were then able to be activated. DG was without effect throughout this time (not shown).

In addition to DG [21,23], other lipid compounds were recently found to exert inhibitory effects on L-type Ca^{2+} channels in a very similar way to those reported here with TG. These lipids include ceramide [26], which was efficient at 10–100 nM, and farnesol [27], which required much higher concentrations (1–10 μM). Finally, this conversion of PE into TG seems not to be specific of ET-1 because PDGF, which acts via a receptor with intrinsic tyrosine kinase activity, was also able to induce accumulation of alkyl-TG from alkyl-PE [3].

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References

[1] Takai, Y., Kishimoto, A., Kikkawa, U., Mori, T. and Nishizuka, Y. (1979) Biochem. Biophys. Res. Commun. 91, 1218–1224.
[2] Comminges, C., Georgeaud, V., Maury, E., Cariven, C., Prévost, M.C. and Chap, H. (1997) Biochim. Biophys. Acta 1355, 69–80.
[3] Comminges, C., Maury, E., Cariven, C., Prévost, M.C. and Chap, H. (1996) Biochem. Biophys. Res. Commun. 220, 1008–1013.
[4] Boden, E.P. and Keck, G.E. (1985) J. Org. Chem. 50, 2394–2395.
[5] Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
[6] McCarthy, R.T. and Tan Piengco, P.E. (1992) J. Neurosci. 12, 2225–2234.
[7] Ferrante, J., Luchowski, E., Rutledge, A. and Triggle, D.J. (1989) Biophys. Res. Commun 158, 149–154.
[8] De Waard, M., Liu, H., Walker, D., Scott, V.E., Gurnett, C.A. and Campbell, K.P. (1997) Nature 385, 446–450.
[9] Hannun, Y.A. (1996) Science 274, 1855–1859.
[10] Strobaek, D., Christophersen, P., Dissing, S. and Olesen, S.P. (1996) Am. J. Physiol. 271, C1463–C1471.
[11] Hartley, S.A. and Kozlowski, R.Z. (1997) Circ. Res. 80, 170–178.
[12] Kester, M., Thomas, C.P., Wang, J. and Dunn, M.J. (1992) J. Cell Physiol. 153, 244–255.
[13] Kiss, Z. and Anderson, W.B. (1989) J. Biol. Chem. 264, 1483–1487.
[14] Kiss, Z. (1992) Eur. J. Biochem. 209, 467–473.
[15] Burke, J.R., Davern, L.B., Gregor, K.R., Todderud, G., Alford, J.G. and Tramposch, K.M. (1997) Biochim. Biophys. Acta 1341, 223–237.
[16] Mattera, R., Hayek, S., Summers, B.A. and Grove, D.L. (1998) Biochem. J. 332, 263–271.
[17] Ford, D.A., Miyake, R., Glaser, P.E. and Gross, R.W. (1989) J. Biol. Chem. 264, 13818–13824.
[18] Mandal, A., Wang, Y., Ernsberger, P. and Kester, M. (1997) J. Biol. Chem. 272, 20306–20311.
[19] Cabot, M.C. and Jaken, S. (1984) Biochem. Biophys. Res. Commun. 125, 163–169.
[20] Ganong, B.R., Loomis, C.R., Hannun, Y.A. and Bell, R.M. (1986) Proc. Natl. Acad. Sci. USA 83, 1184–1188.
[21] Schreur, K.D. and Liu, S. (1996) Am. J. Physiol. 270, C655–C662.
[22] Hallaq, H., Smith, T.W. and Leaf, A. (1992) Proc. Natl. Acad. Sci. USA 89, 1760–1764.
[23] Galizzi, J.P., Qar, J., Fosset, M., Van Renterghem, C. and Lazdunski, M. (1987) J. Biol. Chem. 262, 6947–6950.
[24] Badou, A., Savignac, M., Moreau, M., Leclerc, C., Pasquier, R., Druet, P. and Pelletier, L. (1997) J. Biol. Chem. 272, 32411–32418.
[25] Schuhmann, K. and Groschner, K. (1994) FEBS Lett. 341, 208–212.
[26] Schreur, K.D. and Liu, S. (1997) Am. J. Physiol. 272, H2591–H2598.
[27] Roullet, J.B., Luft, U.C., Xue, H., Chapman, J., Bychkov, R., Roullet, C.M., Luft, F.C., Haller, H. and McCarron, D.A. (1997) J. Biol. Chem. 272, 32240–32246.