Phospholipid analogues: side chain- and polar head group-dependent effects on phosphatidylcholine biosynthesis

Christoph C. Geilen,[1,*] Anne Haase,[*] Thomas Wieder,[*] Dietrich Arndt,[†] Reiner Zeisig,[†] and Werner Reutter[*]

Institut für Molekularbiologie und Biochemie der Freien Universität Berlin,[*] D-14195 Berlin-Dahlem, Germany; and Max Delbrück Centrum für Molekulare Medizin,[†] Berlin-Buch, Germany

Abstract In recent studies we showed that the phospholipid analogue hexadecylphosphocholine inhibits phosphatidylcholine biosynthesis by affecting the translocation of the rate-limiting enzyme of phosphatidylcholine biosynthesis, CTP:phosphocholine cytidylyltransferase (EC 2.7.7.15), to membranes, where it is active (Geilen et al. 1992. *J. Biol. Chem.* **267:** 6719–6724). The present study was performed to investigate the structure-dependency of this effect. It is shown that the inhibitory properties of phospholipid analogues are dependent on their alkyl side chain length (dodecylphosphocholine < tetradecylphosphocholine < hexadecylphosphocholine < heptadecylphosphocholine < octadecylphosphocholine > eicosadecylphosphocholine). Furthermore, it is demonstrated that this inhibition of phosphatidylcholine biosynthesis by phospholipid analogues is also dependent on the polar head group (hexadecylphosphocholine >> hexadecylphosphoethanolamine = hexadecylphosphoserine). These effects result from an inhibition of the CTP:phosphocholine cytidylyltransferase and are not due to an inhibition of choline uptake or differences in the cellular uptake of the phospholipid analogues investigated.—**Geilen, C. C., A. Haase, T. Wieder, D. Arndt, R. Zeisig, and W. Reutter.** Phospholipid analogues: side chain- and polar head group-dependent effects on phosphatidylcholine biosynthesis. *J. Lipid Res.* 1994. **35:** 625–632.

Supplementary key words hexadecylphosphocholine • cell proliferation • cytotoxicity

CTP:phosphocholine cytidylyltransferase (EC 2.7.7.15) (CT) is the rate-limiting enzyme in the biosynthesis of phosphatidylcholine in higher eukaryotes (for review see refs. 1–3). Its activity is regulated by translocation of the enzyme between cytosol and membranes. The cytosolic, lipid-free form is inactive, whereas the membrane-bound form is active. Recently, a third form was described, which is an active, cytosolic enzyme-112 kDa protein aggregate (4). The translocation process has been shown to be regulated by various lipids (5, 6). The lipids that were first reported to regulate CT were glycerophospholipids (7–9). Translocation to membranes is enhanced in particular by the presence in the membrane of anionic glycerophospholipids such as phosphatidylglycerol, phosphatidylserine, or phosphatidylinositol. Free fatty acids have also been reported to regulate CT activity (10–12), and their activating properties are acyl chain length-dependent (6). Furthermore, mono- and diacylglycerols may activate CT (13, 14). It has been suggested that an increased diacylglycerol content of cellular membranes after treatment of cells with phospholipase C (15) or phorbol ester (16) is the underlying mechanism of enhanced phosphatidylcholine biosynthesis in these cells. These data on the activation of CT by naturally occurring lipids in vivo and in vitro suggest that the process of CT-membrane interaction depends on both the surface charge of membranes and the lipid packing of these membranes.

Recently, the synthetic phospholipid, hexadecylphosphocholine (HePC), has been shown to inhibit PC biosynthesis (17, 18). This lipid analogue has also been reported to inhibit cell proliferation and tumor growth (19–21). In the present study, we systematically investigated the effect of this class of phospholipid analogues on phosphatidylcholine biosynthesis and cell proliferation in vivo by using analogues that differ from hexadecylphosphocholine in their alkyl chain length or in their polar head group (**Fig. 1**).

Abbreviations: CT, CTP:phosphocholine cytidylyltransferase; HPLC, high performance liquid chromatography; HePC, hexadecylphosphocholine; PC, phosphatidylcholine; HepPC, heptadecylphosphocholine; OcPC, octadecylphosphocholine; EicPC, eicosadecylphosphocholine; DoPC, dodecylphosphocholine; TePC, tetradecylphosphocholine; HePS, hexadecylphosphoserine; HePE, hexadecylphosphoethanolamine; CMC, critical micellar concentration; HPTLC, high performance thin-layer chromatography; TPA, 12-O-tetradecanoylphorbol-13-acetate; PBS, phosphate-buffered saline.

[1]To whom correspondence should be addressed at: Department of Dermatology, University Medical Center Steglitz, The Free University of Berlin, Hindenburgdamm 30, D-12200 Berlin, Germany.

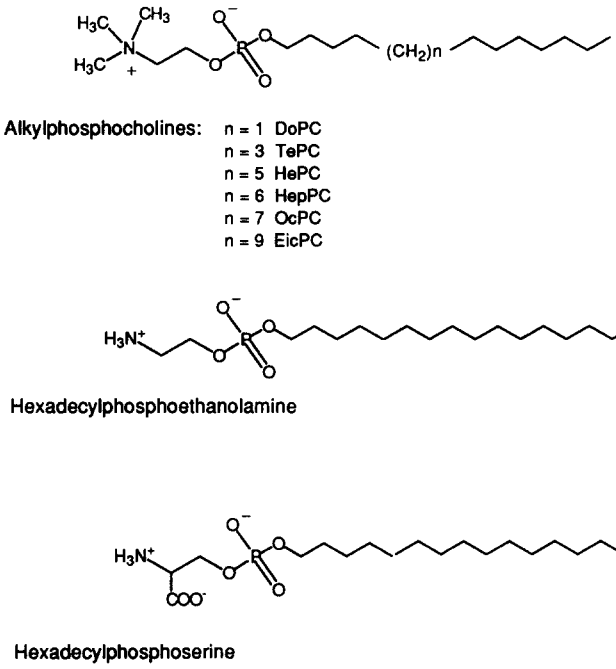

Fig. 1. Chemical structures of different phospholipid analogues.

MATERIALS AND METHODS

Materials

Hexadecylphosphocholine (HePC), heptadecylphosphocholine (HepPC), octadecylphosphocholine (OcPC), and eicosadecylphosphocholine (EicPC) were synthesized as described recently (22, 23). Dodecylphosphocholine (DoPC) was prepared by Prof. J. Mulzer and M. Mickeleit, Institut für Organische Chemie, Freie Universität Berlin. Tetradecylphosphocholine (TePC) was obtained from Novabiochem (Bad Soden, Germany). Hexadecylphosphoserine (HePS) and hexadecylphosphoethanolamine (HePE) were a gift from Asta Pharma (Frankfurt a. Main, Germany). The purity of each analogue was checked by silica gel-HPLC connected with a light-scattering detector Sedex 55 (ERC, Regensburg, Germany) using n-hexane–isopropanol 3:2 (v/v) as eluent. The detection sensitivity was < 100 pmol of the analogue. All analogues investigated had a purity of more than 95%. Additionally, the identity of each analogue was checked by mass spectrometry and ^{1}H-NMR analysis. [Methyl-^{3}H]choline chloride (2.8–3.1 TBq/mmol) was from Amersham (Braunschweig, Germany). Silica gel 60 HPTLC plates, solvents, and reagents (reagent grade) were purchased from E. Merck (Darmstadt, Germany) and Fluka (Neu-Ulm, Germany). The bicinchoninic acid (BCA)-kit for protein determination was obtained from Pierce (Weiskirchen, Germany). Miscellaneous lipids and phosphatidylcholine precursors were from Sigma (München, Germany). For quantification of radioactivity, a Berthold LB 2821 HR thin-layer chromatography scanner (Berthold; Wildbad, Germany) was used.

Determination of the critical micellar concentration (CMC)

The critical micellar concentration of alkylphosphocholines with different chain lengths was determined by the method of Saski and Shah (24). Briefly, each sample was dissolved in aqueous, saturated iodine solution in a concentration range of 0.0005% to 0.1% (weight per volume). The light transmission of the samples at 360 nm was compared with that of iodine solution without additions. The concentrations were plotted half-logarithmically versus transmission. The intersection of the two straight lines indicated the CMC value of the respective substance.

Cell culture

MDCK cells were grown in plastic culture dishes (Nunc, Wiesbaden, Germany) containing Dulbecco's minimal essential medium supplemented with 10% calf serum, 0.56 g/l L-glutamine, 100,000 IU penicillin, and 0.1 g/l streptomycin. Media and culture reagents were obtained from Gibco (Berlin, Germany); penicillin and streptomycin were from Boehringer (Mannheim, Germany). Confluent cells were subcultured every 3 days after detaching the cells with a trypsin/EDTA solution. For experimental purposes, cells were used on the third day of culture. All phospholipid analogues were dissolved in PBS, pH 7.4, and then added to the culture medium.

Cytotoxicity assay

Cytotoxicity of the different phospholipid analogues to MDCK cells was estimated according to Culvenor et al. (25) by measurement of the alkaline phosphatase activity of the cells. After incubation for 24 h with different concentrations of the respective phospholipid analogue, the cells were centrifuged at 1000 *g* for 10 min, and the supernatant was discarded. The reaction mixture containing 20% diethanolamine, 2 μmol/l $MgCl_2$, 2 μmol/l *p*-nitrophenol-phosphate, and 10% Triton X-100 was added. After 1 h at 37°C the reaction was stopped with 0.5 mol/l NaOH. The amount of *p*-nitrophenolate was determined photometrically at 410 nm.

Cell proliferation assay

Cell proliferation was measured by determination of the cell number according to the method of Gilles, Didier, and Denton (26). Cells were fixed by treatment with 1% glutaraldehyde for 15 min, stained for 30 min in 0.1% crystal violet solution, then washed with deionized water for 15 min. Crystal violet absorbed by cell nuclei was solubilized with 0.2% Triton X-100 and the amount released was quantitated photometrically at 590 nm.

Choline uptake assay

MDCK cells were grown to confluence on collagen-coated plastic foils. For the assay, cells were treated with medium containing 50 μmol/l of the respective lipid analogue for 2 min and then were immersed in pulse medium containing 28 μmol/l choline and 3 μCi/ml [^{3}H-methyl]-choline as radioactive tracer. After different times, as indicated in the legends, cells were washed thoroughly twice with 580 μmol/l choline in PBS. For quantification of the choline uptake, cells with the entire plastic foils were taken for scintillation counting. Uptake was calculated by the incorporated tracer radioactivity.

Measurement of cellular uptake of the different phospholipid analogues

MDCK cells were grown to confluence and treated with medium containing 50 μmol/l of the respective lipid analogue for 6 h. Then, cells were washed twice with PBS, harvested with a cell lifter (Costar; Cambridge, MA), and lyophilized. The pellets were extracted by modified lipid extraction according to Bligh and Dyer (27): 50 μl methanol, 25 μl chloroform, and 20 μl water were added. Samples were stirred for 2 min on a vortex-mixer and centrifuged at 13,000 *g* for 10 min. Phase separation was accomplished by addition of 25 μl chloroform and 25 μl water. The suspension and centrifugation steps were repeated. Then 15 μl of both the organic and the aqueous phases were applied to silica gel 60 HPTLC plates using an HPTLC applicator (Linomat III; Camag, Berlin, Germany). Lipids were separated according to Touchstone, Chen, and Beaver (28), using the solvent chloroform–methanol–triethylamine–water 30:35:34:8 (by vol). Phospholipid analogues were stained with cupric sulfate in phosphoric acid and quantified by use of a video-densitometer (Biotec-Fischer, Reiskirchen, Germany). Staining was linear in the range of 0.2 to 2.0 nmol of each analogue.

Measurement of phosphatidylcholine biosynthesis

After incubation with pulse-medium ([^{3}H]choline; 1 μCi/ml) supplemented with 50 μmol/l of the different phospholipid analogues, cells were washed twice with ice-cold PBS (pH 7.2) and harvested with a cell lifter (Costar; Cambridge, MA) followed by modified lipid extraction according to Bligh and Dyer (27) as described above. Then 10 μl of the chloroform phase was taken for scintillation counting and 15 μl was applied to silica gel 60 HPTLC plates using an HPTLC applicator (Linomat III; Camag, Berlin, Germany). Lipids were separated according to Touchstone et al. (28) as described above. Radioactivity was quantified by radioscanning (LB 2821 HR, Berthold, Wildbad, Germany). Phospholipids were identified by calibrating the scanner with known standards. Approximately 95% of the radioactivity co-chromatographed with the phosphatidylcholine standard.

For determining PC biosynthesis under stimulating conditions, cells were simultaneously treated with 5 nmol/l 12-O-tetradecanoylphorbol-13-acetate (TPA) and 50 μmol/l of the different alkylphosphocholines.

Permeabilization of cells by digitonin

Confluent MDCK cells were incubated for 6 h in medium containing no supplement (control) or 50 μmol/l of the respective phospholipid analogue. Cells were then washed two times with ice-cold PBS, and all the following steps were carried out at 4°C. Three hundred μl buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 2 mM dithiothreitol, 0.025% NaN_3, 1 mM PMSF, and 0.5 mg/ml digitonin was added to the cells. After 3 min the digitonin supernatant was removed. Undissolved digitonin was pelleted at 13,000 *g* for 2 min and the remaining cell ghosts were washed with 1 ml of PBS, harvested in 50 μl of buffer as described above (the harvesting buffer contained 0.03% Triton X-100 and no digitonin) and then homogenized by a Dounce homogenizer. Cytidyltransferase activity was measured in the digitonin supernatants and in the cell ghost homogenates as described below. From the data, the ratio of cytosolic and membrane-bound CT activity was calculated for each dish.

Cytidylyltransferase assay

Cytidylyltransferase activity was measured by a modified method of Sohal and Cornell (29). The reaction mixture contained 50 mmol/l Tris-HCl, pH 7.4, 0.03% Triton X-100, 100 mmol/l NaCl, 10 mmol/l $MgCl_2$, 3 mmol/l CTP, 1.5 mmol/l [methyl-^{14}C]phosphocholine (sp act 20 Bq/nmol), liposomes (400 μmol/l phosphatidylcholine–400 μmol/l oleic acid) and 10 μl of enzyme preparation in a final volume of 55 μl. After incubating for 30 min at 37°C, the reaction was stopped by freezing the samples in liquid nitrogen. The samples were lyophilized, dissolved in 20 μl methanol–water 1:1 (v/v) and applied to HPTLC plates. After developing the plates in a solvent system containing methanol–0.6% NaCl–25% aqueous NH_3 8:5:1 (by vol), the radioactivity was determined by radioscanning. One unit of enzyme activity is defined as one nmol of CDP-choline formed per min.

Other procedures

Cellular protein was determined by the BCA-assay (30) using bovine serum albumin as a standard. Statistical analysis was performed by the Student's *t*-test.

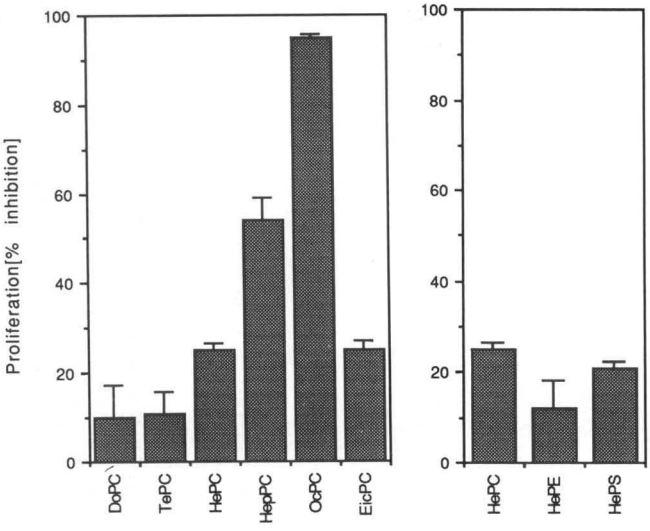

Fig. 2. Antiproliferative effect of different phospholipid analogues. MDCK cells were incubated with 100 μmol/l of the respective phospholipid analogue. For control experiments no supplements were added. After 16 h of incubation the proliferation rate was determined by crystal violet assay as described in Materials and Methods. Values are given as % inhibition of cell proliferation compared with control values ± SD (n = 4).

RESULTS

Dose-dependent effect of different phospholipid analogues on cell proliferation and cell toxicity

The dose-dependent effect of phospholipid analogues with different chain length and various polar head groups on the proliferation of MDCK cells was investigated. Concentrations of 10 μmol/l, 50 μmol/l, 100 μmol/l, and 200 μmol/l were used and the cells were incubated for 16 h. All phospholipid analogues investigated inhibited the proliferation of MDCK cells in a dose-dependent manner, but the inhibitory activity of the different phospholipid analogues varied over a wide range. At a concentration of 100 μmol/l, the phospholipid analogues with a serine and ethanolamine head group, HePS and HePE, showed an inhibition rate of less than 20% versus controls (IC_{50} > 200 μmol/l) (**Fig. 2**).

The effect of the choline derivatives was dependent on the length of the alkyl group (Fig. 2). Short phospholipid analogues such as DoPC and TePC were weak inhibitors of cell proliferation with IC_{50} values above 200 μmol/l. Potent substances were HePC, HepPC, and OcPC, which showed IC_{50} values of about 180 μmol/l, 100 μmol/l, and 75 μmol/l, respectively. A further increase in chain length resulted in a decrease of the antiproliferative effect. The IC_{50} of EicPC was above 200 μmol/l.

To examine the cytotoxic effect of the different phospholipid analogues, confluent MDCK cells were incubated with phospholipid analogue concentrations of 10 μmol/l–300 μmol/l for 6 h. Subsequently, viability of the cells was determined. A dose-dependent increase of the cytotoxic effect occurred for all substances examined. With exception of HepPC, no significant cytotoxic effects occurred at concentrations ≤ 50 μmol/l (viability ≥ 90%) for all lipid analogues tested. In general, the results were similar to those of the cell proliferation assay. However, at 50 μmol/l the most cytotoxic agent was HepPC (**Fig. 3**).

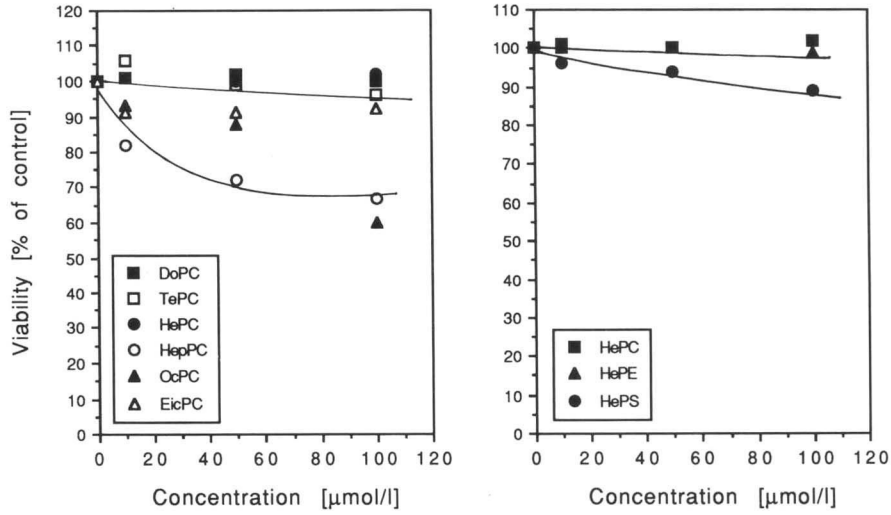

Fig. 3. Cytotoxic effect of different phospholipid analogues. MDCK cells were incubated for 6 h with different concentrations of the respective phospholipid analogues as indicated in the figure (DoPC; TePC; HePC; HepPC; OcPC; EicPC; HePS; HePE). Alkaline phosphatase activity was then determined as described in Materials and Methods. Values are the mean of four separate determinations (SD < 5%).

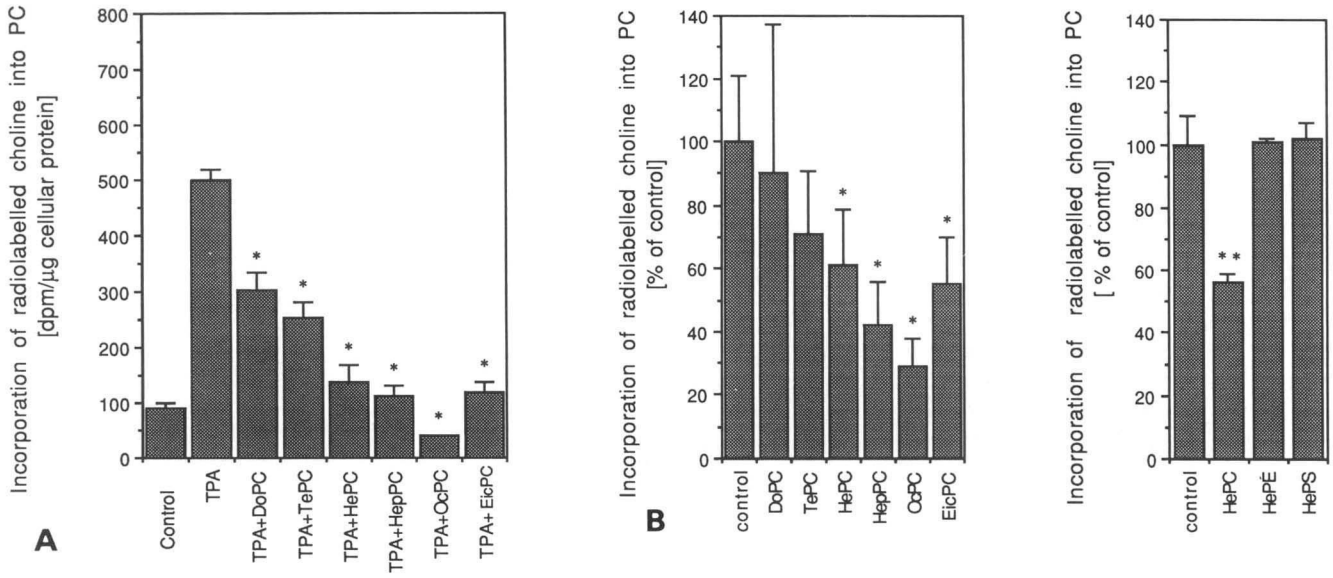

Fig. 4. A: Effect of different phospholipid analogues on choline incorporation into PC under phorbol ester-stimulated conditions. For the experiments under TPA-stimulated conditions, cells were pretreated with 5 nmol/l TPA and 50 µmol/l of the respective phospholipid analogues simultaneously for 3 h. Then radiolabeled choline (2 µCi/ml) was added and the cells were incubated further for 2 h. The subsequent steps were carried out as described for the experiments under non-stimulated conditions (Fig. 4B). The values of incorporated radioactivity are given in dpm/µg protein ± SD (n = 3). (Significantly different from TPA-stimulated control: $^*P \leq 0.05$.) B: Effect of different phospholipid analogues on choline incorporation into PC under non-stimulated conditions. MDCK cells were incubated for 6 h with 50 µmol/l of the respective phospholipid analogue and [methyl-^{3}H]choline (2 µCi/ml). The cells were harvested, lipids were extracted, and the choline incorporation into phosphatidylcholine was determined as described in Materials and Methods. For control values no phospholipid analogue was added. The values of incorporated radioactivity are given in % of control and are the mean of three separate determinations ± SD. (Significantly different from control: $^*P \leq 0.05$; $^{**}P \leq 0.01$.)

Effect of alkylphosphocholines with different alkyl side chains on TPA-stimulated and non-stimulated phosphatidylcholine biosynthesis

MDCK cells were pretreated for 3 h with 5 nmol/l TPA, or with 5 nmol/l TPA plus 50 µmol/l of the respective phospholipid analogue, or with no supplements as control. Then [methyl-^{3}H]choline [2.5 µCi/ml] was added. After 2 h of incubation, cells were harvested and PC was analyzed as described in Materials and Methods. The results shown in **Fig. 4A** indicate that antagonization of TPA-stimulated PC biosynthesis is dependent on the length of the alkyl side chain, with a maximum effect at C-18 (OcPC). Nearly the same results were obtained with non-stimulated MDCK cells. After 6 h of incubation with different phospholipid analogues, incorporation of [methyl-^{3}H]choline into PC was inhibited. The inhibition depended on chain length (Fig. 4B), and OcPC was the most efficient inhibitor (70% inhibition). Beside the fact that DoPC has no effect on non-stimulated PC biosynthesis, it abolished TPA-stimulated PC biosynthesis by approximately 60%. All phospholipid analogues investigated had no effect on choline uptake (data not shown).

Effect of phospholipid analogues with different polar head groups on phosphatidylcholine biosynthesis

To determine the effect of different polar head groups on choline incorporation, HePC, HePS, and HePE were investigated. The experiments were carried out as described in the previous section. The results shown in Fig. 4B indicate that the choline head group is essential for the inhibitory effect on PC biosynthesis. This is also true for the antagonization of phorbol ester stimulation (data not shown).

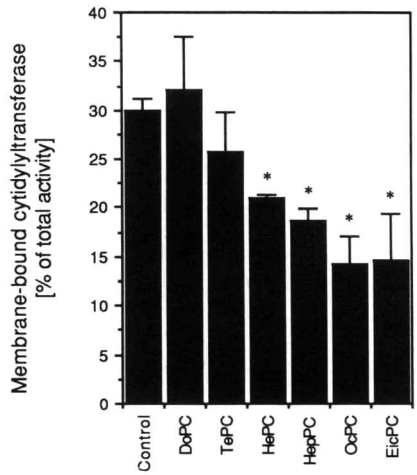

Fig. 5. Effect of different alkylphosphocholines on the subcellular distribution of the CTP:phosphocholine cytidylyltransferase. MDCK cells were incubated for 6 h with 50 µmol/l of the respective phospholipid analogue. Then the subcellular distribution of CT was determined by digitonin release as described in Materials and Methods. The values of membrane-bound enzyme are given in % of the total activity of CT ± SD (n = 3). (Significantly different from control: $^*P \leq 0.05$.)

Effect of alkylphosphocholines on the subcellular distribution of CTP:phosphocholine cytidylyltransferase

By permeabilizing MDCK cells with digitonin, which causes cytosolic contents to leak into the culture medium, it is possible to distinguish between the cytosolic and the membrane-bound form of CT. In order to examine the effect of different alkylphosphocholines on the translocation process, the distribution of CT in control and alkylphosphocholine-treated cells (6 h) was measured by determination of CT activity in the digitonin supernatant and in the cell ghosts. **Fig. 5** shows the side chain-dependent effect of different alkylphosphocholines on the distribution of CT activity after a digitonin release of 3 min, which is in accordance with the results of the inhibition of PC biosynthesis described above.

Cellular uptake of different phospholipid analogues

To exclude the possibility that differences in the action of phospholipid analogues investigated in this study were due to differences in their cellular uptake, this cellular uptake was determined as described in Materials and Methods. As shown in **Table 1,** all phospholipid analogues, with the exception of DoPC, were taken up to a similar extent. There was no significant correlation between uptake of phospholipid analogues and inhibition of cell proliferation ($r = 0.41$), and between uptake of phospholipid analogues and inhibition of PC biosynthesis ($r = 0.70$).

DISCUSSION

In this study, we investigated the effect of different phospholipid analogues on phosphatidylcholine biosynthesis and cell proliferation in vivo. Previous studies showed that the synthetic phospholipid analogue, hexadecylphosphocholine, disturbs the translocation process of the key enzyme of PC biosynthesis, CTP:phosphocholine cytidylyltransferase, and finally leads to an inhibition of PC biosynthesis in vivo (18). Furthermore, different studies have shown that HePC inhibits the cell proliferation of various cell lines (19–21).

TABLE 1. Cellular uptake of different phospholipid analogues

Phospholipid Analogue	Uptake
	nmol analogue/10^6 cells
DoPC	2.1 ± 0.1
TePC	7.3 ± 2.8
HePC	7.3 ± 0.7
HepPC	6.4 ± 0.5
OcPC	11.0 ± 0.8
EicPC	16.2 ± 1.2
HePE	10.4 ± 1.8
HePS	5.2 ± 0.6

Confluent MDCK cells were treated with medium containing 50 μmol/l of the respective phospholipid analogue for 6 h. Cells were harvested, extracted, and cellular uptake was measured by HPTLC as described in Materials and Methods (n = 3).

CT is an amphipathic protein and its activation is lipid-dependent. In most reported studies on lipid requirements, naturally occurring lipids such as glycerophospholipids, fatty acids, or diacylglycerols were used, and the results of these studies have led to several conclusions about CT-membrane interactions (5, 6). In the present study, we tested these conclusions using synthetic phospholipid analogues. By comparing our data with published results, the following points were confirmed.

First, it was reported that lipids that act as translocators have an optimum chain length of > 14 carbon atoms. For example, oleic acid has the strongest stimulatory effect on PC biosynthesis (9). In our results, OcPC showed the best inhibitory effect on choline incorporation into PC as well as on the CT distribution, where OcPC reduced the membrane-bound form by over 50% compared to control cells. It is possible that a lipid that modulates CT activity must have an optimum chain length to be integrated into the membrane bilayer and its effect on the regulation of CT translocation depends on its polar head group. Choline head group-containing lipids, such as alkylphosphocholines, alkyl-lysophosphocholines, or PC, inhibit CT translocation to membranes, whereas only lipids without a choline headgroup, such as oleic acid, oleoyl alcohol, or diacylglycerol, could activate the membrane translocation of CT. Very recently, Jamil, Hatch, and Vance (31) suggested that the binding of CT to membranes is modulated by the ratio of bilayer-forming lipids (e.g., PC) to non-bilayer-forming lipids (e.g., oleic acid or diacylglycerol). The phospholipid analogues investigated are non-bilayer-forming lipids (32), but differed in their action on CT translocation and PC biosynthesis. From our data, we therefore suggest that the ratio of choline head group-containing lipids to non-choline head group-containing lipids modulates CT translocation. However, an effect on choline uptake could be excluded, which is in accordance with our previous findings in studies with HePC (18). Furthermore, no differences occurred in the cellular uptake of the phospholipid analogues that could explain their differences in action.

In a recent study, we showed that HePC antagonizes phorbol ester-stimulated PC biosynthesis by inhibiting the translocation of CT (33). Therefore, we investigated the effect of the different alkylphosphocholines in TPA-stimulated MDCK cells. The chain length-dependent effect was similar to that found in experiments with non-stimulated cells. However, it is obvious that the response to DoPC is different in stimulated and non-stimulated cells. DoPC has no effect on PC biosynthesis in non-stimulated cells, but it antagonizes the TPA-induced effect by about 60%.

Furthermore, the observation that lipids with the same polar head group such as HePC, OcPC, and 1-palmitoyllysophosphatidylcholine, have different effects on CT translocation has to be investigated in more detail. It was suggested that changes in membrane properties, like fluidity and packing of lipids, influence the type of regulative action; but HePC, OcPC, and LPC have nearly the same biophysical properties. For example, all alkylphosphocholines investigated have critical micellar concentrations in the range between 170 and 280 μmol/l measured by the method of Saski and Shah (24). Our results on the effect of this class of phospholipid analogues with different polar head groups support the suggestion that the structure of the polar head group (e.g., charge, space requirements) of the lipid influences its modulatory properties. HePS and HePE do not inhibit PC biosynthesis in TPA-stimulated and non-stimulated MDCK cells.

Concerning CT-membrane interaction, it is interesting that CT activity depends on the state of the lipid phase. It was shown in in vitro studies that fluid-phase lipids are better activators of CT than gel-phase lipids. We demonstrated that more than 95% of the incorporated HePC is associated with cellular membranes (34). Therefore, we suggest that phospholipid analogues such as HePC are incorporated into cellular membranes and inhibit the CT-membrane fluidity. At least, we demonstrated that the antiproliferative effect of alkylphosphocholines is paralleled by their ability to inhibit PC biosynthesis. There is a good correlation between the effect of lipid analogues on cell proliferation and incorporation of radiolabeled choline into PC ($r = 0.96$) and between the effect on CT translocation and incorporation of radiolabeled choline into PC ($r = 0.92$). In contrast, no significant correlation occurred between the effect on cell viability and incorporation of radiolabeled choline into PC ($r = 0.69$), and between cell viability and cell proliferation ($r = 0.60$).

In previous studies, it was suggested that the antiproliferative effect of HePC is due to an inhibition of protein kinase C (35, 36). From our data, we suggest that the antiproliferative effect of alkylphosphocholines might also be mediated by inhibition of PC biosynthesis. In further studies, we will focus our interest on the relationship between PC biosynthesis and cell proliferation.

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