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# Lipid-induced changes in vascular smooth muscle cell membrane fluidity are associated with DNA synthesis

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**Abstract.** In the present study, we examined whether changes in the membrane fluidity of vascular smooth muscle cells (VSMCs) alter their DNA synthesis For this purpose, the membrane fluidity of the cells was modulated after treatment of VSMCs with 1,2-dioleoyl phosphatidylcholine (PC) Treatment of VSMCs with 1,2-dioleoyl PC-rich medium containing 10% heat-inactivated human serum and 3 mg/ml 1,2-dioleoyl PC for 24 h resulted in an increase in VSMC membrane fluidity at all temperatures from 15° to 40°C as well as a 51% inhibition of DNA synthesis, compared with untreated cells Remarkably, enrichment of VSMCs with 1,2-dioleoyl PC/cholesterol-rich medium containing 10% human serum, 3 mg/ml 1,2-dioleoyl PC and 2 mg/ml cholesterol restored both membrane fluidity and DNA synthesis to the levels of untreated cells The present findings show an inverse association between increased membrane fluidity and cellular DNA synthesis

## INTRODUCTION

The most common membrane lipids in mammalian cells are cholesterol and phospholipids Both play an important role in the regulation of membrane fluidity The fluid mosaic model is the predominant model used for the understanding of membrane structure In this model, proteins are immersed in a phospholipid bilayer and are able to accomplish various types of movement Phase transition between the 'liquid crystalline' and 'crystalline' state is determined by phospholipids and temperature Under physiological conditions, modulation of the membrane fluidity of mammalian cells is achieved by changes in the molar ratio of cholesterol to phospholipid or by changes in the overall degree of unsaturation of the phospholipid acyl chains In this context, membrane phospholipids such as 1,2-dioleoyl phosphatidylcholine (PC) increase membrane fluidity In direct contrast, membrane cholesterol causes a more rigid, ordered membrane structure (for review see Shinsky & Barenholz 1978) It is well established that the growth of VSMCs plays a major role in the pathogenesis of cardiovascular diseases such as atherosclerosis and hypertension (for review see Ross 1993) Several previous studies have demonstrated that cholesterol enrichment of VSMCs modulates the functional activity of some integral proteins, including ion channel activity (Gleason *et al* 1991, Sen *et al* 1992) The purpose of the present study was to investigate whether increasing membrane fluidity results in changes in cell DNA synthesis

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## MATERIALS AND METHODS

### Cell culture

Rat aortic VSMCs were isolated from the thoracic aortae of Wistar–Kyoto rats (6–8-week-old, Charles River Wiga, Sulzfeld, Germany) by enzymatic dispersion as described previously (Sachinidis *et al.* 1995). Cells were cultured at 37°C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum, nonessential amino acids, 100 IU/ml penicillin and 100 μg/ml streptomycin.

### Enrichment procedure

Preparation of heat-inactivated serum was performed as described previously (Shinitzky 1978). The cells from healthy human blood were separated by centrifugation at 3000 ***g***. The removed plasma was incubated at 56°C for 30 min and the heat-inactivated serum was then separated by centrifugation at 3000 ***g***. Heat-inactivated serum was used for the enriching media. Enrichment was performed as described previously (Shinitzky 1978). Briefly, a mixture of 5 : 1 (v/v) tetrahydrofuran (THF) and 0.6% aqueous KCl was used as a dispersing solvent for 1,2-dioleoyl PC enrichment. This mixture was called THF medium. A solution of 3 mg/ml 1,2-dioleoyl PC or 3 mg/ml 1,2-dioleoyl PC/2 mg/ml cholesterol in THF medium was added into 10 volumes of vigorously stirred serum medium consisting of 10% heat-inactivated serum in DMEM supplemented with nonessential amino acids (L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, glycine, L-proline, L-serine), 100 IU/ml penicillin and 100 μg/ml streptomycin. The mixture was frozen and then lyophilized to complete dryness under sterile conditions. The lyophilized material was then dissolved in the correct volume of sterile distilled water (enriching medium). Enrichment was performed after incubation of confluent VSMCs for 24 h with the following media: (1) enriching medium containing 1,2-dioleoyl PC; (2) enriching medium containing 1,2-dioleoyl PC/cholesterol; (3) control medium without either lipid compound.

### Fluorescence polarization studies

After enrichment the media were removed and the VSMCs were washed 3 times with phosphate-buffer saline (PBS). Control, 1,2-dioleoyl PC and 1,2-dioleoyl PC/cholesterol-enriched VSMCs (75 $cm^2$ flasks) were scraped off in 10 ml PBS and underwent sonification (50 W, 30 s). Total cellular membranes were labelled with the lipid-soluble fluorophore 1,6-diphenyl-1,3,5-hexatriene (DPH)(10 μm) after vigorous stirring and dispersion for 3 h at 37°C as previously described (Medow & Segal 1987, Gleason *et al.* 1991). The steady-state fluorescence anisotropy (r) measurements were performed in a Perkin-Elmer LS50 luminescence spectrofluorometer (Überlingen, Germany) equipped with polarizing filters in the excitation and emission beams at an excitation wavelength of 365 nm and at an emission wavelength of 430 nm. Fluorescence anisotropy was calculated from the degree of fluorescence polarization using the Perrin equation. Membrane fluidity was expressed in the form of Arrhenius plots (fluorescence polarization term $((r_0/r) - 1)^{-1}$ vs. $1/T(°K) \times 10^3$). The upper limit of the r is $r_0$ (for DPH, $r_0 = 0.362$) (Medow *et al.* 1987). Higher values of the term $((r_0/r) - 1)^{-1}$ indicate a lower membrane fluidity.

### Determination of DNA synthesis

After enrichment of VSMCs in 24-well culture plates, the media were removed and VSMCs were washed 3 times with PBS. Then the medium was replaced by serum-free medium (DMEM supplemented with nonessential amino acids, 100 IU/ml penicillin and 100 μg/ml streptomycin). Twenty hours later [$^3$H]thymidine (111 kBq/ml) was added. Four hours after this the experiments were terminated as described previously (Sachinidis *et al.* 1995).

**Materials**

DMEM and PBS were obtained from Gibco BRL, Eggenstein, Germany FCS was obtained from Boehringer Mannheim (Mannheim, Germany) [Methyl-$^3$H]thymidine was obtained from Amersham, Little Chalfont, UK Cholesterol, 1,2-dioleoyl PC and DPH were obtained from Sigma Chemical Co , Deisenhofer, Germany

**Statistics**

Values are expressed as the arithmetic mean ± SD Statistical analysis of the data was performed using the one factor ANOVA-Scheffe *F*-test A value of $P < 0\ 05$ was considered statistically significant

## RESULTS AND DISCUSSION

Figure 1 shows a representative Arrhenius plot of cell membrane fluidity, expressed as $((r_0/r) - 1)^{-1}$, as a function of temperature in untreated, 1,2-dioleoyl PC and 1,2-dioleoyl PC/cholesterol-enriched cells Low values of $((r_0/r)-1)^{-1}$ indicate a high membrane fluidity VSMCs membrane fluidity was increased in 1,2-dioleoyl PC-enriched VSMCs at all temperatures between 15° and 40°C Interestingly, membrane fluidity in 1,2-dioleoyl PC/cholesterol-enriched VSMCs was decreased toward the values of untreated VSMCs Using the enrichment procedure of Shinitzky (1978), enrichment of VSMCs with 1,2-dioleoyl PC

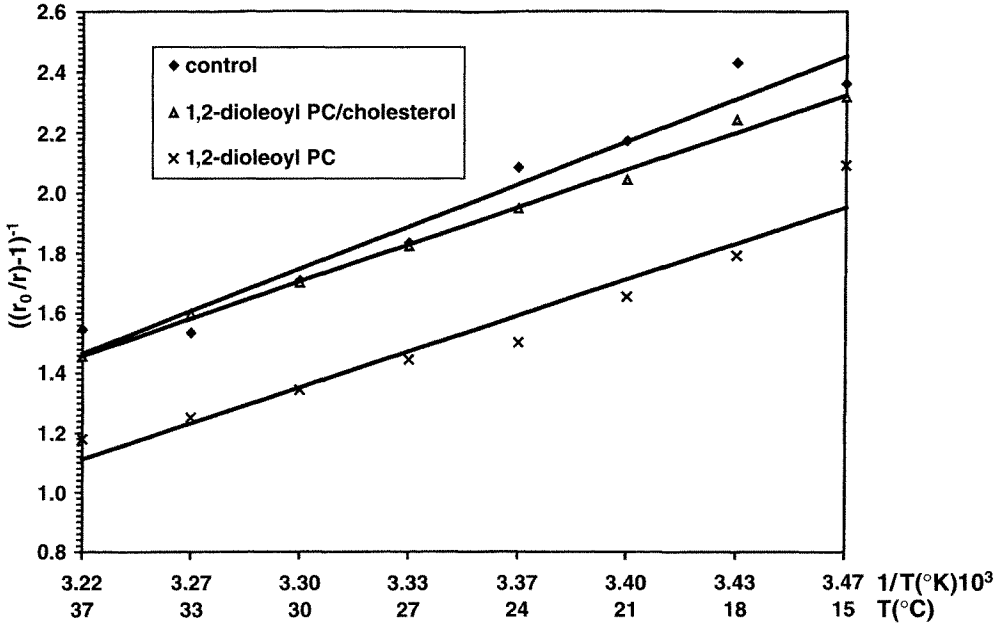


**Figure 1.** Representative Arrhenius plot of the fluorescence anisotropy (r) of 1,6-diphenyl-1,3,5-hexatriene (DPH) in VSMCs membranes VSMCs were seeded in 75 cm$^2$ flasks and cultivated in culture medium until confluence VSMCs were treated with 1,2-dioleoyl phosphatidylcholine (PC), 1,2-dioleoyl PC/cholesterol and control medium for 24 h After labelling of the cell membranes with DPH measurements were performed at an excitation wavelength of 365 nm and an emission wavelength of 430 nm

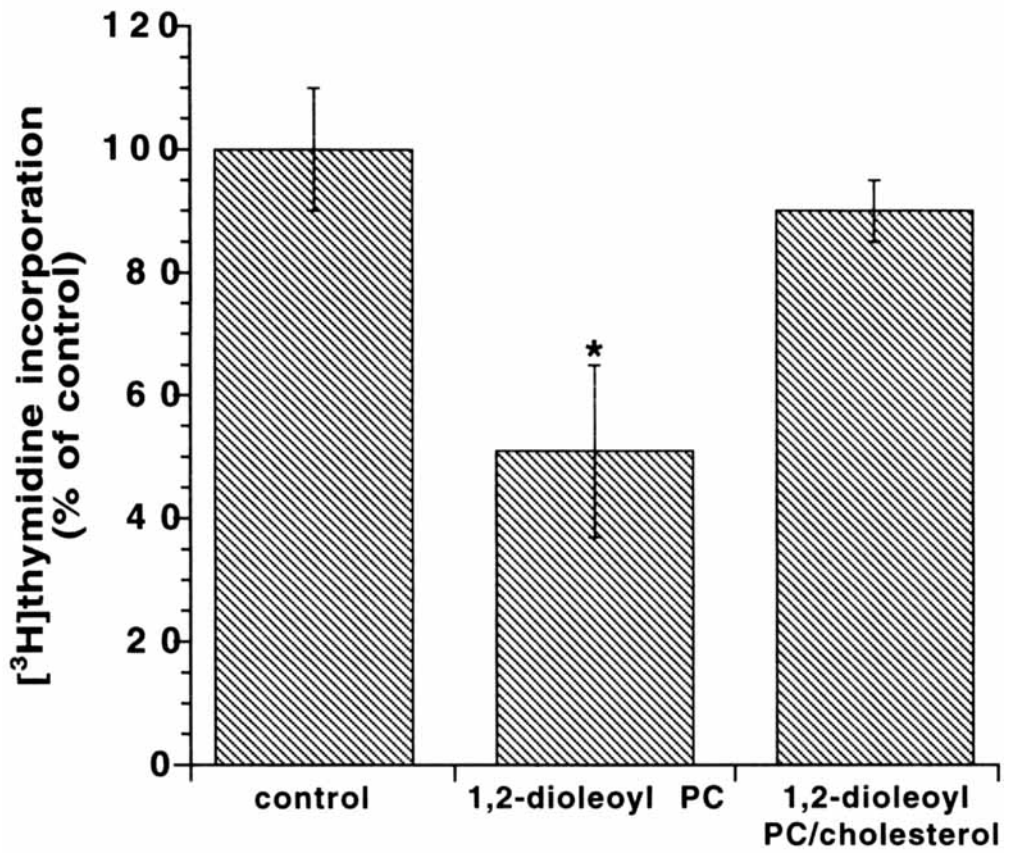


**Figure 2.** DNA synthesis in 1,2-dioleoyl phosphatidylcholine (PC)-and 1,2-dioleoyl PC/cholesterol-enriched VSMCs. Confluent cells (24-well plates) were treated with 1,2-dioleoyl PC, 1,2-dioleoyl PC/cholesterol and control medium for 24 h. Then the medium was replaced with serum-free medium and after 20 h of incubation, wells were exposed to 111 kBq/ml [$^3$H]thymidine. Four hours later the reaction was terminated and cell protein and [$^3$H]thymidine incorporation into cell DNA were quantified. Values from two representative experiments performed in triplicate wells were expressed as percentage of control (100%) (means $\pm$ SD, $^*P < 0.05$ for 1,2-dioleoyl PC vs. control).

resulted in a marked increase of membrane fluidity. In striking contrast, concomitant enrichment with 1,2-dioleoyl PC and cholesterol inverses the effect of 1,2-dioleoyl PC towards the control membrane fluidity values.

The [$^3$H]thymidine incorporation into 1,2-dioleoyl PC-enriched VSMCs was decreased by 51% compared to untreated VSMCs (100 $\pm$ 5%) (Figure 2). In contrast, there was no significant difference between the [$^3$H]thymidine incorporation in untreated and 1,2-dioleoyl PC/cholesterol-treated VSMCs (90 $\pm$ 6%). Another remarkable finding of the present work was that the restoration of membrane fluidity to control values by concomitant enrichment with cholesterol abrogated the inhibitory effects of 1,2-dioleoyl PC on cellular DNA synthesis. Although the exact mechanisms whereby 1,2-dioleoyl PC-enrichment of VSMCs results in an inhibition of DNA synthesis are unknown it may be hypothesized that inhibition occurred due to the increase in membrane fluidity. Under physiological conditions, membrane fluidity is at an equilibrium necessary for the optimal functional activity of several proteins involved in the regulation of DNA synthesis. It is widely believed that membrane fluidity

markedly affects either the enzymatic activity or function of membrane proteins Such an effect on calcium channels has been shown to result in an increase in the intracellular $Ca^{2+}$ in VSMCs (Gleason *et al* 1991, Sen *et al* 1992, Sachinidis *et al* 1997) Changes in membrane fluidity may occur through the distribution of phospholipids or cholesterol between serum and the cell membranes Membrane fluidity may be modulated by diet, pharmacological manipulations or by age (Medow *et al* 1987) Our findings provide evidence that changes in the membrane fluidity of VSMCs result in a modulation of DNA synthesis and might be involved in VSMCs growth processes

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