Sphingosine-mediated electroporative DNA transfer through lipid bilayers

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Abstract When the cationic sphingosine is present in planar lipid bilayers, the adsorption of pDNA is enhanced and the electroporatability of the bilayer is facilitated. Furthermore, pDNA, adsorbed to lipid bilayers composed of diphytanoyl lecithin and D-sphingosine causes electroporative channel-like events of conductance 1 to 7 pS, provided the voltage polarity is correct and the voltage is high enough, $V_m \geq +30$ mV. The data suggest electrotransport of pDNA through the bilayer, mediated by transient complexes between DNA and the lipids in the pore edges of elongated, electropercolated hydrophilic pore zones. The results are the basis for the optimisation of electroporative transfer of DNA or oligonucleotides to cells and tissue.

Key words: Cationic lipid; Plasmid DNA; Membrane electroporation; Patch clamp; DNA electrodiffusion

1. Introduction

In the mitogenic signal transduction cascade, the activity of protein kinase C requires the presence of anionic lipids. Cationic monoalkyl lipids such as D-sphingosine are neutralising the anionic ones and thereby modulate the kinase activity [1]. Cationic lipids have been found to be involved in other cellular functions such as eukaryotic transcription and replication processes, modulation of surface receptor activities in cell-cell communication as well as differentiation and neoplastic transformation by protein kinase C-independent pathways [2–4]. On the other hand, cationic liposomes are used as carriers for antisense oligonucleotides which regulate specific gene products [5–7]. Despite abundant appearance of cationic monoalkyl components and cationic lipids, the physicochemical properties of the complexes between nucleic acids and cationic lipids are not well understood.

The binding of DNA to dimyristoylphosphatidylcholine liposomes containing sphingosine was studied, among others, by differential scanning calorimetry. It was proposed that DNA changes the thermal phase transition behaviour of the phospholipids [8,9] by association with several positively charged micelles to yield larger aggregates or that DNA is within larger fused vesicles [10].

Undoubtedly, there is a need to further characterise the DNA-sphingosine interactions. Here we provide results suggesting that cationic monoalkyl lipids like sphingosine facilitate the electro-diffusive transport of DNA across locally electroporated membrane patches. The initial stage of the electroporative transfer of DNA into the bilayer surface is of electrophoretic nature, causing electro-insertion provided that the polarity of the membrane voltage permits electrodiffusion into the bilayer [11,12]. It appears that cationic lipids play a similar role as Ca^{2+} or Mg^{2+} in mediating the adsorption of DNA [12] to planar lipid bilayer surfaces causing ionic current pulses (P. Siemens, data not shown).

2. Materials and methods

Diphytanoyl lecithin (DPhL, $M_r = 846.3$; 3 mg/ml) from Avanti Polar Lipids, Birmingham (Alabama) and D-sphingosine (Sph, $M_r = 299.5$; 1 mg/ml) from Sigma were used in the weight ratio 5:1 for the preparation of lipid bilayer membranes. The plasmid DNA Yep351 (5.6 kbp, $M_r = 3.6 \times 10^6$) was isolated and purified in supercoil form according to Maniatis et al. [13]. The buffer conditions were 150 mM KCl, 1 mM Tris-HCl, pH 7.0, 293 K (20°C). The bilayer was formed at the tip of a patch pipette from a lipid monolayer spread on an air/water interface by the methods of Corronado and Latorre [14] and Schuerholz and Schindler [15]. Petri dishes of 10 cm^2 area filled with 3 ml of the buffer were used and the lipid monolayers were formed by spreading a 3 μl solution of DPhL and Sph in chloroform over the electrolyte surface.

The pipettes were pulled from borosilicate glass (World Precision Instruments, Inc.). When filled with the electrolyte solution, the pipette had a resistance in the range of 6 ± 1 MΩ which corresponds to a tip diameter of 1.5 ± 0.5 μm. The electric seal resistance of the bilayer was typically in the order of 10 GΩ. DNA (0.5 mg/ml) is added to Petri dishes in 6 μl aliquots. The effective length of the twisted pDNA molecule is 0.61 μm [11]. The average distance between pDNA molecules in the solution is about 1.8 μm. The interaction of DNA with lipid membranes causes current pulses, provided the voltage is large enough and of the right polarity [11]. Membrane voltage (V_m) by convention bears the sign of the electrode inside the pipette.

The transmembrane currents were recorded using a patch clamp amplifier (1–3 kHz). The current and voltage outputs were connected to an oscilloscope and to a two-channel analogue-digital converter lined on an IBM PC compatible computer. For data analysis, the software package programs PAT (gift of Dr. J. Dempster, University of Strathclyde, UK) and HUND by Ivan Bonev (Sofia, Bulgaria) were used. All data were recorded at 293 K (20°C). In all experiments the concentration of pDNA was 2.7 nM.

3. Results

In Fig. 1A it is seen that there is zero current in the absence of pDNA at $V_m = +30$ mV. The high ohmic seal is not changed after the addition of 2.7 nM pDNA to the solution (data not shown). DPhL is highly fluid at temperatures above $T \cong -120$°C and the phase transition temperature of Sph is $T \cong +39$°C. It is recalled that the adsorption of pDNA to liposomes, formed by phospholipids with phase transition temperatures between 0°C and 30°C and sphingosine changes the fluidity and phase transition behaviour of the bilayer [8,9]. In the case of the DPhL-Sph mixture, however, the phase

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Abbreviations: DPhL, diphytanoyl lecithin; Sph, D-sphingosine

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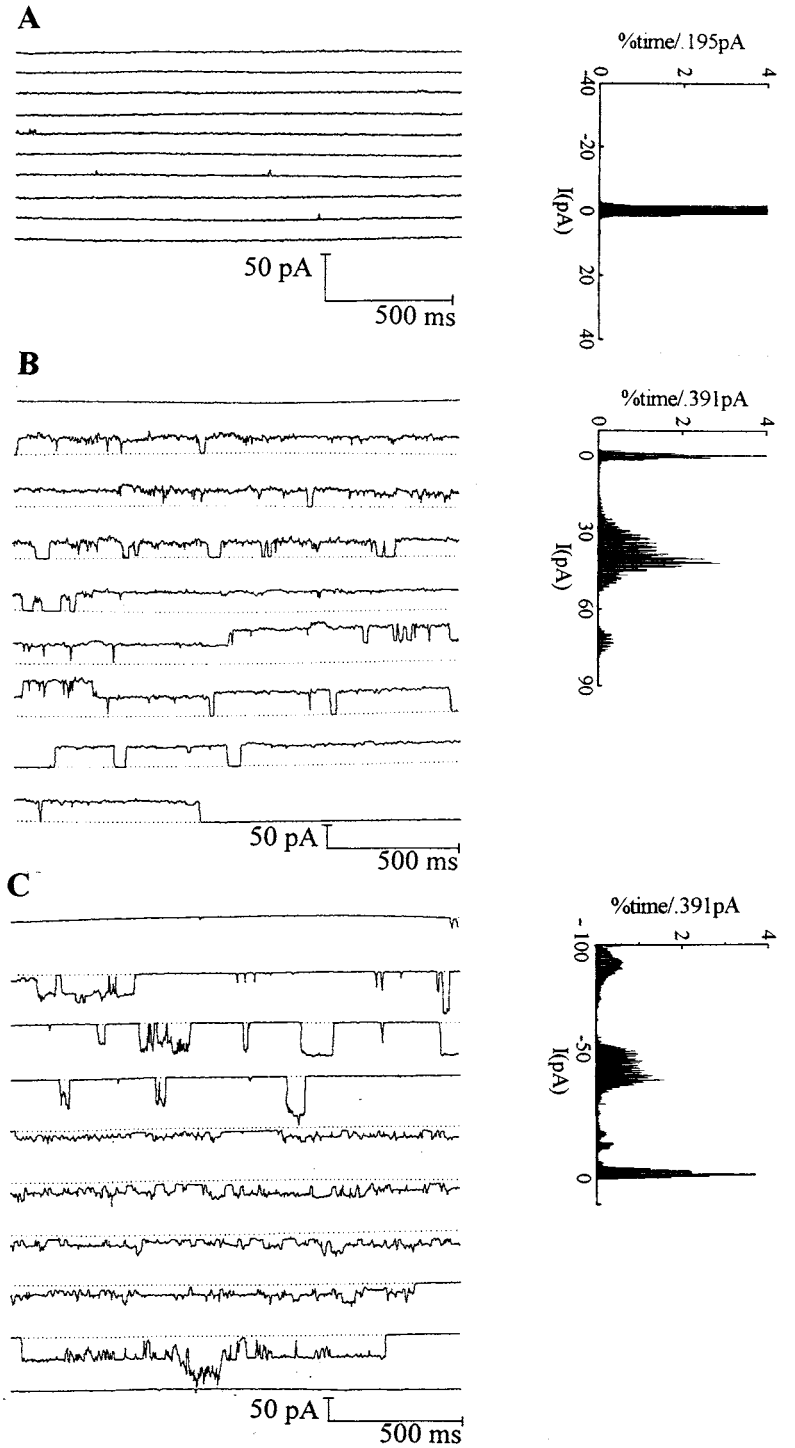

Fig. 1. Current intensity (I) as a function of time at constant voltage under Neher-Sakmann patch clamp conditions. A: $V_m = +30$ mV, DPhL:Sph (weight ratio 5:1) bilayer (control without DNA, $I = 0$). B: $V_m = +30$ mV followed by C: $V_m = -30$ mV. The same bilayer is exposed to pDNA added to the outside solution; [pDNA] = 2.7 nM. The records A are at 100 mV/pA, B and C are at 50 mV/pA, respectively. The histogram A refers to a record of 20.5 s, B and C of 18.4 s and 20.5 s, respectively.

transition temperature appears to be not changed when pDNA is adsorbed on the bilayers because DPhL is in the fluid state down to −120°C. As a consequence, the good seal in the patch pipette after the addition of DNA is maintained.

As known from similar studies [11], electric current pulses initially occur only at positive larger voltages, i.e. when the negative electric potential is at the membrane side of pDNA addition and the positive potential on the other membrane side. In Fig. 1B it is seen that the sequence of open, i.e. conductive, states lasts 15.2 s. Appreciably large current pulses develop only at $V_m \geq +30$ mV, which is rather low compared with bilayers from pure DPhL (in the presence of pDNA, but in the absence of Sph [14]). The currents show fluctuations and rather broad amplitude distributions and the conductance

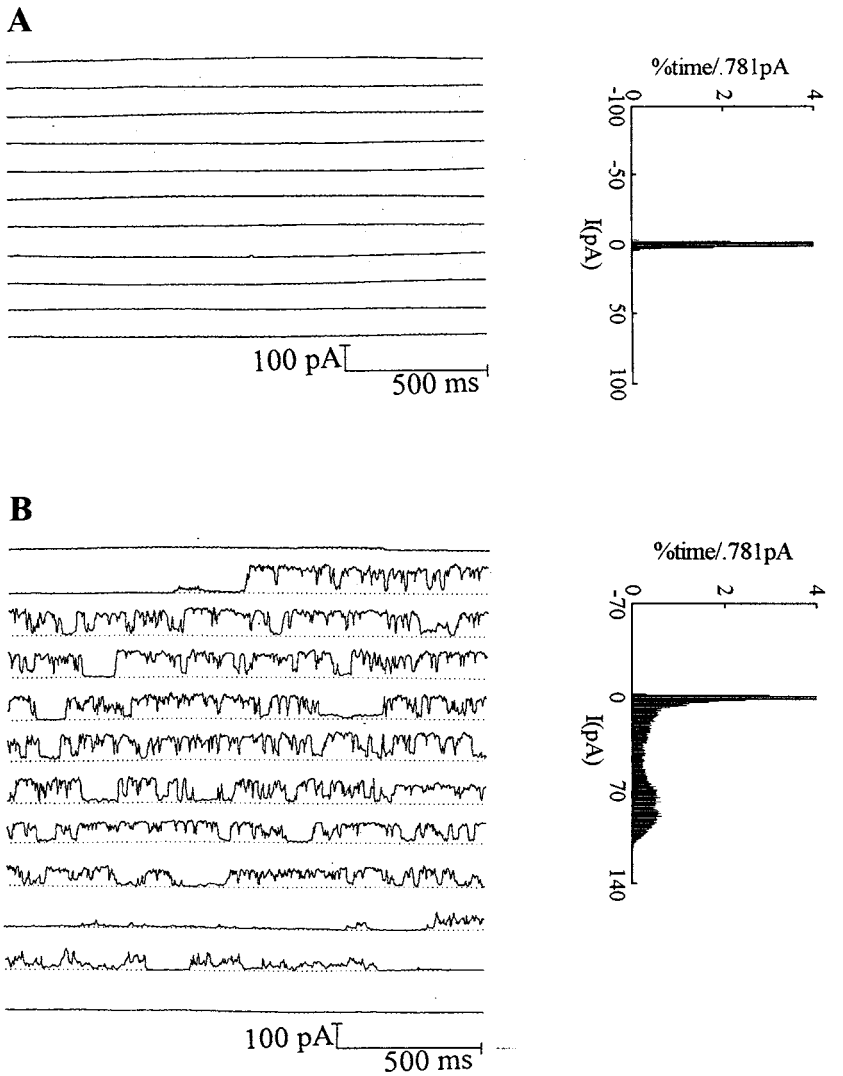

Fig. 2. Current intensity (I) as a function of time at constant voltage $V_m = +100$ mV of the same bilayer as in Fig. 1. A: Control without pDNA at $V_m = +100$ mV; record is at 25 mV/pA; B: in the presence of pDNA and (positive) voltage $V_m = +100$ mV, record is at 25 mV/pA and followed by C (for C and D see p. 84): at $V_m = -100$ mV, record is at 10 mV/pA. D: The record is at 10 mV/pA, $V_m = -100$ mV and is made 10 min after applying the (minus) voltage ($V_m = -100$ mV). The histogram in D shows that there is no current. The histogram A refers to a record of 22.5 s, those of B, C and D refer to records of 24.6 s, 32.8 s and 20.5 s, respectively.

values are of 1 to 1.5 nS. When, however, after the previous positive voltage ($V_m = +30$ mV) a negative voltage ($V_m = -30$ mV) is applied, then current pulses appear (Fig. 1C); their main conductance values are 1.3 to 1.7 nS and 3 nS. Here the sequence of open states lasts 16.2 s.

In Fig. 2B it is shown that at the applied positive voltage $V_m = +100$ mV the open state starts with a slow increase in the current for about 320 ms before a sequence of fluctuating open states of 0.7 to 1 nS occurs lasting 19.6 s. At the negative voltage $V_m = -100$ mV (Fig. 2C) applied after the positive voltage the dominant conductance value is 4.0 nS. The sequence of open states lasts 32.7 s.

In all records (Figs. 1 and 2) the current at the negative voltage fluctuates more frequently than at the positive voltage and the duration of fluctuations is longer. After about 10 min the current returns to zero (Fig. 2D) as in Fig. 2C ($V_m = -100$ mV) and as the control (Fig. 2A) in the absence of pDNA. The return to the zero current level in Fig. 2D after the previous voltage manipulations is suggested to reflect that the pDNA, which has entered the pipette volume at $V_m = +100$ mV, has left the pipette interior back to the outside.

Frequently, even at a low positive voltage ($V_m = +30$ mV), the current activity starts with small short pulses lasting 1 s before the sequence of larger open states reaches a maximum conductivity of 7 nS (data not shown).

In Fig. 3 it is seen that the presence of pDNA at the positive voltage $V_m = +30$ mV causes a current time pattern with very many short opening-closing events.

4. Discussion

The cationic sphingosine is likely to be the counterionic partner for the interaction of the lipid bilayer with the anionic DNA polyelectrolyte. The interaction is electrically visible provided the voltage drop favours *electrodiffusive* entrance of DNA into the bilayer surface. The transport is preceded by a stronger electrostatic adsorption leading perhaps to an embedding of pDNA into bilayer surface. Upon adsorption of

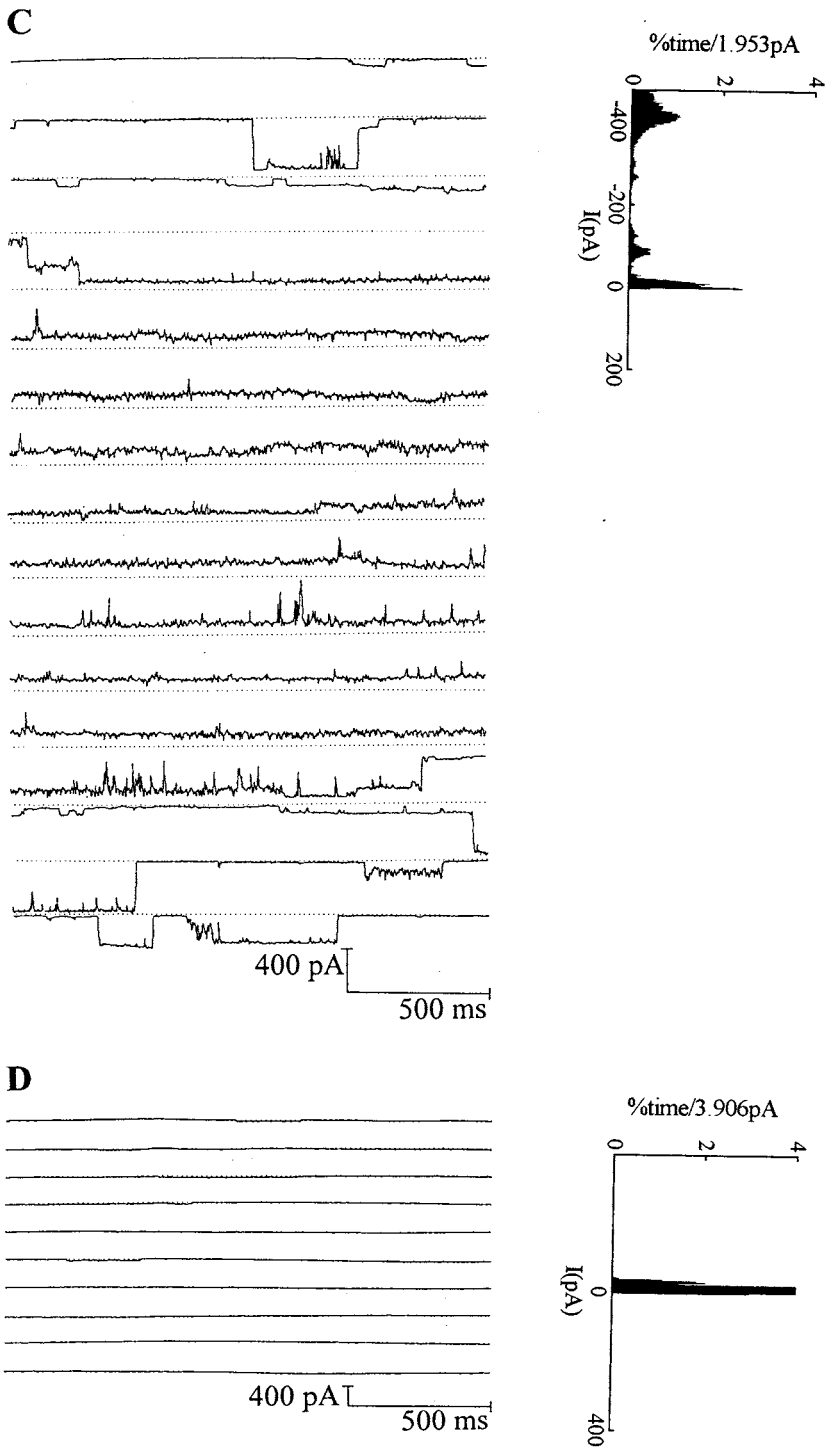

Fig. 2 (*continued*).

the anionic DNA, the cationic sphingosine head groups are probably accumulated in the area of attachment of the adsorbed parts of the long nucleic acid molecules. If plasmid DNA is adsorbed, local ridge-like contact zones with higher curvature may be formed (Fig. 4). Higher curvature facilitates electric pore formation [16] and pore percolation [17] along the attached parts of the adsorbed pDNA.

As discussed previously in detail [11], the current is carried by the small ions of the salt KCl; the contribution of a possible permeating DNA is negligibly small; quantitative estimates suggest that the long lasting current pulses are not due to large pores through which DNA electro-diffuses freely.

The porous patches of inserted and embedded DNA probably are elongated small zones topologically favoured in the presence of positively charged wedge-like monoalkyl lipids such as sphingosine. Wedge-like molecules in low concentra-

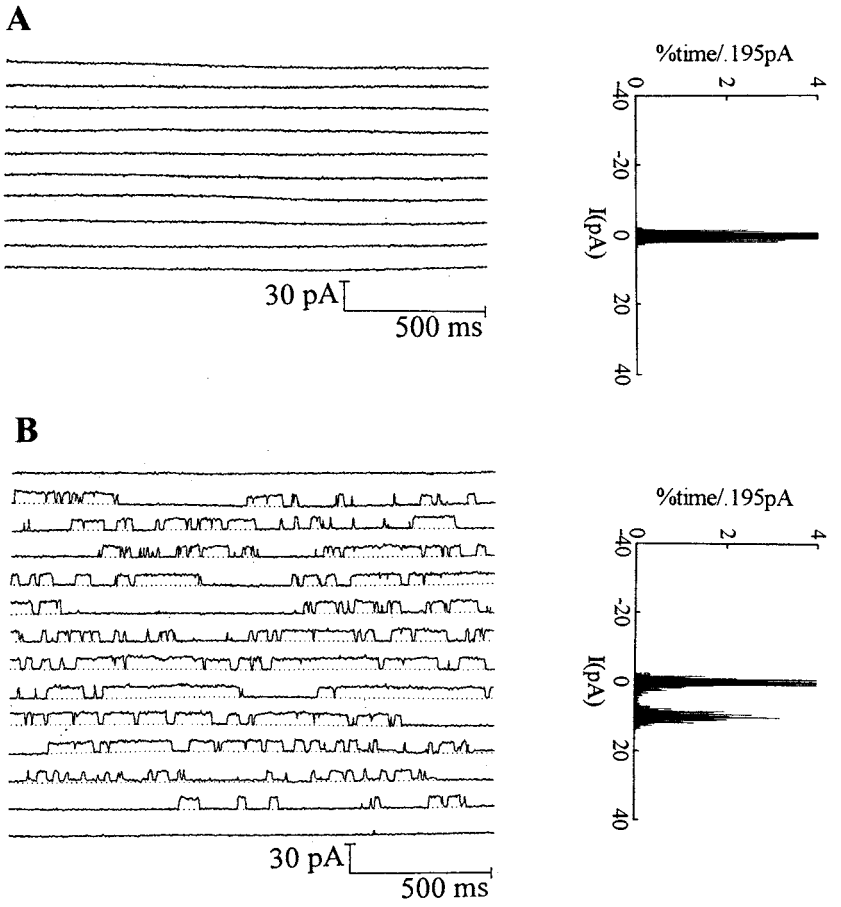

Fig. 3. Current intensity (I) as a function of time at $V_m = +30$ mV. Both records are at 100 mV/pA. A: Control at $V_m = +30$ mV, without pDNA and B: in the presence of pDNA at $V_m = +30$ mV; the sequence of open states lasts 24.5 s. The current histogram for trace B exhibits one type of open state (12 pA). The histogram A refers to record of 20.5 s and B of 28.7 s, respectively.

tion in the bilayer will be dominantly found in the wall edges of probably hydrophilic pores (Fig. 4). Although our data can presently be only qualitatively analysed, they suggest, together with the quantitative analysis given previously [11], a simple sequence of steps for the electroporative DNA transport facilitated by sphingosine.

In summary, the channel-like current pulses, caused by pDNA interacting with the cationic lipids, occur only when the DNA is originally at the negative electrical potential side of the bilayer. All estimates are consistent with the interpretation that the adsorbed pDNA is electro-diffusively inserted in, and slowly (within seconds) pulled through, an electroporated bilayer zone along the attached. The electrotransport does apparently not require large open pores with free DNA diffusion. The main new result is that cationic lipids like sphingosine are highly catalytic, facilitating both the attachment of DNA, probably acting at first as counterionic reaction partners for the anionic DNA, and secondly, by providing locally limited 'half-micellar'-like ridges in the contact zones with DNA. The relatively large current pulses, reflecting channel events up to conductance values of 7 nS, may indicate that the presence of cationic monoalkyl lipids renders the bilayer particularly flexible for interactions with anionic polyelectrolytes like DNA.

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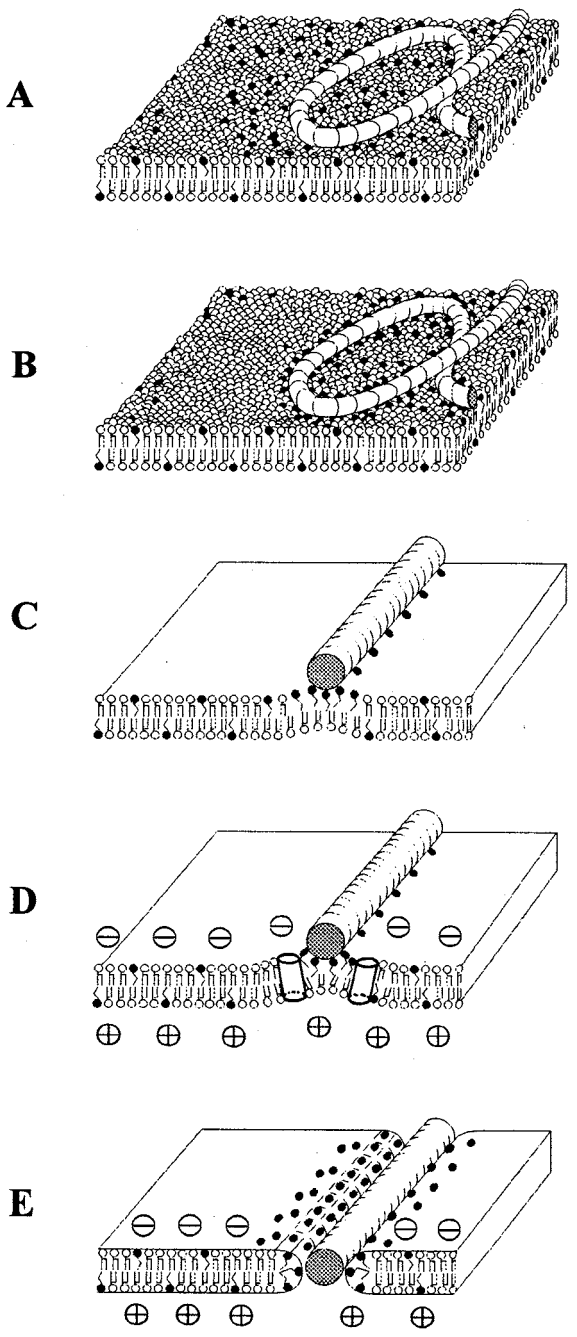

Fig. 4. Scheme for the sequence of events suggested by the data on the electroporative transport of DNA, facilitated by the presence of sphingosine in the bilayer membrane. A: Partial adsorption of a section of pDNA on the bilayer surface. B: The DNA polyanion causes partial phase separation of the cationic sphingosine molecules accumulating near the contact zone of the adsorbed DNA. C: The tendency of monoalkyl surfactants like Sph to form micelles might lead to 'micellar ridges' with higher local curvature. D: Application of a voltage of the correct polarity and of a minimum magnitude (30 mV) leads to local membrane electroporation, i.e. the formation of perhaps hydrophobic pores in the curved parts. A transient intermediate state for the insertion of the DNA into the surface is probably an elongated hydrophilic porous zone. E: Passage of DNA through a hydrophilic percolated porous zone. The DNA largely blocks the 'leaky' macropores. Therefore, these elongated pores are not stable without the presence of DNA, they probably close immediately after the passage of DNA. (Large permanently open electropores would discharge the membrane thereby reducing the polarising field which is necessary to drive water and ions to form the porous zone.)
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