BIOPHYSICS AND BIOCHEMISTRY

Effect of Leukemia-Inhibiting Factor on Bilayer Lipid Membrane

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We studied the effect of leukemia-inhibiting factor on bilayer lipid membranes. Leukemia-inhibiting factor in a concentration of 10 ng/ml nonspecifically increased membrane permeability for ions. Leukemia-inhibiting factor acts as a surface-active substance on bilayer lipid membranes.

Key Words: *cytokines; leukemia-inhibiting factor; bilayer lipid membranes*

Leukemia-inhibiting factor (LIF) is the major component of media for culturing of mammalian stem cells (SC) [2,3,10]. The mechanisms underlying the effect of this cytokine on SC include the interaction with specific receptors and transmembrane protein gp130, which results in activation of tyrosine kinases and transduction factor STAT3 [6,9,11,13]. LIF initiates proliferation of SC and provides their pluripotent state without morphological signs of differentiation [4,5, 10]. In the absence or low activity of LIF, SC develop into embryoid bodies or undergo spontaneous differentiation. Therefore, the content of this cytokine in the medium should be maintained at a relatively high level (10 ng/ml) during culturing. Changes in cell membranes after long-term exposure to LIF and relationship between the state of these structures and pluripotency of SC remain unclear.

Here we studied the effect of LIF on cell membranes. Experiments were performed with the bilayer lipid membrane (BLM).

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MATERIALS AND METHODS

BLM were prepared from azolectin (Sigma) dissolved in n-decane (20 mg/ml). Membranes were formed on 70-100 μ pores in Teflon membranes separated with solutions of 1 M KCl with 40 mM Tris-HEPES buffer (pH 7.2) [8]. The formation of BLM and measurements were performed at room temperature. The results were analyzed using BLM software developed by A. Ya. Zil'bershtein. Patch-clamp recordings were performed at a membrane potential of 100 mV.

LIF (PeproTech Inc.) was used in a concentration of 10 ng/ml. Fibroblast-conditioned medium (FCM) was obtained after 48-h culturing of primary embryonic fibroblasts (isolated from mouse embryos gestational age 12 days) in DMEM containing 10% fetal bovine serum (ICN) at 37°C and 5% CO_2. The culture medium was separated from fibroblasts by centrifugation and sterilized by passing through Millipore filters (pore size 0.22 μ).

RESULTS

Appreciable changes in BLM conductance were observed 20-30 min after addition of LIF to the electrolyte from one or both sides of the membrane. When

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LIF was added from one side, only insignificant changes in BLM conductance (20-140 pA, Fig. 1, *a*) were recorded. During prolonged exposure of BLM to LIF spontaneous variations in conductance (100-600 pA) attaining 1400 pA were observed (Fig. 1, *b*, *c*).

In the presence of LIF on both sides of BLM, conductance was one order of magnitude higher than after addition of this agent only on one side the membrane (Fig. 2). It should be emphasized that the increase in conductance was similar at the same concentration of LIF. The stationary state was not observed up to complete destruction of membranes. This "behavior" of BLM in the presence of LIF did not allow evaluation of the concentration dependency.

These data show that ion conductance was not selective. The results obtained after addition of 10 mM $CaCl_2$ suggested that Ca^{2+} ions are involved in the interaction of LIF with BLM (Fig. 2, *d*). The increase in positive charge of the membrane in the presence of Ca^{2+} was accompanied by a decrease in conductance under the influence of LIF. Short current clamps disappeared, which indicates that Ca^{2+} ions produced a stabilizing effect.

Our results show that LIF possesses surface-active properties and can bind to the lipid matrix and modulate membrane conductance via the formation of nonspecific channels. Previous studies revealed the presence of LIF in FCM [1]. Therefore, we evaluated the effect of LIF on BLM permeability (Fig. 3). FCM changed BLM permeability (similarly to LIF). It should be emphasized that FCM was active in relatively high dilutions (1:40 and more). After increasing FCM concentration in the electrolyte solution the lipid bilayer was not formed on a Teflon membrane, which attested to high surface activity of FCM. It is important that FCM contains not only LIF, but also other substances capable of forming ion channels (Fig. 3, *c*). The recording obtained after removal of FCM from the solution demonstrates high hydrophobicity of active agents.

In high dilutions (1:20 and more) FCM compensates LIF deficiency in the medium for culturing of mouse SC. However, in high concentrations FCM impairs adhesion and suppresses (or even blocks) proliferative activity [1]. These data are consistent with the results of electrophysiological assay (Fig. 3) and confirm the presence of surface-active factors (*e.g.*, LIF) in FCM.

Structurally, LIF is a glycoprotein consisting of 6 amphiphilic α-helixes that contain mainly hydrophobic and positively charged amino acids [7,12]. LIF binds to the lipid matrix and increases ionic permeability of cell membranes via the formation of nonspecific channels of various diameters. Substances with relatively high molecular weight can pass through these channels.

Electrophysiological assay of BLM shows that LIF affects SC not only via the corresponding complex of receptors and transmembrane protein gp130. LIF acts as a surface-active substance and interacts with the lipid matrix. LIF possesses hydrophobic properties and binds to cell membranes, which indicates that the concentration of this cytokine in the medium

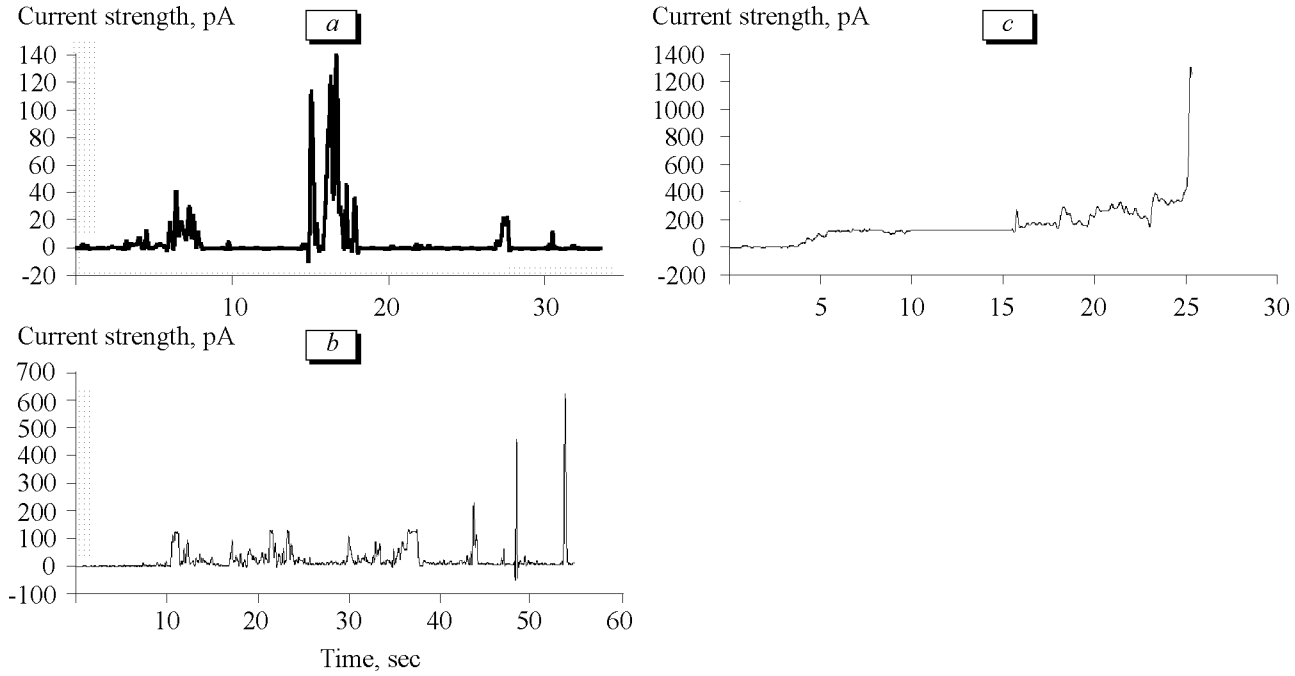

Fig. 1. Fragments of recording of current through the bilayer lipid membrane 20 (*a*), 45 (*b*), and 60 min (*c*) after the addition of 10 ng/ml LIF in the solution of 1 M KCl and 40 mM Tris-HEPES (pH 7.2) on one side of the membrane.

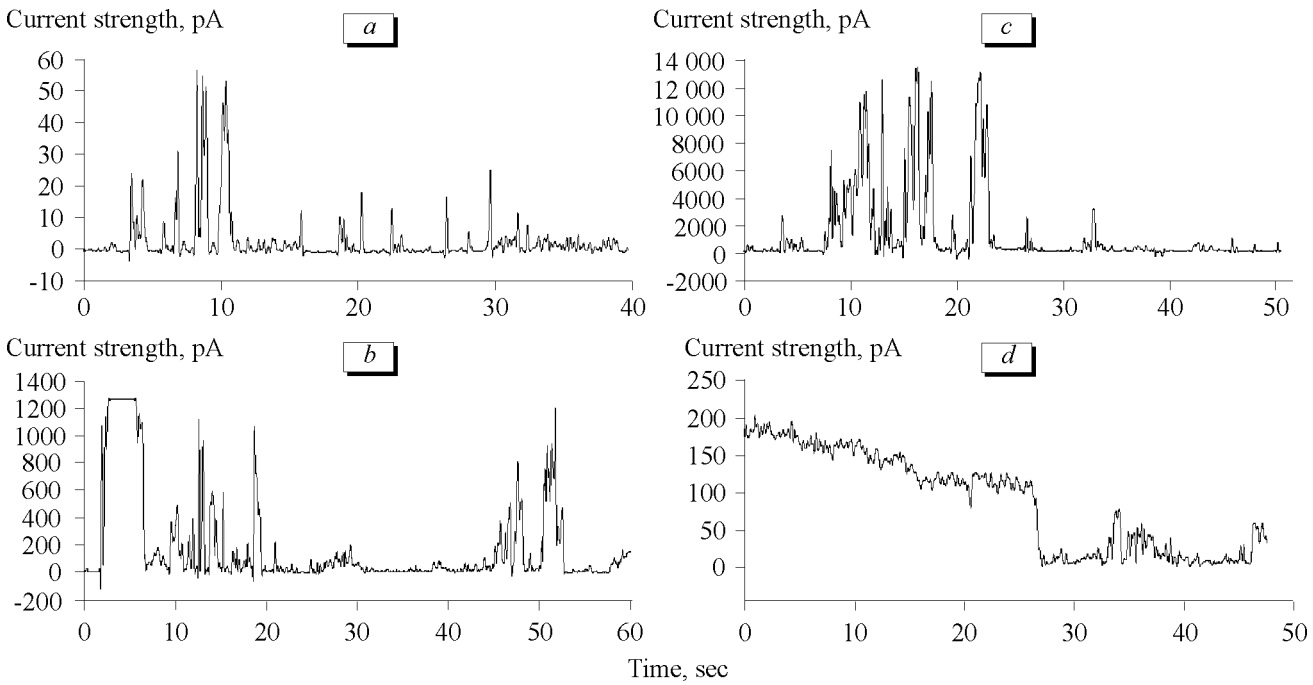

Fig. 2. Fragments of 40-min recording of current through the bilayer lipid membrane 10 (*a*), 20 (*b*), and 40 min (*c*) after the addition of 10 ng/ml LIF on the outer and inner side of the membrane; changes in current on the membrane produced by 10 mM $CaCl_2$ (*d*). The surrounding solution contained 1 M KCl and 40 mM Tris-HEPES (pH 7.2).

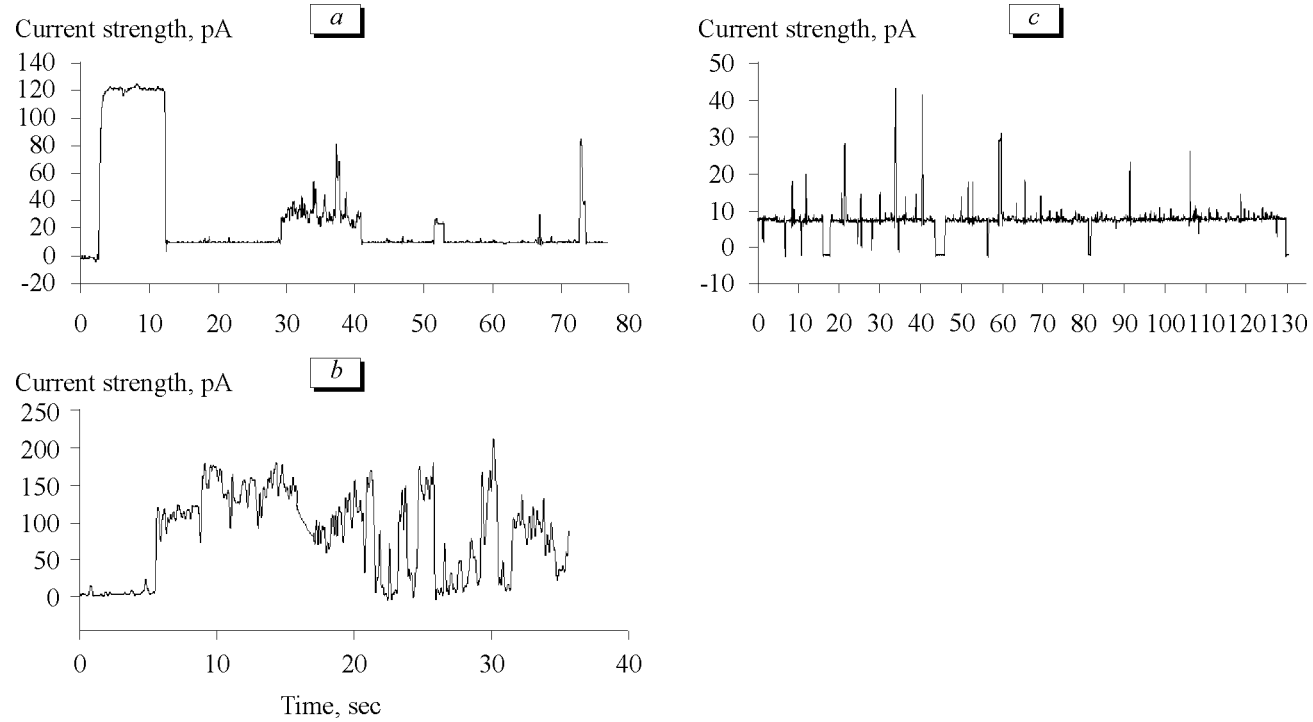

Fig. 3. Changes in conductance of the bilayer lipid membrane immediately (*a*) or 20 min after the addition (*b*) and after removal of 27 μl/ml conditioned medium from the surrounding solution (*c*).

for SC should be maintained at a constant level throughout culturing.

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