ABSTRACT

A miniaturized technique was developed based on the electric-field-induced permeabilization (electroporation) of biological membranes using a low-voltage dc pulse generator and 5-μm-diameter microelectrodes controlled by high-graduation micromanipulators. A spatially highly focused electric field allowed for selective chemical and biological manipulation of single targeted cells in confluent cultures by cytoplamic introduction of polar cell-impermeant solutes.

The method was characterized by using patch clamp recordings and fluorescence microscopy. From transmembrane current responses, the electric field strength necessary for pore-formation, pore-opening and closing kinetics, as well as pore open times were determined. The electroporation pulse preceded pore-formation, and analyte entry into the cells was dictated by concentration, and membrane resting potential driving forces.

Examples of electroporation are shown where Fluorescein, and Fluo-3 are electroporated into the soma of cultured single progenitor cells derived from adult rat hippocampus. Fluo-3 was also introduced into individual sub-micrometer diameter processes of progenitor cells, and a plasmid vector cDNA construct (pRAY 1), expressing the green fluorescent protein, was electroporated into single cultured Cos 7 cells.

It is demonstrated that microelectroporation is a suitable and powerful method for introducing antisense agents into single cells in complex cellular networks. By electroporation of antisense oligonucleotides against signal transducer and activator of transcription 3 (STAT3) into single adult progenitor cells, we demonstrate that ciliary neurotrophic factor (CNTF) is an instructive signal for astroglial type 2 cell fate specifically mediated via the activation of STAT3.

The miniaturized electric-field-induced permeabilization method was also modified for initiation of chemical reactions in individual phospholipid vesicles. Reactions in vesicles, 1-to-5 micrometers in diameter, containing a single reagent or a complete reaction system, were initiated either by electroporation or electrofusion of a pair of vesicles. Product formation was monitored by far-field laser fluorescence microscopy. For example, formation of fluorescein by the enzymatic hydrolysis of its diphosphoester precursos by alkaline phosphatase was monitored in a single liposome. The ultrasmall characteristic of this reaction volume led to rapid diffusional mixing that permits the study of fast chemical kinetics. This technique is also well suited for the study of reaction dynamics of biological molecules within lipid-enclosed nanoenvironments that mimic cell compartments.

A method for cell-cell and cell-liposome fusion at the single-cell level is also described. Spatially selective microelectrofusion of a cell-cell or cell-liposome pair was achieved by the application of a highly focused electric field over the fusion partners. The ability to fuse together single cells opens new possibilities in the manipulation of the genetic and cellular makeup of individual cells in a controlled manner. Fusion of a single liposome with a target cell allows the introduction of the liposomal content into the cell interior as well as the addition of lipids and membrane proteins onto the cell surface. This cell-liposome fusion represents an approach to the manipulation of the cytoplasmic contents and surface properties of single cells. As an example, we introduced a membrane protein (γ-glutamyl-transferase) reconstituted in liposomes into the plasma membrane of a single cultured cell.