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In vivo electroporation of the urinary bladder in mice

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Cell membrane is the major obstacle to incorporation of different substances into cells. Especially in the urothelium of the mammalian urinary bladder, plasma membrane of superficial cells acts as a strong and thick barrier, preventing penetration of exogenous molecules into the cytosol. Electroporation is one of the methods which enable access of different cytochemical labels to the cytosol; so far, however, it has not been used on the urothelial cells in vivo. Therefore, the aim of this study was to determine whether in vivo electroporation of the urinary bladder is a suitable method for introduction of labels into the cytosol of urothelial cells. Labels of various molecular masses were introduced: trypan blue, TRITC-phalloidin and FITC-labelled antibody (IgG). The results demonstrated that electroporation in vivo was a suitable method for introduction of labels into the cytosol of urothelial cells and could be used as a technique for detection of intracellular molecules and studying biochemical reactions.

Key words: bladder; electroporation, trypan blue, fluorescent dyes

Introduction

Electroporation is a method used for introduction of different molecules into the cytosol *in vitro* and *in vivo*.[1,2] Exposure of cells or tissues to short intense electric pulses induces transient electropores in the membrane, which, under suitable conditions, does not affect viability of cells. Recently, electroporation has been used for drug delivery as well as gene delivery *in vivo*.[2,3] Based on preclinical studies, successful employment of electroporation for delivery of chemotherapeutic drugs such as bleomycin and cisplatin has been confirmed also in clinical trials on cancer patients.[4,5] Electroporation is widely used for *in situ* biochemical studies in cells. It has proved its efficiency for introducing molecules of different molecular weight either for labelling intracellular components or for studying biochemical pathways.[6,7]

Urothelial cell membrane is asymmetric, with specific protein uroplakins on the outer surface of the membrane. This asymmetric unit membrane protects the underlying epithelium from the toxic urine and is believed to play a role in strengthening the urothelial apical surface in order to prevent tissue rupture during bladder distension.[8] Functioning as a strengthening and a barrier, the plasma

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membrane of uroepithelial cells is highly impermeable. In order to penetrate this strong barrier, several immunocytochemical as well as electron microscopical methods were employed.[9,10] Due to its broad application electroporation could also be used for introduction of molecules into the urothelial cells. Further, this method has potential application in the treatment of urothelial tumors since introduction of chemotherapeutic drugs into the tumor cells by electric pulses (electrochemotherapy) was demonstrated to be effective. However, those tumors were not grown *in situ*, but transplanted subcutaneously into the back of the mice.[11]

The aim of this work was to determine whether *in vivo* electroporation of the urinary bladder was a suitable method to introduce molecules of various molecular masses into the cytosol of urothelial cells. If electroporation proves suitable in the present study, it could also be used for cytochemical studies, i.e. the introduction of labels into the cells to detect molecular constituents as well as chemotherapeutic drugs.

Material and methods

Mice

In the experiments, an inbred strain of NIH mice was used, purchased from Krka d.d. (Novo Mesto, Slovenia). Mice were maintained at a constant room temperature (24 °C) and natural day/night light cycle, in a conventional animal colony. Before the experiments, the mice were subjected to an adaptation period of at least 10 days.

In vivo electroporation

Adult female mice were anesthetised with an intraperitoneal injection of a mixture of Ketanest 150µg, Rumpun 10µg and atropine 0.1µg per 1g of body weight (Parke-Davis GMBH). Abdomen of the mice was opened and the urinary bladder exposed. After rinsing the bladder with saline, 0.2 ml of the label was injected intravesically. As labels, molecules of various molecular masses were used: 10 mg/ml trypan blue (MW 961) (Sigma), 7.8 µg/ml TRITC-phalloidin (MW 1305) (Sigma) and 10µg/ml FITC-anti rabbit IgG (155 kDa) (Sigma).

Application of electric pulses was performed as described previously.[12] Briefly, electric pulses were delivered by two flat parallel stainless-steel electrodes, 8 mm apart from each other (two stainless-steel strips, length 35 mm, width 7 mm with rounded edges) to the exposed bladder. Eight square wave electric pulses of 720 V or 1040 V amplitude, pulse width 100 µs and repetition frequency 1 Hz, were generated by an electropulsator Jouan GHT 1287 (Jouan, France).

Electron microscopy

Ten minutes after the application of electric pulses, the bladders were fixed with 4% paraform aldehyde and 2% glutaraldehyde in cacodylate buffer, postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer, preembedding stained with uranyl acetate, dehydrated and embedded in Epon. Ultrathin sections were stained with lead citrate and examined in a Jeol 100 CX electron microscope.

Light microscopy

Ten minutes after the application of electric pulses, the bladders were fixed in 4% formaldehyde. After rinsing, the tissue was frozen and sectioned with a cryotom. The sections mounted in a Slow fade mounting solution (Molecular Probes) were examined under a conventional or fluorescent microscope (Laborlux Leiz).

Results

Electroporation

Light microscopy examination of tissue in the bladder exposed to 8 electric pulses of amplitude 1040 V established that large areas of the urothelium were detached from the basal lamina. Undetached urothelial cells were loosely connected to each other (Figure 1).

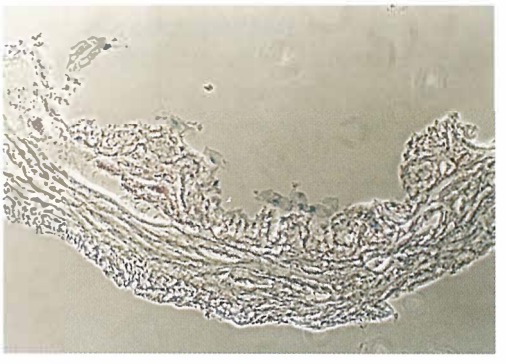

Figure 1. The bladder exposed to electric pulses at an amplitude of 1040 V. Large areas of the urothelium were detached from the basal lamina, 32x.

It was obvious that on the ultrastructural level the most severe changes took place at the layer of basal cells. Many of these cells were split into half, parallelly to the basal lamina (Figure 2). In undetached superficial urothelial cells, facing the bladder lumen, the membranes of fusiform vesicles were torn at the edges where the normal membrane linked two asymmetric unit membrane plaques.

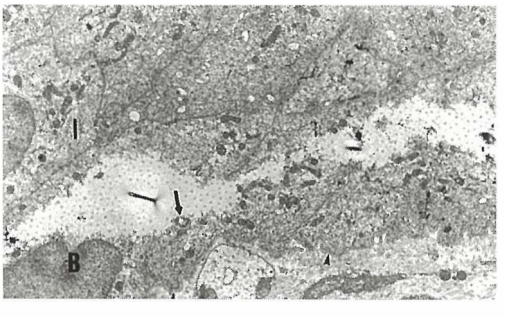

Figure 2. In the tissue, electroporated at 1040 V, tearing of basal cells (B) was evident (↑). In intermediate cells (I) no morphological changes were seen. (V) basal lamina, 2600x.

In order to avoid damage to the cells by electroporation, lower amplitudes of electric pulses were tested in the second set of experiments. No morphological changes were observed in the bladder urothelium when the bladder was exposed to electric pulses of amplitude 720 V (Figure 3). Therefore, all the following experiments were performed at this amplitude of electric pulses.

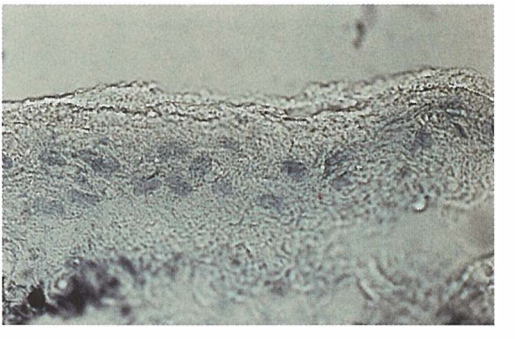

Figure 3. Trypan blue staining of urothelial nuclei (↑). The bladder was exposed to the electric pulses, at an amplitude of 720 V, 128x.

Labelling of the urothelial cells

In the preliminary experiments of this study, it was proved that none of the labels that we used could penetrate the urothelial cells.

After the application of electric pulses to the bladder (amplitude 720 V), trypan blue (MW 961) dye entered the urothelial cells and was evenly distributed over the whole urothelium. Nuclei of all the three epithelial layers were stained intensively. In the connective tissue only sporadic nuclei were stained (Figure 3). TRITC-phalloidin (MW 1305) entered only the superficial urothelial cells and labelled the actin filaments at the basolateral membranes. The actins in the intermediary and basal layer of cells were not stained (Figure 4). Loading of cells with FITC-IgG (MW

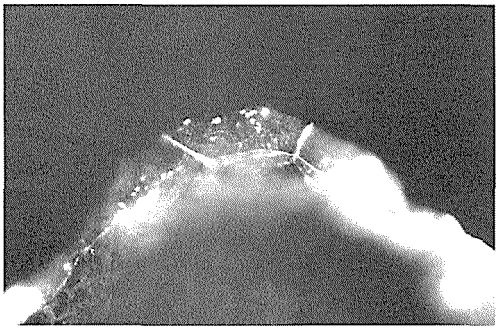

Figure 4. Phalloidin labelling of actin observed mainly at the cell borders of superficial cells. The bladder was exposed to electric pulses at an amplitude of 720 V, 128x.

155 kDa) was successful in approximately 2/3 of superficial cells. The fluorescence was distributed equally in heavy loaded cells. No labelling was found in the cells beneath the superficial ones (Figure 5).

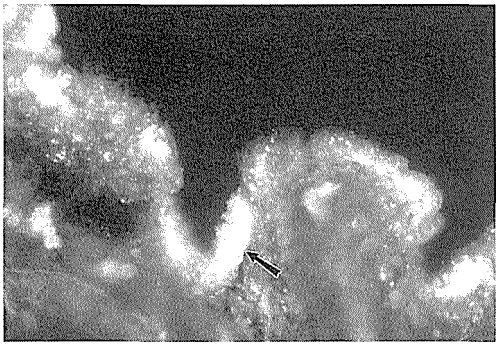

Figure 5. FITC- IgG entered most superficial cells (↑) after the application of electric pulses at an amplitude of 720 V to the bladder, 128x.

Discussion

In this study labels of various molecular masses, trypan blue, TRITC-phalloidin and FITC-labelled antibody (IgG), were introduced into the urothelial cells by electroporation. Our results demonstrate that electroporation enables penetration of the label through a thickened plasma membranes and could therefore be used as an additional technique for the detection of molecular constituents inside the cells and studying biochemical reactions.

Electroporation *in vitro* has already proved its usefulness for labelling intracellular constituents with specific labels which can not enter intact cells.[1] For example, actin network and intermediate filaments were visualized after the introduction of FITC-phalloidin and FITC-vimentin by electroporation of human gingival fibroblasts.[6] Our results demonstrate that electroporation is also a suitable method for introduction of labels into the urothelial cells *in vivo.*

All the labels entered the superficial cells at electric pulses amplitude 720 V without visible changes in cell morphology. The effectiveness of label penetration depended on their molecular masses. The smallest label trypan blue (MW = 961) entered all the urothelial cells, but was mainly prevented from staining nuclei of fibroblasts in the lamina propria. In such cases, basal lamina may act as a barrier. Phalloidin, a molecule that is much larger (MW 1305), labelled the actin filaments only in superficial cells. The label was bound to actin filaments only in basolateral region. Such pattern of actin filaments distribution is also known in urothelial cells which have not been electroporated. The results show that phalloidin has not entered intermediate and basal cells. We can not exclude the possibility that actin from superficial cells has bound most of the phalloidin and so reduced the concentration of phalloidin passing through this dense layer of actin towards intermediate cells, below the sensitivity of fluorescence microscope. Loading of most superficial cells with FITC-IgG provides the possibility of using electric pulses as a new method for labelling cell's constituents in vivo with labels that have a high molecular weight.

In conclusion, this study expands the area of application of electric pulses *in vivo* from electrogene therapy,[3] electrochemothera-

py,[2,4,5,12] transdermal drug delivery[1,3] to *in situ* studies of cellular constituents and biochemical pathways in the bladder.

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