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Stefan Corvin · Simone T. Bösch · Christoph Maneschg · Georg Bartsch · Helmut Klocker

An in vitro model for videoimaging of human bladder smooth muscle cell contractions

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Abstract Knowledge regarding human bladder smooth muscle cell (SMC) physiology is very limited. Only a few specific medical therapies for bladder disorders have therefore been established. The objective of this study was to develop a model for videomicroscopy of bladder SMC contractions. Cells were isolated from human cystoprostatectomy specimens and cultured in a modified EMEM medium. These cells were identified as SMCs by means of immunohistochemistry. For videomicroscopy, the culture flasks were coated with a viscous agent to allow cell contraction. Contractions were visualized by means of a cell culture microscope with a time-lapse videosystem. For cholinergic stimulation of the cells, acetylcholine, in concentrations ranging from 100 μM to 10 mM, was applied. The percentage of contracting cells within the observation field was evaluated for quantitative analysis. In control experiments without contractile stimulant 6% of the cells were observed to contract. Stimulation with acetylcholine induced a significant dose-dependent increase to 47% in contracting cells. These results demonstrated that videomicroscopy is an appropriate tool to investigate the contraction mechanisms of bladder SMCs. This model offers the possibility of studying drug effects on the human detrusor in vitro.

Key words Videomicroscopy · Smooth muscle cell · Contraction · Acetylcholine

Introduction

Human detrusor dysfunction leads to a severe loss in quality of life. Detrusor instability may cause urge symptoms and incontinence, whereas hypocontractility can induce residual urine formation leading to recurrent urinary tract infections. The exact mechanisms of bladder smooth muscle cell (SMC) contractility are still not completely understood. Therefore, only a few specific pharmacological agents have been established in the therapy of human detrusor disorders. Most of these drugs deal with the cholinergic activation of the bladder muscle. Anticholinergic substances, for example, are used in the treatment of bladder spasticity, whereas cholinergic stimulants can improve bladder emptying in the hypocontractile detrusor. However, these agents are unspecific and therefore present side effects in other organ systems with a cholinergic innervation. Most in vitro research has been performed on isolated bladder muscle tissue strips [6, 7, 13, 14, 22, 23]. In these models, however, influences from neighboring cells and connective tissue components make interpretations very difficult sometimes. Only a few studies exist on the investigation of bladder SMC contractions on a cellular level [5, 8, 10, 11, 21]. A cell culture model of human detrusor cells may lead to a better understanding of the contraction mechanisms. The aim of this study was to develop a model for videomicroscopy of SMC contractions. Using this model, contraction mechanisms as well as pharmacological effects can be investigated in vitro.

Material and methods

Cells were isolated from human bladder tissue obtained from cystoprostatectomy specimens from patients with bladder cancer. Tissue specimens were examined histologically by fast frozen section to exclude tumor invasion. Cells were cultured using a tissue explant culture technique. Bladder detrusor tissue was cut into small pieces, which were attached to the bottom of the culture flask (Falcon Labware, Meylan, France). A modified Eagle's mimimal essential medium (EMEM) , with 10% fetal calf serum, 1% penicillin–streptomycin solution, and 2.5% EMEM nonessential amino acids, was used. A first cell passage with trypsin was performed, when about 50% of the culture flask was covered with cells. Further passages followed in intervals of 4–7 days depending on growth velocity. Cells were characterized using immunohisto-

S. Corvin (✉)
Department of Urology, Ludwig-Maximilians-Universität, Marchioninistrasse 15, D-81377 Munich, Germany
e-mail: Stefan.Corvin@uro.med.uni-muenchen.de
Tel.: +49-89-7095-3530; Fax: +49-89-70958890

S. T. Bösch · C. Maneschg · G. Bartsch · H. Klocker
Department of Urology, University of Innsbruck, Austria

chemistry for the specific SMC markers: actin, myosin, and desmin. For the identification of fibroblastic features of the cells, a specific fibroblast antibody was used (Dianova, Hamburg, Germany). Genital skin fibroblasts, as well as the prostatic carcinoma cell line LNCaP, served as controls. In contraction experiments, culture flasks were coated with Cell-Tak (Collaborative Biomedical Products, Bedford, Mass.), a mixture of polyphenolic proteins from mussels in a concentration of 4 $\mu g/cm^2$. For videomicroscopy, a cell culture microscope with a magnification of ×200 was used. An incubation chamber around the microscope maintained constant physiological conditions for the cells. Because of the low contraction velocity, a video system with a time-lapse system was mandatory. This system allowed an observation in sequences representing 1.1 sec real time per frame. Nine to twenty-six (mean 15) cells were observed in the video field in each experiment. After a control period of 1 h, acetylcholine dissolved in culture medium in concentrations ranging from 100 μM to 10 mM was added as the contractile cholinergic stimulant. The cells were observed for a further 2 h after addition of the agent. In control experiments an equal amount of culture medium without stimulant was added. For adrenergic stimulation of the cells, phenylephrine was used. Concentrations of 10 and 100 μM, which have previously been shown [3] to induce contractions of prostatic SMCs, were applied. Contraction studies with acetylcholine were also performed in human prostatic SMCs and rhabdosphincter muscle cell cultures. For quantitative analysis, video sequences were observed at 50 times the velocity. Only those cells showing shortenings of at least 25% of initial cell length within 30 sec were defined as contracting. The percentage of contracting cells in the video observation field was evaluated. For statistical evaluation, the Kruskal–Wallis test was used.

Results

After 3–5 days, cells were seen to emerge from the tissue slices. A first cell passage was performed after 10–14 days. Only cells with stromal configuration were observed in the culture. Immunohistochemistry demonstrated these cells to express typical SMC markers, such as actin (Fig. 1), myosin, and desmin. A negative staining was seen with the fibroblast antibody, identifying these cells as SMC. Genital skin fibroblasts, which served as control, were negative for the SMC markers but positive for the fibroblast antibody, whereas prostatic carcinoma cells were negative for all markers. Contraction studies started with the second to third cell passage. Coating of the culture flasks was essential to allow cell contraction. Without coating only sporadic contractions were observed. Contraction velocity, however, was not altered by culture flask coating. The fastest contractions (< 1 sec) were also seen at real time velocity; however, a better documentation was provided by the time-lapse system (Fig. 2). Contractions appeared as shortenings of the cells and could be distinguished clearly from other cell movements, such as pseudopadal extension. A maximum shortening of 80% was observed. The number of contracting cells, however, and not the degree of cell shortening, in contrast to investigations with single-cell electrical stimulation [10], showed significant differences between different dosages of acetylcholine. Under control conditions without contractile stimulant 6% of the cells were seen to contract during the 2-h observation period. Addition of acetylcholine induced a dose-dependent increase of cell contractility. Acetylcholine was shown to be most effective at a concentration of 10 mM, with 47% of cells contracting (Fig. 3). Two hours after the addition of the agent the contraction rate was seen to decrease. Therefore, the observation was not prolonged. Phenylephrine as an adrenergic stimulant had no effect on the contraction rate. In human rhabdosphincter cells acetylcholine showed a similar contractile response [4]. A significant increase in the contraction rate of sphincter cells was observed at a concentration of 10 mM. Pros-

Fig. 1 Immunohistochemistry demonstrates a positive staining for specific SMC markers like α-actin

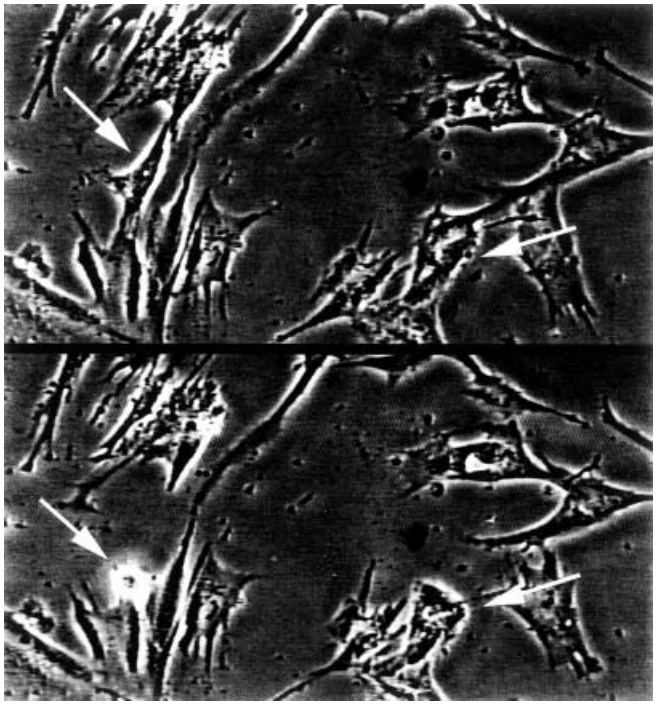

Fig. 2 Videomicroscopy allowed an excellent documentation of cell contractions. The cells marked by *arrows* were seen to contract within this microscopic field

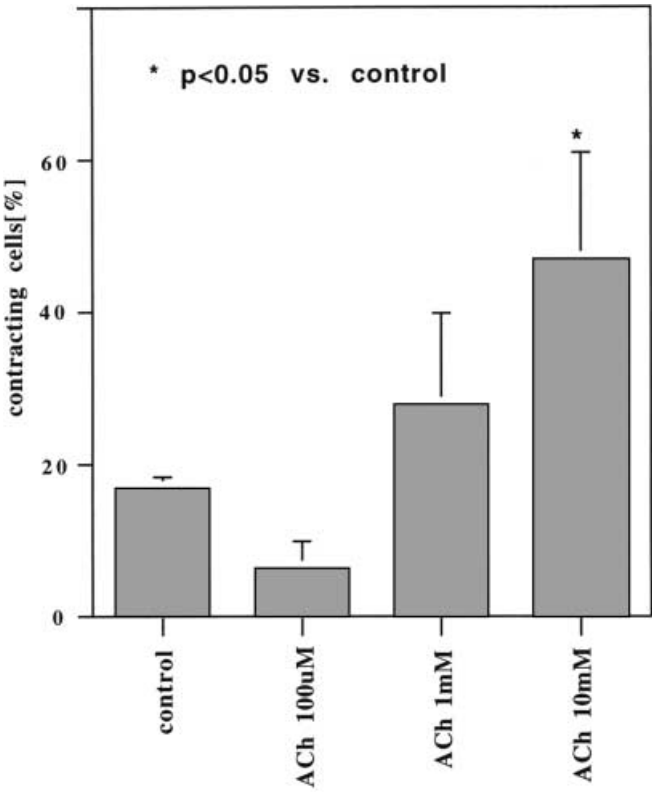

Fig. 3 Cholinergic stimulation of the cells with acetylcholine (*ACh*) induced a dose-dependent increase of contracting cells to a maximum of 47%. The histogram presents values and standard deviations of 22 independent experiments with nine cell strains

tatic stromal cells, however, which have been shown to be activated by adrenergic stimulants such as phenylephrine, did not contract upon stimulation with acetylcholine.

Discussion

Detrusor smooth muscle has not been studied as extensively as that of other tissues, such as the vascular or respiratory system. Owing to this lack of knowledge, medical treatments of bladder dysfunctions like detrusor instability are still far from satisfactory. Understanding the contraction mechanisms of bladder SMCs may be the key to establishing new drugs for the treatment of bladder dysfunctions. Various models have been established for the investigation of detrusor physiology. In vivo studies use mainly laboratory animals, such as cats, rats, and others [9, 12]. However, published work has shown important differences between humans and laboratory animals in detrusor physiology, which make studies on human tissue more relevant [20]. Most investigators studying detrusor physiology use isolated muscle strips obtained from humans or animals. In these studies changes in tissue tension are measured after the application of contractile stimulants in special organ baths [6, 7, 13, 14, 22, 23]. Tissue preparations contain SMCs as well as nonmuscular components representing properties of the whole detrusor muscle. In this model experimental results depend strongly on tissue preparation and quality, making interpretation and comparison of different experiments difficult. Furthermore, experiments must be performed immediately after tissue isolation, when the cells are still viable. The knowledge of detrusor physiology on a cellular level may lead to the development of more selective drugs for the treatment of bladder dysfunctions. Investigations can be performed either with cell culture models or freshly isolated cells [10]. The advantage of a cell culture model compared with isolated cells is the great number of cells available, allowing various experimental assays in the same culture. Primary cell cultures can be obtained from tissue specimens either by enzymatic digestion [19, 24] or a tissue explant culture [1, 11, 18]. Because of the limited amount of tissue available, the explant culture technique was used. Primary cell cultures very often do not completely differentiate to the cell type of the original tissue. Stromal cells like SMCs and fibroblasts do not represent stable cell phenotypes, showing transitions between the two cell types [2, 17]. By using the modified EMEM medium it was possible to culture cells expressing specific features of SMC as shown by the immunohistochemistry. A negative immunohistochemical staining with the specific fibroblast antibody characterized the cells as well-differentiated SMCs. However, conditions in a two-dimensional cell culture are quite different to those found in the natural environment of the human tissue. In a cell culture flask, for example, no supporting extracellular matrix is present. Only few reports exist of experiments with detrusor SMC cultures. In these studies, mainly receptor physiology or intracellular calcium fluctuations have been investigated [5, 8, 11, 21]. Results demonstrate, for example, an increased calcium uptake in cells after cholinergic stimulation. Our results demonstrate that videomicroscopy is an applicable method for the documentation of SMC contractions. As shown in vivo and in vitro, acetylcholine induces cell contractions by activation of muscarinic receptors on detrusor cells [16]. Anticholinergic medication is the main therapeutic option in the treatment of detrusor instability. The maintenance of cholinergic responses in culture adds credibility to this system as a representative model of human detrusor physiology. To identify contractions after cholinergic stimulation as a specific phenomenon, cells were also stimulated with the adrenergic agent phenylephrine. This agent has been shown to be an effective contractile stimulant for prostatic SMCs [3]. In the present study, however, phenylephrine did not increase the contraction rate of bladder SMCs. Acetylcholine was also applied to other muscle cell cultures. In prostatic SMCs, for example, which have previously been shown [3] to be stimulated by adrenergic agents (phenylephrine), no contractile response was observed after the application of 100 μM to 10 mM acetylcholine. Acetylcholine, however, significantly increased contrac-

tility of cultured human rhabdosphincter cells [4]. These results demonstrate that videomicroscopy of cultured bladder SMCs is a reliable and specific model for the investigation of detrusor physiology and pathophysiology. This easy model allows an investigation of drug effects on a cellular level and might help to reduce expensive clinical studies or animal experiments. Investigation of additional contraction stimulants might offer promising therapeutic innovations in the treatment of detrusor dysfunctions [18].

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