509

Wood D[1], Fry C [1]
1. Institute of Urology

THE ISOMETRIC CONTRACTILE CHARACTERISTICS OF CULTURED HUMAN DETRUSOR SMOOTH MUSCLE CELLS AND THEIR RESPONSE TO CARBACHOL.

Aims of Study

The use of cell cultures as a means of generating urological tissue implants (1) and models is a reality (2). However, in order for cultured cells to be appropriately deployed it is crucial to characterise the functional phenotype of these cells for both applications. Using a cultured force monitor, previously used to assess cells involved in wound contraction (3) we have measured the force generated by cultured human detrusor smooth muscle cells when embedded in a collagen gel. A further aim was to develop an experimental system that would measure tension changes generated by these cells in the presence of physiological agonists. Other authors have not been able to demonstrate this latter feature (4).

Methods

Cultured human detrusor smooth muscle cells from human bladder biopsies were cultured in a D-valine supplemented culture medium (4). Biopsies were obtained with Local Regional Ethical Committee approval and informed patient consent. Cultured myocytes were trypsinised and the resulting suspension was added to rat's tail collagen solution (First Link, Brierley Hill, West Midlands, UK), and solidified at 37°C and bathed in culture medium, supplemented with antibiotics and 10% foetal bovine serum. The gel was attached to metal A-frames linked to a Cu-Be strain gauge (the variable arm of a Wheatstone-bridge network). Following calibration the data were used to give a force measurement generated by the gel and the cells. The curves for a control gel (i.e. containing no cells) and a cell containing gel were initially tested and found to be different by more than two standard deviations of the control gel data throughout the duration of the experiment. The final measurements for force generated by cells were obtained by subtraction of a mean value of control gel force from that of cell-containing gels at each time point.

Results

Cell-free control gels generated an initial exponential increase of tension (time constant 122s) followed by a small additional creep. For inter-experimental comparison values were sampled at 1000 minutes. Cell-free gels generated 0.120±0.028 mN at this time.

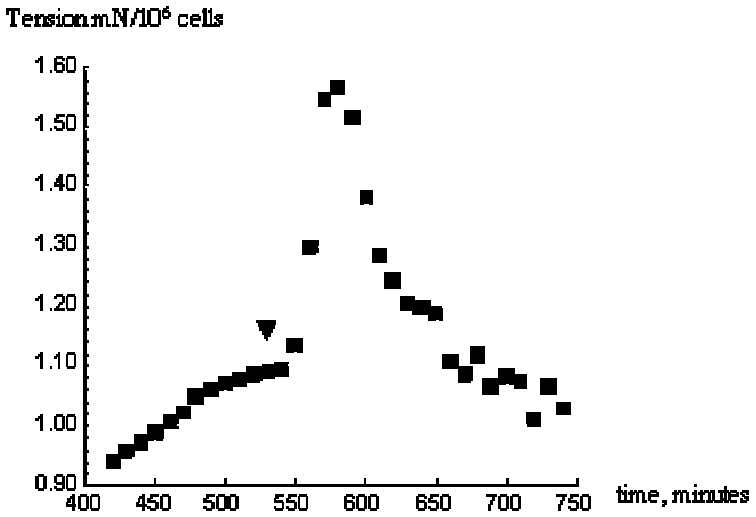

Figure 1 The change of force generated by the application of 200µM carbachol to the culture medium bathing a cell-containing collagen gel. Carbachol was added at the time indicated by the arrow.

Cell force at 1000 minutes (cell-containing minus cell-free force) was 0.264 mN/10^6 cells (0.159–0.579, n=10), representing a median force of $2.64.10^{-7}$ mN per cell. During the initial period cell-force developed with a rate of rise of 2.51±1 87 µN.min^{-1} per 10^6 cells over a period of 253±157 minutes (n=10) after which it stabilised to a constant plateau. The addition of 200 µM carbachol during the constant phase increased cell-force by 0.35±0.13 mN/10^6 cells, which was 60±23% above the plateau level. Figure 1 shows a typical experimental response. Addition of an equivalent volume of culture medium elicited no response indicating the experimental response was not an addition artefact.

Conclusions

Cultured human detrusor smooth muscle cells are capable of generating a contractile response in a collagen gel which was altered by carbachol. The requirement for high concentrations of carbachol may be due to the diffusion properties of the gel. Experiments with a lower serum content in the culture medium may reduce the required concentration of carbachol due to increased muscarinic receptor expression (6).

References

1. Falke G et al. *World J. Urol*. 2000. **18:** 36-43
2. Oberpenning et al *Nat Biotiech* 1999; **17**: 149-155
3. Eastwood M et al *BBA* 1994: **1201**; 186-192
4. Sui GP *et al*., *J Urol* 2001; **165**: 627-632
5. Yang CM *J.receptor res.* 1990; **10**: 235-247
6. Kropp B. et al *J.Urol*. 1996. **155**: 2098-2104