Wednesday 25 June 14.30–15.30
Poster Session 10: Physiology
Chairmen: M. Drake and N. George

P090

Long-term *in vitro* culture and characterization of human urinary tract smooth muscle cells

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BACKGROUND

Cultured human urinary tract smooth muscle cells (SMC) are useful for investigating urinary tract dysfunction and in the regeneration of urological organs using tissue-engineering techniques. Methods for culturing SMC are not well described and currently two main approaches are used, i.e. explant and enzymatic culture. Explant culture provides a continuous source of cells, although the initial yield is poor, while enzymatic extraction provides a high initial yield of SMC, but lacks a continuous source of cells, and the SMC isolated have been reported to loose their phenotype after four passages [*Neurourology* 2002; **21**: 71–9]. The objective of this study was to develop an improved method of isolating and culturing urinary tract SMC that provides a high yield of SMC which retain their phenotype after multiple passages.

METHODS

Human SMC were isolated from the stroma of the urinary tract by mincing the tissue into 1 mm specimens before incubating for 2 h with collagenase IV (100 U/mL) on a shaker. The resultant explants were seeded in a minimum amount of culture medium to initiate culture. Immunostaining was used at each passage to assess SMC phenotype.

RESULTS

In culture the cells had a spindle-shaped morphology with a centrally located oval nucleus and formed the 'hill and valley' formation at confluence. Immunostaining revealed that the cells were strongly positive for α-smooth muscle actin, calponin and desmin, and weakly positive for smooth muscle myosin. The cells expressed extracellular matrix components including fibronectin, laminin, collagen types I, III and IV. Cells retained this pattern up to the 10th passage.

CONCLUSION

We describe an efficient method of propagating SMC that retain their phenotype for prolonged periods in culture. Current work is directed towards applying these cells in urology tissue engineering.

Funding: British Urological Foundation

P091

Effect of nitrergic stimulation and inhibition on ureteric peristalsis in the pig model: a chronic study

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INTRODUCTION

We tested the hypothesis that stimulation and inhibition of nitrergic receptors leads to a decrease and increase, respectively, of ureteric contractile function *in vivo*.

MATERIALS AND METHODS

Twelve female pigs (68–76 kg) were studied; an electronic pressure-monitoring catheter in the right ureter was left in situ. Nephrostomy, arterial, venous and cystostomy catheters were placed. Ureteric peristalsis was studied before and after administering NΩ-nitro-L-arginine (LNNA) and L-arginine.

RESULT

There were systemic effects of the agents; LNNA resulted in an increased amplitude of ureteric peristaltic force in the distal ureter and of the hydrostatic pressure in the pelvicalyceal system. L-arginine did not affect the contractility of ureter but did result in a significantly higher diuresis. The frequency of peristalsis and the length of the contracted segment of ureter were unaffected by either the agonist or antagonist of nitric oxide synthesis.

CONCLUSION

Biological effects of nitric oxide on ureteric motility are regionally localized. Functional effects of nitric oxide correspond to its morphological distribution. Inhibition of the nitric-oxide synthase-positive nerves modulates phasic contraction of the distal ureter. It also increases tonic activity of the ureteric muscle, resulting in a higher hydrostatic pressure in the renal pelvis.

Funding: The Netherlands Organisation for Scientific Research

P092

The measurement of force generated by cultured human detrusor smooth muscle cells and their contractile response to carbachol

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INTRODUCTION

Cell culture is a means of generating tissue implants [*World J Urol* 2000; **18**: 36–43] and models; it is crucial to characterise cells for both applications. Using a cultured force monitor, previously used to assess cells involved in wound contraction [*BBA* 1994; **1201**: 186–92] we measured the force generated by cultured human detrusor smooth muscle cells (CHDM) and modified that force using carbachol.

MATERIALS AND METHODS

CHDM from human bladder biopsies were cultured as described previously [*J Urol* 2001; **165**: 627–32]. Biopsies were obtained with ethical committee approval and patient consent. The suspension was added to rat's tail collagen solution (First Link, Brierley Hill, West Midlands, UK), solidified at 37°C and bathed in culture medium. The gel was attached to metal A-frames linked to a Cu-Be strain gauge (the variable arm of a Wheatstone-bridge network).

RESULTS

The mean (interquartile range) force at 1000 min was 0.264 (0.159–0.579) mN/10^6 cells, representing a median force of 2.64 x 10^{-10} N per cell. Force develops with an initial mean (SD) rate of rise of 2.51 (1.87) μN/min/10^6 cells over 253 (157) min (10 samples). Carbachol increased the force by 60 (23)% above the pre-addition tension. An increase of 0.35 (0.13) mN/10^6 cells (three replicates).

CONCLUSION

CHDM are capable of generating a contractile response which was altered by carbachol. Previous authors have not reported this [*J Urol* 1996; **155**: 2098–104).

Funding: BUF/Blackwell Science, St Peters Trust

P093

Features of ATP-release from the wall of pig and human urinary bladder

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INTRODUCTION

The purinergic system is involved in both the sensory and motor bladder functions in animals, and may be important in pathological conditions in man. This study investigated ATP release from the pig and human bladder dome.

MATERIALS AND METHODS

Bladder strips were placed under 1 g tension in baths containing Krebs solution and were subjected to varying degrees of stretch (up to 50%) and electrical field stimulation (10–40 Hz). Experiments used urothelium, muscle and full-thickness bladder wall. A luciferase assay was used to quantify ATP release. The effect of the neurotoxin tetrodotoxin on ATP release to estimate non-neuronal ATP was also assessed. The results were analysed statistically using Student's *t*-test.

RESULTS

There was a significant release of ATP over basal level by both mechanical and electrical stimulation (both $P < 0.05$). The ATP release from pig bladder, at a mean (SD) of 37.6 (13.46) pmol/g of tissue with electrical stimulation and 12.25 (3.03) pmol/g with stretch, was comparable to the release from the human bladder, at 49.07 (22.1) pmol/g of tissue with electrical stimulation and 12.77 (1.65) pmol/g with stretch, under similar conditions. The main source for ATP release was the urothelium, at 13.3 (2.96) pmol/g of tissue, and not the muscle, at 2.14 (0.72) pmol/g ($P < 0.05$). Release of ATP by electrical stimulation but not stretch was sensitive to tetrodotoxin.

CONCLUSION

Human and pig bladder appeared to be similar in releasing ATP; this appears to be principally neuronally mediated with an additional non-neuronal component. These findings support the view that ATP released primarily from the urothelium may have an important functional role in both sensory and motor detrusor function in humans.

P094

Loss of modular autonomous activity with ageing in the isolated mouse bladder

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INTRODUCTION

Modular autonomous activity in the bladder has been identified as a possible contributory factor in detrusor overactivity. We studied the isolated whole mouse bladder, to ascertain whether altered modular activity could be a causal factor in detrusor dysfunction associated with ageing.

MATERIALS AND METHODS

Two groups of nulliparous female C57bl mice were studied, i.e. 'young' (aged 3–4 months, six) and 'ageing' (14–18 months, seven). The

bladders were microsurgically removed and mounted in whole-organ tissue baths. Intravesical pressure and simultaneous registration of intramural contractions were recorded under standard conditions.

RESULTS

Modular autonomous activity, consisting of localised contractions and propagating waves, were recorded after equilibration in all the young mice but in none of the ageing group. The specific M3-agonist arecaidine elicited a compound response in young mice, comprising a shift in baseline pressure caused by direct muscle stimulation, with superimposed fluctuations resulting from non-myogenic stimulation. During the agonist response, the bladder surface manifested dramatically complex contractile activity. The ageing mice showed a baseline pressure shift, with no superimposed fluctuations and none of the complex contractile activity seen in young mice. Tetrodotoxin did not influence autonomous activity or the arecaidine responses.

CONCLUSIONS

Ageing is associated with reduced modular autonomous activity in the isolated bladder, with loss of phasic pressure fluctuations and modular contractile activity evoked by muscarinic stimulation in young mice, while the direct muscle response is preserved. These observations provide a possible basis for age-related bladder dysfunction, including detrusor failure and increased postvoid residual volumes.

Funding: Special Trustees of the Royal Victoria Infirmary

P095

Electromyographic detection of purinergic signalling in isolated unstable human bladder

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INTRODUCTION

Nerve-evoked, atropine-resistant contractions can be generated in detrusor muscle samples taken from overactive bladders in humans, and probably originate from purinergic (ATP-activating P2X receptors) neurotransmission. In contrast to cholinergic mechanisms, purinergic activation involves membrane depolarisation and may be detectable using extracellular electrical recording techniques. We tested the hypothesis that dysfunctional detrusor has a greater propensity to generate electromyographic activity than normal by virtue of purinergic mechanisms.

MATERIALS AND METHODS

Focal electrical recordings were made using validated suction electrodes from field-stimulated human detrusor strips taken at operation from 24 clinically evaluated bladders. The evoked signals were pharmacologically characterised and compared with the expression of atropine-resistant contractions in the same samples.

RESULTS

A nerve-evoked, artefact-free electromyographic signal was recorded from five detrusor strips. These all originated from idiopathically overactive or obstructed but not hyper-reflexic or stable bladders. The signal was consistently abolished after desensitising purinoceptors but unaffected by atropine, and was recorded from strips that expressed significantly greater atropine-resistant contractile activity than those strips from which no electrical signal was recorded.

CONCLUSIONS

Detrusor from functionally normal human bladders appears to be electromyographically inert. In contrast, the expression of purinergic contractile mechanisms in idiopathically overactive and obstructed, but not hyper-reflexic, isolated human detrusor is associated with an electromyogram. These studies show for the first time electromyographic recordings from human detrusor smooth muscle. Moreover, they provide direct electrophysiological evidence for purinergic signalling in the dysfunctional bladder, and a technique capable of evaluating clinically, and differentiating from neuropathy, this putative purinergic myopathy.

Funding: Research into Ageing Fellowship

P096

The influence of host immune factors in the development of UTI

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INTRODUCTION

UTI is a common and potentially life-threatening illness. Although much is known about the bacterial factors that enhance pathogenicity, little is known about host factors that influence outcome. The host immune response, including complement activation, is vital to clear infection but has been implicated in the development of scarring. We therefore investigated the influence of C3 deficiency in experimental UTI.

MATERIALS AND METHODS

Ascending infection was induced in mice by inoculating *Escherichia coli* into the bladder (urethral catheterization). Infection was induced in mice deficient in C3, the pivotal complement component, and control mice. The adherence of fluorescent-labelled bacteria to cells of the urinary tract was determined by fluorescence-activated cell sorting. Internalisation of bacteria was determined using the gentamicin protection assay.

RESULTS

Ascending infection developed in 75% of complement-sufficient mice but in < 10% of the C3-deficient mice. During infection the concentration of C3 in the urine increased 400-fold, reaching sufficient concentrations to opsonise bacteria, as shown by immunofluorescence. Opsonization did not alter the adherence of bacteria to either bladder or renal epithelial cells. However, internalisation of bacteria by both bladder and renal epithelial cells was increased 10-fold when bacteria were opsonized with C3. In addition, C3 protein produced by renal tract epithelium was sufficient to enhance internalisation.

CONCLUSION

Internalisation of bacteria into epithelium has been implicated as a mechanism by which bacteria increase infectivity. We provide evidence that bacteria use host defence proteins to increase internalisation. We hypothesise a C3-receptor interaction promoting active uptake of bacteria and establishing infection.

Funding: Wellcome Trust

P097

A novel nitric-oxide-independent soluble guanylate cyclase activator (BAY 41-2272) relaxes human and rabbit corpus cavernosum

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INTRODUCTION

In cavernosal smooth muscle nitric oxide (NO) activates the enzyme soluble guanylate cyclase (sGC), which catalyses the synthesis of cGMP. This in turn induces smooth muscle relaxation, facilitates increased blood flow and elicits penile erection. A pyrazolopyridine, BAY 41-2272, has been identified to stimulate sGC in a NO-independent manner with no effect on cGMP breakdown. Our aim was to investigate the effect of BAY 41-2272 on the tone of human and rabbit cavernosal smooth muscle.

MATERIALS AND METHODS

Cavernosal strips from male New Zealand White rabbits and humans were mounted in superfusion chambers. Nonadrenergic non-cholinergic relaxation responses elicited by electrical field stimulation. The effect of BAY 41-2272 was investigated in the absence or presence of 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), an sGC inhibitor, or N-nitro-L-arginine methyl ester (L-NAME), a NO-synthase inhibitor. The potency of BAY 41-2272 was compared with that of a currently available sGC activator, YC-1, and a NO-releasing compound, spermine-NONate.

RESULTS

BAY 41-2272 resulted in a concentration-dependent relaxation of both human and rabbit cavernosum, with mean (SD) EC_{50} values of 300.3 (33.8) and 407.5 (22.07) nmol/L, respectively. ODQ caused a concentration-dependent significant rightward parallel shift in the dose-response curve; L-NAME resulted in a small rightward parallel shift only; the EC_{50} with BAY41-2272 was 429.1 (30.9) and 836.7 (46.7) nmol/L in human and rabbit tissues, respectively. BAY 41-2272 was significantly more potent than spermine-NONate, with an EC_{50} of 843.3 (54.4) nmol/L, and YC-1, at 13173.2 (2018.4) nmol/L, at inducing relaxation of rabbit corpus cavernosum.

CONCLUSION

BAY41-2272 causes relaxation of human and rabbit corpus cavernosum more potently than currently available sGC activators (YC-1) and NO donors. This group of compounds may be significant in managing patients with erectile dysfunction with a lack of endogenous NO.

Funding: St Peter's Andrology Trust, The International Society for Sexual and Impotence Research

P098

The effects of ischaemia on corporal smooth muscle contraction and recovery using an *in vitro* pharmacological model

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INTRODUCTION

Ischaemic priapism results in the formation of an hypoxic microenvironment and reduces the availability of glucose for cellular metabolism. Glucopenia and acidosis occur at a late stage in this condition, as indicated by corporal blood analyses from patients with acute priapism. This study aims to assess the effects of these conditions on corporal smooth muscle tone.

MATERIALS AND METHODS

Strips of rabbit corpus cavernosum were mounted in organ baths, precontracted and superfused with Krebs solution bubbled with carbogen. Electrical field stimulation was commenced (50V, 5Hz, duration 0.3 ms). The tissue strips were perfused for up to 24 h and the smooth muscle tone recorded simultaneously in hypoxic, acidotic (pH 6.9) and glucopenic conditions. Experiments were repeated using a 4-h period of ischaemia followed by reoxygenation and superfusion with Krebs solution (pH 7.4).

RESULTS

The results were expressed as a mean (SD) percentage of the initial tone (100% at t_0). Hypoxia alone significantly reduced smooth muscle tone at 4, 8 and 12 h. Using a 4-h perfusion period, hypoxia significantly reduced tone compared with control tissue, to 40.4 (3.3)% (six samples, $P < 0.05$). Corporal smooth muscle tone was significantly reduced further when hypoxia was combined with acidosis, to 20.6 (2)%, or glucopenia, to 16.6 (7.7)%. Reversal of combined hypoxic and acidotic conditions after 4 h allowed corporal smooth muscle to regain the initial tone, at 101.7 (4.23)%.

CONCLUSION

Hypoxia produces a rapid and sustained reduction in smooth muscle tone. Concomitant acidosis or glucopenia produces an even greater reduction in smooth muscle tone, suggesting smooth muscle dysfunction increases with longer periods of ischaemic priapism.

P099

Impaired endothelium-dependent cavernosal relaxation in hyperhomocysteinaemic rabbits: a new model for erectile dysfunction

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INTRODUCTION

It has been suggested that elevated plasma levels of homocysteine may be a risk factor for erectile dysfunction (ED), and that its vasculopathic impact may be mediated via the inhibition of nitric oxide (NO) activity by superoxide (O^{2-}). To investigate the effect of sustained hyperhomocysteinaemia (HHC) on erectile function, a novel animal model was developed using a diet rich in methionine, a precursor for homocysteine.

MATERIALS AND METHODS

In an controlled study, six rabbits were fed a chow supplemented with methionine (20 g/kg) for 1 month. Serial serum homocysteine measurements were taken. Cavernosal strips were mounted in an organ bath and relaxation studied when stimulated with carbachol (10^{-9} to 10^{-4} mol/L), sodium nitroprusside (SNP, 10^{-9} to 10^{-4} mol/L) and nonadrenergic, noncholinergic (NANC)-mediated relaxation (0.5-20 Hz). A23187-stimulated cGMP production was also assessed using cavernosal segments and ELISA. Tissue O_2- production was assessed using the cytochrome c assay.

RESULTS

HHC was confirmed in the methionine-rich diet group. There was a significant inhibitory effect on carbachol-stimulated cavernosal relaxation in the HHC group, but not in SNP-elicited relaxation. NANC-mediated relaxation was also unaffected by HHC. There was a corresponding significant reduction in cavernosal cGMP production (1.95 vs. 4.28 fmol/mg/min), with a significant increase in cavernosal tissue O_2-production (195.7 vs 33.6 nmol/mg/h).

CONCLUSIONS

Methionine-rich diet-induced HHC for 1 month promotes a marked inhibition of endothelium-dependent relaxation and cGMP formation, but not NANC-mediated relaxation, in the isolated rabbit corpus cavernosum.

Funding: Educational grant from Abbott Laboratories