Benign prostatic hyperplasia (BPH) is a stromal disease that subsequently affects epithelial growth. Growth factors activate downstream intracellular pathways, but the linkage to cellular proliferation is unclear. In many cells, growth factors raise intracellular Ca\(^{2+}\) and some calcium channels are not evident in differentiated cells. Ca\(^{2+}\) channels have not been characterized in human prostate smooth muscle, and the aim was to carry out an analysis prior to an investigation of the cellular functions of growth factors.

Prostate samples were obtained, with Ethical Committee approval, from patients undergoing either transurethral resection of the prostate (TURP) or radical prostatectomy. They were stored in Ca\(^{2+}\)-free Heps-Tyrode solution. Isolated cells were prepared by collagenase-based digestion and, in general, this was easier with prostatectomy samples as retrieved tissue was less damaged. Experiments were carried out in HCO\(_3\)/CO\(_2\)-buffered Tyrode solution at 37°C. Whole-cell recordings used patch-electrodes, and whole-cell capacitance was measured on membrane rupture to scale membrane currents to unit capacitance. Ca\(^{2+}\)-based electrode solutions, to block outward currents, were used to record inward currents, and K\(^{+}\)-based solutions to record resting potentials. Data are means±S.D., and differences between data sets \(P<0.05\) were tested using Student’s unpaired \(t\) test.

The resting potential was \(-63\pm11\) mV \((n=8)\) and action potentials were elicited with Ca\(^{2+}\)-filled electrodes; maximum upstroke velocity was about 0.8 V s\(^{-1}\). Inward current was dependent on superfusate Ca\(^{2+}\) with peak current at 0 mV. Current density (holding potential, \(V_h\), \(-100\) mV) was 1.56±0.47 pA pF\(^{-1}\) \((n=15)\). The L-type current was the larger component. At their respective maximum voltages L-type current density was 1.56±0.47 pA pF\(^{-1}\) \((+10\) mV) and T-type current was 0.83±0.16 pA pF\(^{-1}\) \((-20\) mV).

This is the first study to characterize systematically inward current in human prostate smooth muscle. Mean peak net inward current is about 60 % of that in detrusor (Sui et al. 2001) and has two components that have properties of L-type and T-type channels. Of interest is the Ni\(^{2+}\) dependency of the T-type component as there are several isoforms of the \(\alpha\)-subunit. The \(\alpha_{1C}\) subtype has a low affinity for Ni\(^{2+}\) compared to the \(\alpha_{1H}\) subtype (Lee et al. 1999). In detrusor the low affinity subtype is present (Sui et al. 2001) but in prostate the low [NiCl\(_2\)] (20 \(\mu\)M) used for block suggests the \(\alpha_{1H}\) subtype. Thus any manipulation of prostate channel activity could have selective effects over detrusor.
of excitable cells and responded to ATP so that they could form an electrical network to distribute an electrical signal over a reasonable area upon focal depolarisation. These properties are consistent with the hypothesis that these cells form an intermediate stage in the modality of bladder sensation and could act as a variable gain integrating stage.

Sui GP et al. (2002). BJU Int 90, 118–129.

We thank the Wellcome Trust for financial assistance.

All procedures accord with current local guidelines and the Declaration of Helsinki.

C8
Colocalization and expression of β2-adrenergic receptor and large conductance calcium-activated potassium channel in human myometrium

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β2-Agonists have been shown to modulate K+ currents and stimulate the activity of large conductance calcium-activated potassium (BKCa) channels in myometrium (Anwer et al. 1992; Hamada et al. 1994). However, it is not known whether the mechanism of this activation involves a direct or indirect interaction between the β2-adrenergic receptor (AR) and BKCa channels. The aim of this study was to investigate the nature of this association using confocal immunofluorescence and co-immunoprecipitation studies.

This study was approved by the Southern Derbyshire Ethics Committee and written informed consent was obtained from all tissue donors. Myometrial biopsies were obtained from singleton term pregnant women (gestational age >37 weeks) undergoing labour (cervical dilatation >3 cm; n = 6). Tissue was divided into two parts. One half for confocal microscopy was processed to yield cytopins. Thus, isolated myometrial cells were obtained following enzymatic dispersal, and fixed in 2% (w/v) paraformaldehyde for double staining immunofluorescence with the avidin–biotin complex technique. The remaining tissue was stimulated with 1 mM ritodrine (a β2-sympathomimetic) for immunoprecipitation with either β2-AR or BKCa channel antibody. Proteins were resolved by 10% SDS–PAGE then blotted with either β2-AR or BKCa antibody as appropriate.

Confocal microscopic visualization demonstrated the colocalization of β2-AR and BKCa channel proteins in the plasma membrane of human myometrium from both labouring and non-labouring women. Co-immunoprecipitation experiments revealed that β2-AR antibody was able to immunoprecipitate BKCa channel protein and that BKCa antibody was able to immunoprecipitate β2-AR protein in both groups of women.

This study demonstrated that β2-AR and BKCa channel are colocalized in the human myometrium. They are also apparently linked by a direct protein–protein interaction. This close association suggests the synergistic role of these two membrane proteins in myometrial relaxation. Further studies are needed to examine the functional correlation between the β2-AR and BKCa channel in pregnant human myometrium particularly at the onset of labour.


We would like to thank the staff and patients in the Department of Obstetrics and Gynaecology, Derby City General Hospital who assisted with this study.

All procedures accord with current local guidelines and the Declaration of Helsinki.

C9
Role of P1-receptors in the contractile function of guinea-pig and human detrusor smooth muscle

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Nerve-mediated contractions of detrusor smooth muscle are mediated through the release of two neurotransmitters, acetylcholine and ATP (Burnstock et al. 1978). These are rapidly broken down in the synapse by specific extracellular enzymes. The breakdown product of ATP, adenosine, reduces contractions in other smooth muscle to reduce contractures (Lynch & Huddart 1991). Adenosine may modulate contraction either by acting on the muscle or the motor-nerve ending and is believed to act through P1-receptors that are divided into four subtypes (A1, A2a, A2b, A3). The site and subtypes of these receptors in detrusor smooth muscle are unclear and this study has addressed these questions.

Detrusor strips were obtained from guinea-pig bladders or human biopsy samples, the latter obtained with local ethical committee permission and informed patient consent. Guineapigs were humanely killed. Strips were superfused at 37°C in HCO3−/CO2–Tyrode solution (pH 7.35). Contractions were evoked either by field-stimulation of embedded motor nerves (3 s train of 0.1 ms pulses, 1–40 Hz, abolished by 1 mM TTX) or direct activation of the muscle with the acetylcholine analogue carbachol (10 mM). All chemicals except ZM241385 were obtained from Sigma UK. Data are expressed as means ± S.D. (n), where n is the number of experiments. Student’s unpaired t test was used to test for significance between data sets (P < 0.05).

Adenosine (1 mM), the non-selective P1-agonist 5′-(N-ethylcarboxamido)adenosine (NECA) (10 mM) and the A1-selective agonist N6-cyclopentyladenosine (CPA) (10 mM) reduced nerve-mediated contractions in guinea-pig tissue. (63 ± 11% (4), 61 ± 11% (6), 71 ± 11% (10) of control, respectively, 8 Hz stimulation). The reduction by CPA was significantly less than that induced by adenosine or NECA. Concentrations were maximal and were determined for each agent previously (pEC50 values were 3.88 ± 0.83 (4), 6.44 ± 0.29 (6) and 7.01 ± 0.86 (4), respectively). The A2a- and A2b-antagonists ZM241385 (100 nM) and alloxazine (1 mM) had no significant effect. Carbachol contractions were not significantly altered by any P1-agonist. There was a frequency-dependent action of P1-agonists, with greater attenuation at low stimulation frequencies. Nerve-mediated contractions with human detrusor strips from stable and unstable bladders were not reduced by any of the above P1-agonists. Carbachol contractions were also unaffected. Force–frequency relationships in the presence of 2-AR or 2-sympathomimetic) for

2-AR antibody was able to immunoprecipitate

methylene-ATP (10 mM) reduced contractions to a greater proportion at lower stimulation frequencies.
We interpret these data to signify that adenosine and analogues with A1-activity exert a presynaptic action on guinea-pig detrusor preparations, but there is little effect postsynaptically. In human there was little effect by the P1-receptor agonists pre- and postsynaptically. P1-receptor agonists are more effective at reducing contractions at lower stimulation frequencies that coincide with a proportionally higher release of ATP.


We thank St Peter’s Trust for financial assistance and Pfizer Ltd for the gift of ZM241385.

All procedures accord with current UK legislation, local guidelines and the Declaration of Helsinki.

C10

Prostaglandin F$_2\alpha$-induced contraction and increased intracellular calcium in small pulmonary arteries of the rat

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The mechanisms by which prostaglandin F$_2\alpha$ (PGF$_2\alpha$) induces increases in [Ca$^{2+}$], and activates smooth muscle contraction are unclear. We have studied the effects of PGF$_2\alpha$ on contraction and [Ca$^{2+}$] in the rat pulmonary artery using fura PE-3 AM-loaded isolated intrapulmonary arteries.

Rats were humanely killed by cervical dislocation.

Low concentrations of PGF$_2\alpha$ (10–100 nM) caused a transient increase in [Ca$^{2+}$], followed by a sustained plateau but did not induce contraction. Further increases in [PGF$_2\alpha$] (3–30 μM) caused a dose-dependent increase in tension but no further increase in [Ca$^{2+}$]. In nominally Ca$^{2+}$-free solution, the transient component of the Ca$^{2+}$ response was still present but the [Ca$^{2+}$], plateau was abolished. Even without further elevation in [Ca$^{2+}$], tension still increased in a dose-dependent manner. Tension dose–response curves show that $V_{\text{max}}$ in Ca$^{2+}$-free physiological salt solution (PSS) was 70% of that in Ca$^{2+}$-containing PSS, i.e. significantly smaller ($P<0.05$, Student’s paired t test). However, EC$_{50}$ Values were similar: 3.4 ± 0.7 μM ($n=7$, mean ± S.E.M.) in Ca$^{2+}$-containing PSS and 5.1 ± 0.8 μM ($n=7$) in Ca$^{2+}$-free PSS. The [Ca$^{2+}$] transient and plateau could be reproduced with the selective PGF$_2\alpha$ receptor (FP) receptor agonist fluprostenol. However, no increase in tension was caused by fluprostenol even at 10 μM. The [Ca$^{2+}$], transient and plateau evoked by both fluprostenol and PGF$_2\alpha$ could be inhibited with the selective FP receptor antagonist AL-8810, or with thapsigargin. In contrast the thromboxane A$_2$ analogue U-46619 caused a sustained dose-dependent increase in [Ca$^{2+}$], and tension. The selective thromboxane A$_2$ receptor (TP) antagonist SQ29584 completely blocks increases in [Ca$^{2+}$], and tension caused by U-46619, and abolished the rise in tension, but not the [Ca$^{2+}$], response, induced by PGF$_2\alpha$.

These results suggest that PGF$_2\alpha$ elevates [Ca$^{2+}$], via FP receptor-induced Ca$^{2+}$ release from intracellular stores with subsequent capacitative calcium entry. However, this is not sufficient to cause contraction, which requires simultaneous activation of TP receptors.

This work was supported by the Wellcome Trust.

All procedures accord with current UK legislation.

Sphingosyolphosphorylcholine-mediated vasoconstriction of rat small pulmonary arteries

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Bioactive sphingolipids and their metabolites such as sphingosyolphosphorylcholine (SPC) are believed to play an important role in regulating various cellular processes, primarily via receptors of the EDG family. Recent evidence has suggested a role for sphingolipids in the control of vascular tone, but the signalling pathways involved may differ significantly depending on the vascular bed, with calcium entry, release from stores, and activation of RhoA and Rho kinase having variable degrees of importance (Cousin et al. 2002; Shirao et al. 2002). The action of sphingolipids has not been investigated in the pulmonary circulation, where there is evidence that Rho kinase may be of particular importance. We therefore examined the effect of SPC on small (300–500 μm i.d.) intrapulmonary arteries (IPA) of the rat.

Rats were humanely killed by cervical dislocation. IPA were mounted on a small vessel myograph. In some cases intracellular calcium was estimated simultaneously using Fura-PE3. Data are given as means ± S.E.M. and were tested for significance using ANOVA.

SPC (3–100 μM) caused a relatively slowly developing, concentration-dependent vasoconstriction in IPA, which reached a plateau after ~30 min. The maximum tension was 111 ± 13% of the response to 80 mM KCl ($n=7$), with an EC$_{50}$ of 18 ± 6 μM. Removal of the endothelium or application of 100 μM L-NAME caused ~20% increase in tension without altering the EC$_{50}$ ($n=4$ and 7). Simultaneous recording of tension and intracellular calcium showed that the development of tension was associated with a rise in intracellular calcium, although tension continued to increase after calcium reached a steady state. Removal of calcium from the bathing solution shifted the concentration–response curve to the right (EC$_{50}$: 97 ± 43 μM, $n=6$, $P<0.05$), without having a significant effect on maximum tension (135 ± 39%, $n=6$). Once tension had reached steady state following application of 10 μM SPC, application of the L-type calcium channel blocker diltiazem (10 μM) had a relatively minor effect on tension and intracellular calcium. However, the Rho kinase inhibitor Y27632 (10 μM) caused partial relaxation of ~35% ($n=5$).

We conclude that SPC-induced constriction of rat IPA is mediated through both calcium entry, primarily via a voltage-independent pathway, and Rho kinase-dependent calcium sensitisation, but that different receptors may be responsible for these two actions.


This work was funded by the Wellcome Trust.

All procedures accord with current UK legislation.