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RESEARCH PAPER

K^+ channel modulation of slow wave activity in the guinea-pig prostate

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Background and purpose: The aim of this study was to investigate the role of different K^+ channel populations and the inhibitory effect of various exogenously applied K^+ channel openers in the regulation of slow wave activity in the guinea-pig prostate.
Experimental approach: Recordings of membrane potential were made using intracellular microelectrodes.
Key results: Tetraethylammonium (TEA 300 μM and 1 mM), iberiotoxin (150 nM) and 4-aminopyridine (4-AP 1 mM) increased the frequency of slow wave discharge. Apamin (1–200 nM) and glibenclamide (1 μM) had no effect on slow wave activity. Lemakalim (1 μM) and PCO-400 (1 μM) abolished the slow waves, as did sodium nitroprusside (SNP 10 μM) and calcitonin gene-related peptide (CGRP 100 nM). The inhibitory effect of these agents was independent of a significant change in membrane potential. In the presence of 4-AP (1 mM), TEA (1 mM) or glibenclamide (1 μM) the inhibitory actions of SNP (10 μM) were attenuated. The inhibitory actions of CGRP (100 nM) were also reversed by glibenclamide (1 μM). In contrast, isoprenaline (1 μM) did not alter the frequency of slow wave discharge.
Conclusions and implications: These results demonstrate that BK_{Ca} and 4-AP-sensitive K^+ channels regulate the frequency of prostatic slow wave discharge. SNP and CGRP abolish slow waves in a hyperpolarisation-independent manner, partially via opening of K_{ATP} channels. BK_{Ca} and 4-AP-sensitive K^+ channels also play an important role in the SNP-induced inhibition of slow wave activity. The lack of membrane hyperpolarisation associated with the SNP- and CGRP-induced inhibition implies that the channels involved in this action are not predominantly located on the smooth muscle cells.
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Keywords: smooth muscle; prostate; slow waves; K^+ channel blockers

Abbreviations: 4-AP, 4-aminopyridine; BK_{Ca} channel, large conductance Ca^{2+}-activated K^+ channel; CGRP, calcitonin gene-related peptide; EFS, electrical field stimulation; K_{ATP} channel, ATP-dependent K^+ channel; PCO-400, (-)-(3*S*,4*R*)-3,4-dihydro-3-hydroxy-2,2-dimethyl-4-(3-oxo-cyclopent-1-enyl-1-oxy)-2*H*-1-benzopyran-6-carbonitrile; PIC, prostatic interstitial cells; PSS, physiological salt solution; SK_{Ca} channel, small conductance Ca^{2+}-activated K^+ channel; SNP, sodium nitroprusside; TEA, tetraethylammonium; TTX, tetrodotoxin

Introduction

It is well established that the prostate gland is supplied by sympathetic, parasympathetic and sensory nerves, which contribute to the maintenance of stromal muscle growth, production of secreted materials and neurotransmitters released upon electrical stimulation. In particular, the importance of the sympathetic innervation in the regulation of smooth muscle contraction in prostate gland is now well established (Andersson, 1996; Hieble and Ruffolo, 1996). Electrical field stimulation (EFS) evokes tetrodotoxin (TTX)-sensitive contractions in many animal species including the rat (Lau *et al*., 1998), guinea-pig (Ohkawa, 1983) and rabbit (Seki and Suzuki, 1989). These contractions are markedly attenuated by α_1-adrenoceptor antagonists and guanethidine, suggesting that, in part, endogenously released noradrenaline mediates the contractions. Accordingly, α_1-adrenoceptor antagonists are used to manage the symptoms associated with benign prostatic hyperplasia by reducing the smooth muscle tone of the prostatic stroma (Hieble and Ruffolo, 1996), although α_1-adrenoceptor antagonists are associated with numerous cardiovascular side effects.

The participation of other transmitters that are either colocalized with noradrenaline or contained within other nerve fibres supplying prostatic smooth muscle is less certain. Candidate substances include acetylcholine, calcitonin gene-related peptide (CGRP), nitric oxide (NO) and

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adenine nucleotides. For example, CGRP immunoreactive fibres have been detected in the rat, guinea-pig and human fibromuscular stroma (Tainio, 1995; Pennefather *et al*., 2000). These fibres not only branch to the smooth muscle cells but also to the epithelial and sub-epithelial cells, indicating that CGRP may regulate contraction and secretion. Exogenously applied CGRP has been found to inhibit the nerve-mediated contractile responses recorded in the rat, in a NO and ATP-dependent K^+ channel (K_{ATP}) channel-independent manner, but not the guinea-pig prostate (Ventura *et al*., 2000). In contrast, NO relaxes the phenylephrine-induced contractions of human cultured prostatic smooth muscle cells via stimulation of PKG and the subsequent activation of K_{ATP} channels and iberiotoxin-sensitive, large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels (Cook *et al*., 2002). NO donors have also been shown to inhibit the nerve-mediated contractions in the prostate of many species including rabbit (Aikawa *et al*., 2001) and dog (Takeda *et al*., 1995).

We have recently demonstrated that spontaneous electrical and contractile activity can be recorded in the stromal layer of the guinea-pig prostate (Exintaris *et al*., 2002). The spontaneous electrical activity in the guinea-pig prostatic stroma consists of regular membrane depolarizations (frequency of $5\,min^{-1}$) that trigger several nifedipine-sensitive spikes. Investigations into the membrane channel currents present in freshly dispersed stromal myocytes of the guinea-pig (Kurokawa *et al*., 1998a, b; Oh *et al*., 2003; Lang *et al*., 2004) and human (Eckert *et al*., 1995) prostate report the presence of nifedipine-sensitive 'L-type' Ca^{2+} channels, 4-aminopyridine (4-AP)-sensitive K^+ channels, as well as iberiotoxin-sensitive BK_{Ca} channels. It has been suggested that the opening and closing of these channels defines the time course of the spikes of the slow wave (Lang *et al*., 2004); however, the electrical properties of the slow waves are yet to be fully characterized. This is especially relevant as the prostatic slow wave activity is likely to contribute to the tone of the prostate and may therefore provide a novel avenue of intervention in patients with prostatic disease (Exintaris *et al*., 2002).

In this report, we have recorded different types of spontaneous electrical activity in the stroma of the guinea-pig prostate using a single intracellular microelectrode: slow waves with properties as described previously (Exintaris *et al*., 2002) and 'pacemaker-like' potentials that occurred at the same frequency as slow waves. We have examined the contribution of different K^+ channel populations to the regulation of slow wave activity. In addition we have investigated the effects of various exogenously applied drugs, which have been reported to cause smooth muscle relaxation by opening K^+ channels (sodium nitroprusside (SNP), CGRP and isoprenaline) on the time-course and frequency of slow wave activity.

Materials and methods

Guinea-pigs (250–400 g) were killed by stunning and exsanguination and the dorsal prostate glands removed through an abdominal incision. All experiments were carried out using procedures approved by the Physiology Department Animal Ethics Committee at Monash University. Individual glands (5 mm × 5 mm) of the dorsal lobe were pinned firmly to the bottom of an organ bath (volume 1 ml) mounted on the stage of an inverted microscope and perfused with physiological salt solution (PSS) at $3–4\,ml\,min^{-1}$ (35°C). Recordings of membrane potential were made from the prostate stroma using a standard unity-gain pre-amplifier and microelectrodes with resistances of 60–80 MΩ when filled with 2 M KCl. Changes in the membrane potential were digitized and stored using a TL1 DMA analog-to-digital interface (Axon Instruments, Union City, CA, USA), Axotape software (Axon Instruments) software and a personal computer (Exintaris *et al*., 2002).

Solutions used

The PSS used during the intracellular microelectrode recording experiments was of the following composition (in mM): NaCl 120, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1, NaH_2PO_4 1, $NaHCO_3$ 25 and glucose 11, bubbled with a 95% O_2: 5% CO_2 gas mixture to establish a pH of 7.3–7.4.

Data analysis

Various parameters of the spontaneous slow waves were measured: the membrane potential 1000 ms before the onset of each slow wave, the frequency of slow wave discharge, the overall amplitude consisting of the amplitude of the depolarizing transient and the amplitude of the initial spike of the slow wave, the peak amplitude of the depolarizing transient (not including the superimposed spikes) and its duration measured from when the depolarization was half-maximal (Figure 1a). The parameters of three or four responses were averaged and compared with those measured after 30 s–1 min, 10–20 min or >30 min of exposure to a 'test' drug. A number of similar experiments were then averaged as indicated and values expressed as mean ± s.e.m. In most experiments, a paired Student's *t*-test was used for tests of significance unless otherwise indicated; $P<0.05$ was considered to be statistically significant.

Materials

The following drugs were used: 4-amino pyridine (4-AP), apamin, CGRP, glibenclamide, iberiotoxin, isoprenaline, SNP, tetraethylammonium (TEA) (all from Sigma-Aldrich, St Louis, MO, USA), (-)-(3*S*,4*R*)-3,4-dihydro-3-hydroxy-2,2-dimethyl-4-(3-oxo-cyclopent-1-enyl-1-oxy)-2*H*-1-benzopyran-6-carbonitrile (PCO-400) (Biomol, Plymouth Meeting, PA, USA). The concentration of all stock solutions ranged between 0.1 and 10 mM. Most drugs were dissolved in filtered distilled water and diluted with PSS to their final concentrations as indicated. Nifedipine was dissolved in absolute ethanol. Stock solutions were generally added 1:1000 dilution. During the intracellular microelectrode recording experiments, solutions were vigorously bubbled with the gas mixture to restore any changes of pH. Ethanol at 0.1% or dimethylsulphoxide had no effect on the spontaneous activity of the prostate.

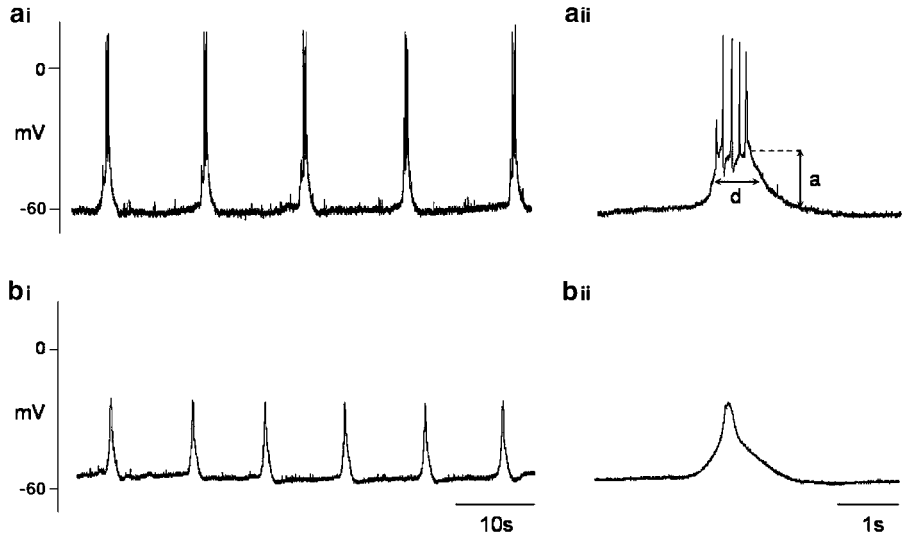

Figure 1 Two types of spontaneous electrical activity were recorded in the guinea-pig prostate. Ninety percent of all electrical recordings exhibited slow wave activity (**a**), while ten percent displayed simple pacemaker potentials (**b**). Various parameters of the spontaneous electrical activity were measured: membrane potential, frequency, the overall amplitude consisting of the peak amplitude of the depolarizing transient and the amplitude of the initial spike of the slow wave, the peak amplitude of the depolarizing transient (a) and its duration measured from when the depolarization was half-maximal (d) (**aii**).

Results

Spontaneous electrical activity in the prostatic stroma

The spontaneous electrical events recorded in the stromal wall of the prostate consisted of two distinct types: slow waves and pacemaker potentials. The majority (90%) of impaled cells ($n=107$ cells) displayed spontaneous slow wave activity, which consisted of a distinct depolarizing phase that triggered one or more spike potentials. These spikes were followed by a repolarizing phase and slight after hyperpolarization of 1–2 mV that slowly decayed before the initiation of the next slow wave (Figure 1a). Slow waves occurred at a frequency of $5.5\pm0.2\,min^{-1}$ ($n=107$) and had an overall amplitude of 55.3 ± 1.4 mV. The averaged membrane potential between slow waves was -57.1 ± 0.6 mV. The mean amplitude and half-amplitude duration of the depolarizing transient of the slow waves were 14.3 ± 1.2 mV and 950 ± 35 ms, respectively. The depolarizations also triggered 2.6 ± 0.1 spike potentials.

A small proportion (10%) of cells displayed spontaneous activity that consisted of potentials, which were biphasic in time course (Figure 1b). In 13 of these 'pacemaker' cells, the mean amplitude of 38.2 ± 1.7 mV was significantly larger, while the half-amplitude duration of the depolarizing transient was significantly shorter, 419 ± 67 ms, than the amplitude and half-amplitude duration of the depolarizing transients of slow waves (unpaired *t*-test, $P<0.05$). In contrast, the membrane potential of -58.0 ± 1.3 mV and frequency of $6.0\pm0.6\,min^{-1}$ of the pacemaker potentials were not significantly different to the slow wave activity (unpaired *t*-test, $P>0.05$) (Figure 1). However, in this study, the electrical properties of the pacemaker potentials were not examined in detail. Further experiments will elucidate the origin and electrical properties of the pacemaker activity.

Effects of K^+ channel openers and blockers

The contribution of various K^+ channel populations to the time course of the prostatic slow wave was examined using TEA (300 μM and 1 mM) and iberiotoxin (150 nM) at concentrations that would be expected to block BK_{Ca} channels; 4-AP (1 mM) that would block 4-AP-sensitive voltage-activated K^+ channels; apamin (1–200 nM) to block small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channels; glibenclamide (1 μM) to block K_{ATP} channels, while both lemakalim (1 μM) and PCO-400 (1 μM) were used to open K_{ATP} channels.

TEA (300 μM) and iberiotoxin (150 nM) (data not shown) significantly increased the frequency of slow wave activity, while other parameters were little affected. In TEA (300 μM for 2–5 min), the frequency of the slow waves was $5.4\pm0.4\,min^{-1}$ compared to $4.7\pm0.5\,min^{-1}$ in the control (paired *t*-test, $P<0.05$, $n=4$), while other parameters were little affected. The membrane potential, number of superimposed spikes, overall amplitude and half-amplitude duration of the slow waves in TEA were -60.6 ± 2.5 mV, 3.5 ± 1.1 spikes, 73.4 ± 1.3 mV and 941 ± 91 ms, respectively, compared with, -59.6 ± 3.0 mV, 3.7 ± 1.2 spikes, 68.7 ± 3.6 mV and 934 ± 127 ms, respectively, in control PSS (all $P>0.05$) (Figure 2a). In 1 mM TEA, slow waves had an amplitude of 65.8 ± 2.7 mV and occurred at a frequency of $7.7\pm0.6\,min^{-1}$ compared with 57.4 ± 1.9 mV and $7.0\pm0.7\,min^{-1}$, respectively, in control PSS (paired *t*-test, $P<0.05$, $n=8$). The average membrane potential, number of spike potentials and half-amplitude duration of the control slow wave activity was -57.0 ± 0.7 mV, 2.3 ± 0.4 spikes and 872 ± 107 ms, compared with -57.2 ± 0.9 mV, 3.0 ± 0.3 spikes and 776 ± 110 ms after 2 min exposure to 1 mM TEA (paired *t*-test, all $P>0.05$). The effect of TEA on the slow wave frequency was reversed upon washout in control PSS.

In 14 experiments, 4-AP (1 mM for 2–5 min) caused a significant increase in the frequency of slow wave discharge

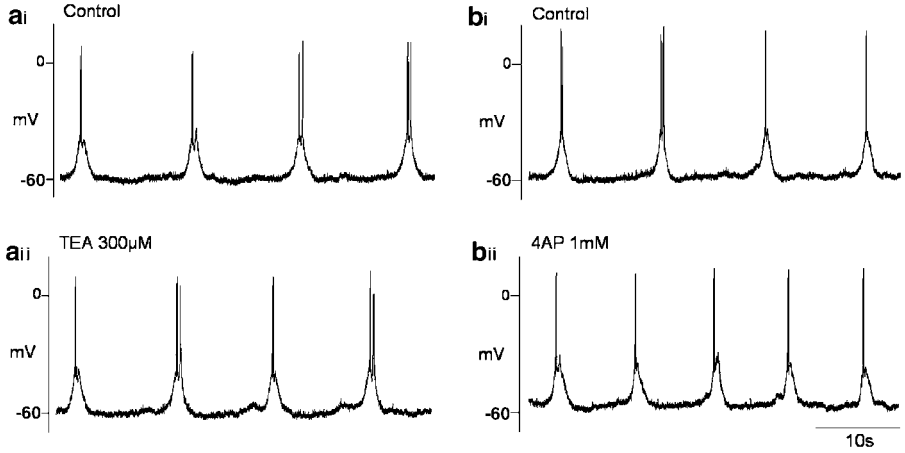

Figure 2 TEA (300 μM, 2–5 min) (**aii**) increased the frequency of the slow wave activity in the guinea-pig prostate by 15% of the control (**ai**). 4-AP (1 mM, 2–5 min) (**bii**) increased the frequency of slow wave discharge by 37% and the half-amplitude duration of the depolarizing transient by 16% when compared to the control (**bi**). TEA, tetraethylammonium.

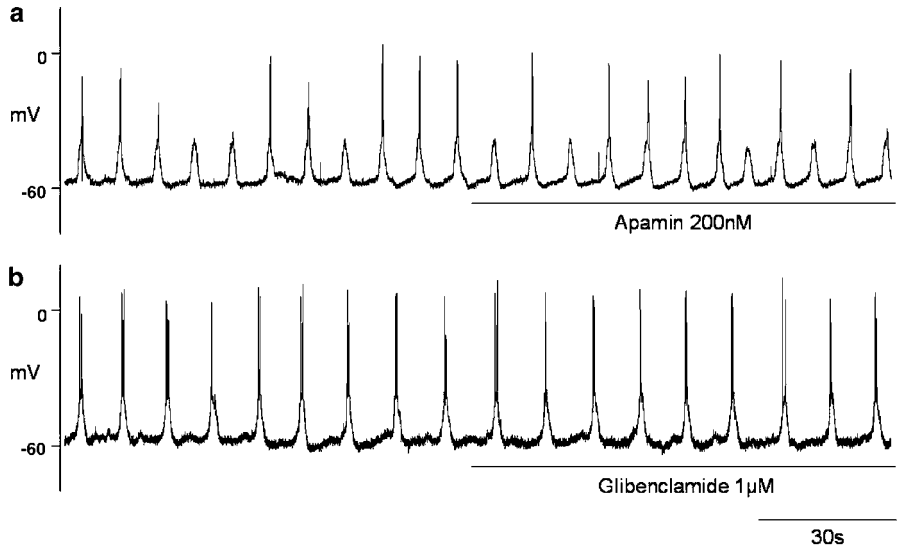

Figure 3 Slow wave activity in the guinea-pig prostate was unaffected by the application of apamin (200 nM, >15 min) (**a**) or glibenclamide (1 μM, 10 min) (**b**).

from $5.4 \pm 0.6\,min^{-1}$ in control PSS to $6.9 \pm 0.5\,min^{-1}$ in the presence of 4-AP. The half-amplitude duration of the depolarizing transient was also increased from 985 ± 76 to 1081 ± 93 ms (paired *t*-test, $P<0.05$) (Figure 2b). Exposure to 4-AP had no significant effects on the remaining parameters: resting membrane potential, number of spikes potentials and overall slow wave amplitude being -56.8 ± 1.7 mV, 2.6 ± 0.3 spikes and 57.1 ± 3.4 mV, respectively, in control PSS compared with -55.7 ± 1.8 mV, 3.3 ± 0.6 spikes and 55.7 ± 2.7 mV after 2–5 min exposure to 4-AP (1 mM) (paired *t*-test, $P>0.05$). The excitatory effects of 4-AP were readily reversed upon washout in PSS.

The membrane potential, number of spike potentials, amplitude and frequency of the spontaneous slow waves were little affected by apamin (1 nM, $n=4$ and 200 nM, $n=8$ for >15 min) (Figure 3a). In the presence of the highest concentration of apamin used (200 nM for >15 min), the membrane potential, number of spikes, duration and frequency were -59.1 ± 0.8 mV, 2.7 ± 0.3 spikes, 708 ± 37 ms and $3.8 \pm 1.8\,min^{-1}$ compared with -60.8 ± 1.0 mV, 2.2 ± 0.1 spikes, 638 ± 107 ms and $3.7 \pm 1.7\,min^{-1}$ under control conditions (paired *t*-test, $P>0.05$, $n=8$).

Similarly, exposure of the preparations to glibenclamide (1 μM for 10 min, $n=10$) had no significant effects on any of the parameters measured: the membrane potential, number of spikes potentials and overall slow wave amplitude being -53.9 ± 1.5 mV, 3.0 ± 0.2 spikes and 55.3 ± 3.6 mV, respectively, in control PSS compared with -54.2 ± 1.6 mV, 3.5 ± 0.4 and 55.1 ± 4.1 mV after 2–5 min exposure to glibenclamide (1 μM) (paired *t*-test, $P>0.05$) (Figure 3b).

In contrast, the K^+ channel openers, lemakalim (1 μM) (Figure 4a) and PCO-400 (1 μM) (Figure 4b) abolished the prostatic slow waves within 1–2 min (paired *t*-test, $P<0.05$, $n=4$) without any significant changes in the resting

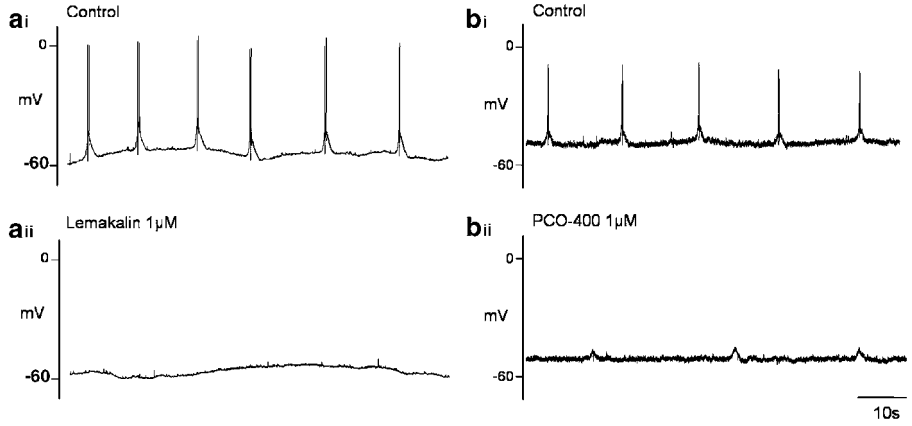

Figure 4 Lemakalim (1 μM) (**a**) and PCO (1 μM) (**b**) abolished slow wave activity recorded in the guinea-pig prostate within 1–2 min of application. PCO, (-)-(3*S*,4*R*)-3,4-dihydro-3-hydroxy-2,2-dimethyl-4-(3-oxo-cyclopent-1-enyl-1-oxy)-2*H*-1-benzopyran-6-carbonitrile.

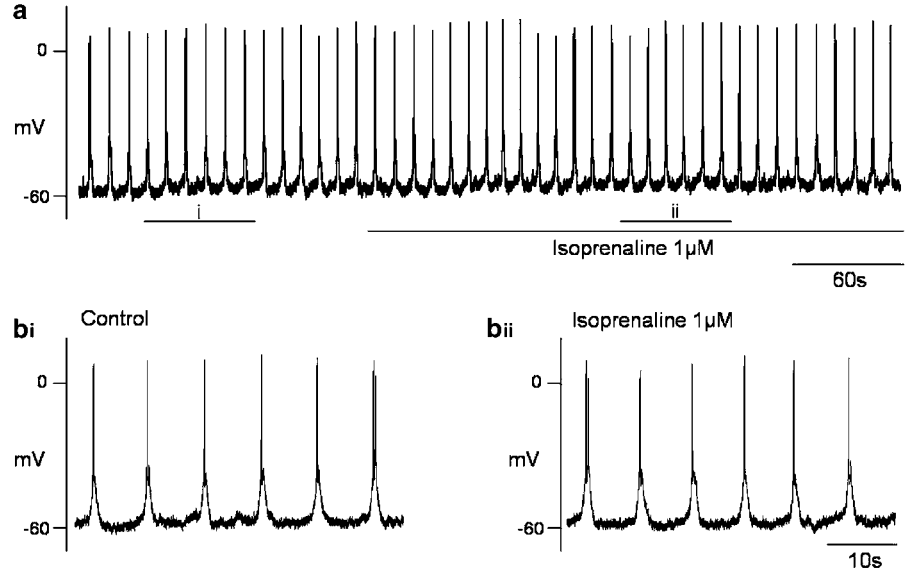

Figure 5 Isoprenaline (1 μM, 10 min) did not have any effects on the slow wave activity in the guinea-pig prostate (**a**). Sections of trace in (**a**) depicted on an expanded time scale (**b**).

membrane potential (paired *t*-test, $P>0.05$, $n=4$). The inhibitory effects of the K^+ channel openers were reversed and slow wave activity returned after 30 min washout in PSS.

Effects of isoprenaline, CGRP and SNP

The β-adrenoceptor agonist, isoprenaline (1 μM) (Figure 5), did not significantly affect any of the parameters measured. The average membrane potential, the number of spikes, total amplitude, half-amplitude duration and frequency were -55.9 ± 1.5 mV, 2.4 ± 0.7 spikes, 58.1 ± 10.5 mV, 1481 ± 464 ms and $5.0\pm0.6\,min^{-1}$, respectively, for control, while in isoprenaline they were -57.2 ± 1.6 mV, 2.6 ± 0.6 spikes, 62.5 ± 5.0 mV, 1436 ± 298 ms and $5.7\pm0.8\,min^{-1}$ (paired *t*-test, $P>0.05$, $n=5$).

CGRP (100 nM) resulted in a time-dependent decrease in the overall amplitude and frequency of the spontaneous electrical events. Within 1–2 min of CGRP's application, slow wave discharge ceased (Figure 6). However, the resting membrane potential of -57.8 ± 0.9 mV was not significantly different to -56.2 ± 1.1 mV in control PSS (paired *t*-test, $P>0.05$, $n=4$). Glibenclamide (1 μM) was able to reverse the effects of CGRP returning the frequency of the slow waves to $5.2\pm0.7\,min^{-1}$, the number of spike potentials per slow wave to 2.1 ± 1.1 spikes, the mean amplitude and half-amplitude duration of slow wave to 56.5 ± 1.7 mV and 1922 ± 715 ms (Figure 6).

In 21 preparations, the NO donor SNP (10 μM) abolished spontaneous slow wave discharge within 1–2 min (Figures 7 and 8a). In most experiments (>80%), SNP caused an initial transient hyperpolarization of the membrane potential of 2–3 mV upon the cessation of slow wave discharge; however, the membrane potential quickly returned to near control values during the inhibitory phase. The inhibitory action of SNP was readily reversed with the addition of glibenclamide (1 μM) (Figure 7) or TEA (1 mM) (data not shown) with all parameters returning to values comparable with the control slow waves (paired *t*-test, $P>0.05$, $n=5$). In four experiments, the addition of 4-AP (1 mM) in the presence of SNP

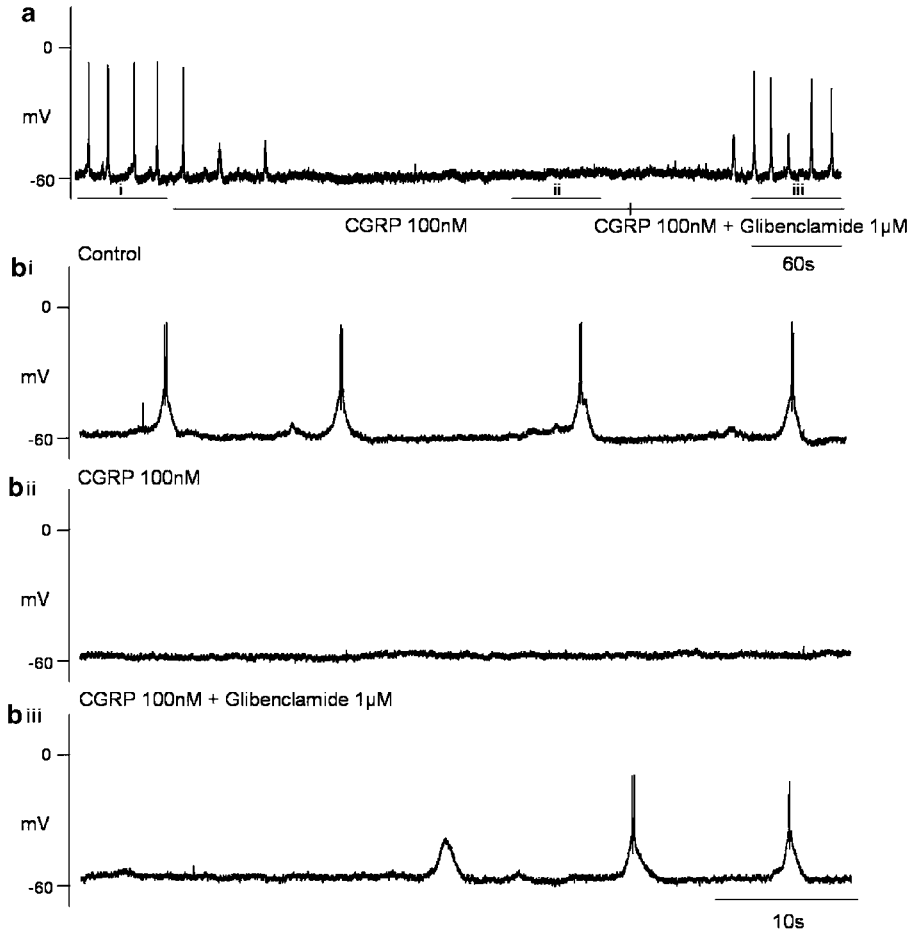

Figure 6 The effects of CGRP (100 nM) on the slow wave activity recorded in the guinea-pig prostate gland (**a**). Sections of trace in (**a**) depicted on an expanded time scale (**b**). CGRP abolished slow wave activity within 1–2 min of application (**a**, **bii**). This inhibitory effect was reversed by glibenclamide (1 μM) (**a**, **biii**). CGRP, calcitonin gene-related peptide.

also restored the slow wave activity to near initial values with the exception to an increased number of spike potentials from 1.7 ± 0.3 spikes in the control to 3.1 ± 0.3 spikes in SNP and 4-AP together (paired *t*-test, $P<0.05$) (Figure 8a and b). Alternatively, the addition of SNP in the presence of TEA or glibenclamide (1 μM) significantly reduced the frequency of slow wave activity within 1–2 min without affecting the remaining parameters (data not shown). Similarly, the addition of SNP in the presence of 4-AP, also significantly reduced the frequency of slow wave discharge to $6.1 \pm 0.5\,\text{min}^{-1}$ compared to $8.6 \pm 0.7\,\text{min}^{-1}$ in 4-AP alone (paired *t*-test, $P<0.05$, $n=5$) (Figure 8c and d). In addition, there was also an increase in the number of spike potentials and duration of the depolarizing transient to 7.5 ± 2.1 spikes and 1831 ± 296 ms, respectively, compared to 4.0 ± 0.9 spikes and 1320 ± 231 ms in 4-AP alone (paired *t*-test, $P<0.05$, $n=5$), while both the resting membrane potential and total amplitude of the slow waves remained unchanged.

Discussion

We believe that the spontaneous slow wave activity in the prostatic stroma contributes to the prostatic tone and may therefore provide a novel avenue of intervention in patients with prostatic disease (Exintaris *et al*., 2002). In addition, although the effects of nerve-mediated agents on the EFS-induced contractions are well characterized, little is known about the effects of these agents on the spontaneous tone of the prostate. Agents that relax the prostate are of particular interest as they could be used clinically to manage the symptoms associated with prostatic disease.

Slow waves and pacemaker potentials

When intracellular recordings are made from the serosal surface of individual prostatic acini, the majority of cells (90%) display slow wave activity which is generated by the smooth muscle cells in the prostatic stroma (Exintaris *et al*., 2002; Lang *et al*., 2004). We have previously shown that the prostatic 'slow waves' recorded in the prostate gland of the guinea-pig closely resemble slow waves recorded in the smooth muscle layers of many organs; including the rabbit urethra (Hashitani *et al*., 1996), guinea-pig mesenteric lymphatics (Van Helden, 1993) and the guinea-pig stomach (Van Helden *et al*., 2000), in their configuration and relative insensitivity to the effects of nifedipine, TTX or blockers of parasympathetic and sympathetic neurotransmission (Exin-

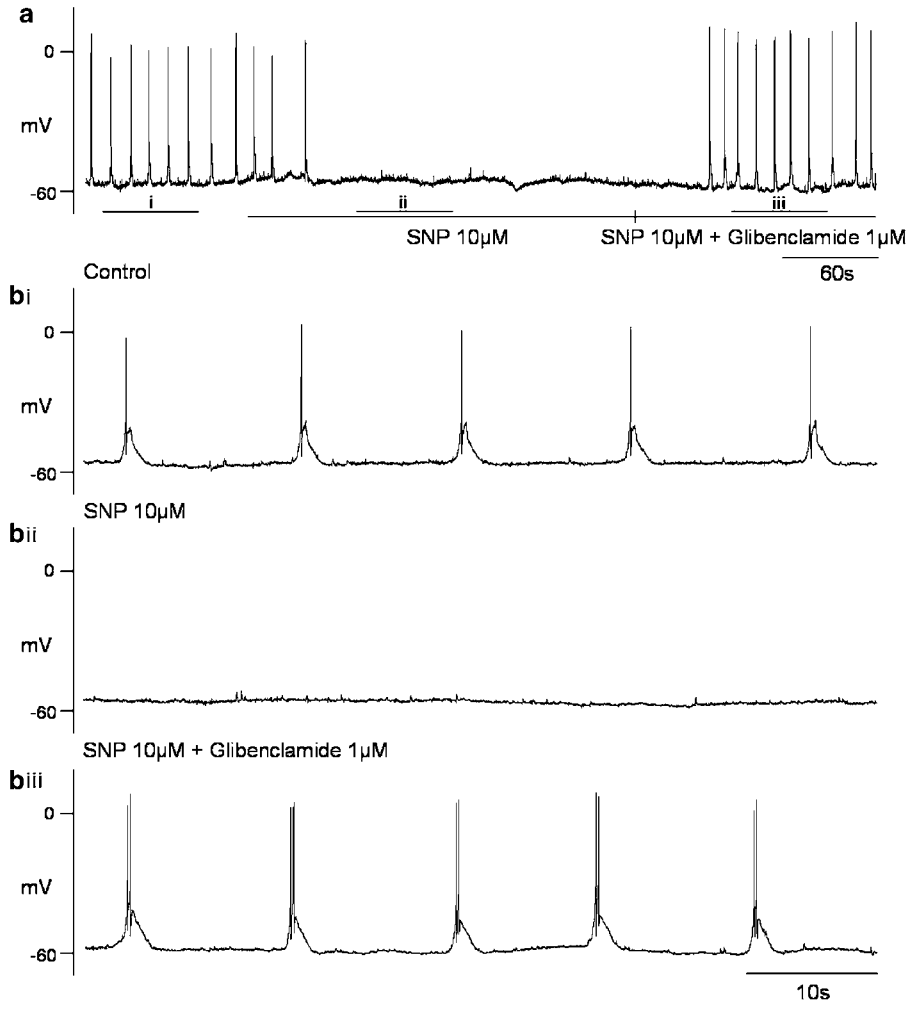

Figure 7 Effects of SNP (10 μM) on the slow wave activity recorded in the guinea-pig prostate (**a**). Sections of trace in (**a**) depicted on an expanded time scale (**b**). SNP abolished slow waves within 1–2 min (**bii**). This effect was readily reversed by glibenclamide (1 μM) (**a, biii**). SNP, sodium nitroprusside.

taris *et al*., 2002). In this study, we have also recorded 'pacemaker-like' activity (10% of all recordings) that consists of a simple waveform of depolarizing and repolarizing phases. 'Pacemaker-like' activity may well arise from our recently identified prostatic interstitial cells (PIC) (Exintaris *et al*., 2002), especially as this activity was recorded by driving the micro-electrode deep into the preparation, presumably between the smooth muscle and epithelial layers where the PIC have been identified previously. The averaged resting membrane potential before the initiation of the pacemaker potential was -58.0 ± 1.3 mV, which was not significantly different to the membrane potential recorded in cells exhibiting slow wave activity -57.1 ± 0.6 mV. Similarly, the frequency of discharge of the 'pacemaker' activity was not significantly different to the corresponding slow wave parameters perhaps suggesting that the two cell types are reasonably well coupled. The waveform recorded in the smooth muscle cells of nifedipine-arrested preparations (Lang *et al*., 2006) may well be a propagated representation of the pacemaker potential as has been demonstrated in smooth muscle preparations of the gastrointestinal system (Hirst and Ward, 2003). Further experiments will elucidate the origin and electrical properties of the 'pacemaker-like' activity.

Slow waves and K^+ channels

This study demonstrated that BK_{Ca} and 4-AP-sensitive K^+ channels regulate the frequency of slow wave activity. It is admittedly difficult to determine whether the effects of TEA, iberiotoxin and 4-AP are on the channels found on the smooth muscle cells, the PIC or both since the modulation of pacemaker activity would also subsequently affect slow wave activity. Previous studies using freshly dissociated smooth muscle cells of the guinea-pig prostate demonstrated that the voltage-activated membrane currents in single stromal myocytes of the guinea-pig prostate consisted of a nifedipine-sensitive Ca^{2+} current, a 4-AP-sensitive, voltage-gated K^+ current and a TEA and iberiotoxin-sensitive whole-cell K^+ current arising from the activation of BK_{Ca} channels (Oh *et al*., 2003; Lang *et al*., 2004). The concentrations of TEA (0.1–1 mM) used in these experiments suggest that the effects of TEA on the slow waves were most likely due to their blocking action of BK_{Ca} channels. These effects were first

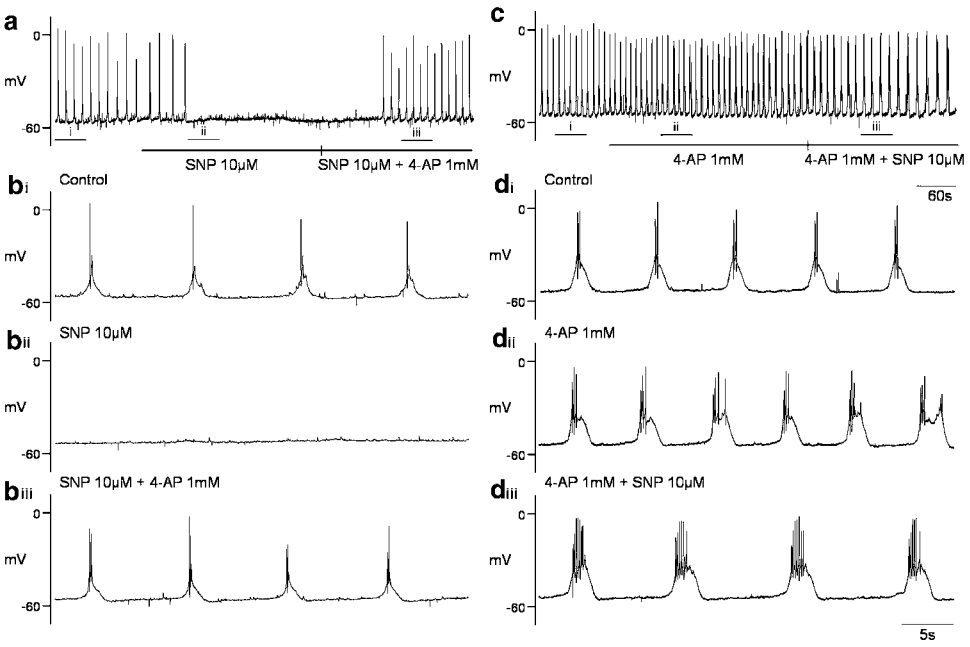

Figure 8 Sections of trace in (**a**) and (**c**) depicted on an expanded time scale in (**b**) and (**d**), respectively. SNP (10 μM) abolished slow waves within 1–2 min (**a**, **bii**). This effect was readily reversed by 4-AP (1 mM) (**a**, **biii**). 4-AP increased both the duration and frequency of slow waves within 2 min (**c**, **dii**). The addition of SNP in the presence of 4-AP reduced the frequency and increased both the duration and number of spike potentials of the slow waves recorded in the guinea-pig prostate (**diii**). SNP, sodium nitroprusside.

reported by Ohkawa (1983). However at the high concentrations (2.5 and 5 mM) that were used, the effects of TEA could have arisen from nonspecific effects on other K^+ channels or the release of neurotransmitters. In this study 4-AP also had an additional effect, increasing the half-amplitude duration of the spontaneous electrical activity suggesting that K^+ flowing through 4-AP-sensitive, voltage-gated K^+ channels is also involved in the repolarization of the prostatic slow waves which is also supported by previous experiments on dissociated smooth muscle cells (Oh *et al*., 2003; Lang *et al*., 2004).

Altogether, the time course of the prostatic slow wave consists of nifedipine-sensitive spikes superimposed on the depolarizing transients which are insensitive to nifedipine, suggesting that the depolarizing transients arise from mechanisms other than the opening of L-type Ca^{2+} channels (Exintaris *et al*., 2002). The repolarizing phase of the slow waves is likely to be determined by the opening of 4-AP-sensitive K^+ channels, while the increased frequency observed with 4-AP, iberiotoxin and low concentrations of TEA suggests the involvement of both 4-AP-sensitive and BK_{Ca} channels in the modulation of slow wave frequency (Figure 2). Although the contribution of intracellular Ca^{2+} stores and chloride channels to the configuration of the pacemaker (Lang *et al*., 2006) and slow waves is likely, it is yet to be fully elucidated.

SNP, CGRP and isoprenaline

Blockade of SK_{Ca} and K_{ATP} channels using apamin and glibenclamide, respectively, had no effect on slow wave activity (Figure 3). In contrast, the K_{ATP} channel openers, lemakalim and PCO-400 abolished the spontaneous slow waves without affecting the membrane potential (Figure 4). Similarly, SNP abolished slow wave discharge (Figures 7 and 8), an effect that was not associated with significant membrane hyperpolarization. Recent studies suggest that NO donors and activators inhibit slow wave and contractile activity in the rabbit urethra by modulating IP_3-dependent Ca^{2+} release from the ICC in a hyperpolarization-independent manner (Hashitani *et al*., 1996; Sergeant *et al*., 2006). Accordingly, the lack of membrane hyperpolarization associated with the SNP (or CGRP)-induced inhibition of the slow waves in the guinea-pig prostate gland could suggest that the channels involved in this inhibitory action are mainly located on the PIC rather than the smooth muscle cells.

It is an interesting observation that the application of glibenclamide to the preparation showed no significant effects on slow wave activity, although was able to reverse the inhibitory effects of SNP and CGRP (Figures 6 and 7). This suggests that ATP-modulated, glibenclamide-sensitive K^+ channels may have an important role in the modulation of slow wave activity in the presence of agents known to cause relaxation, although they seem to have little effect under 'normal' conditions. Glibenclamide-sensitive K^+ channels, as well as iberiotoxin-sensitive BK_{Ca} channels are also involved in both the SNP- and SNAP-induced inhibition of the phenylephrine-induced contractions in human-cultured prostatic stromal cells, via the stimulation of PKG (Cook *et al*., 2002). In our studies, not only did glibenclamide and TEA reverse the inhibitory action of SNP, but so did 4-AP suggesting that the K_{ATP}, 4-AP-sensitive and BK_{Ca} channels are all involved in modulating the SNP-induced inhibitory response of slow wave activity in the guinea-pig prostate gland.

The effects of isoprenaline and CGRP in the prostates of different species are variable. For example, nerves containing CGRP immunoreactivity are sparsely distributed throughout the prostate (Lau *et al*., 1998) and have been shown to cause relaxation in electrically stimulated preparations of the rat (Pennefather *et al*., 2000) but not the guinea-pig (Ventura *et al*., 2000). On the other hand, we have demonstrated that in the guinea-pig prostate, CGRP abolished slow wave activity and that glibenclamide was able to reverse this inhibitory response. This differs from that found in the rat, where the inhibitory effects of CGRP were blocked by the CGRP antagonist (8–37) but unaffected by NO synthase inhibitor N_ω-nitro-L-arginine methyl ester and glibenclamide, suggesting that NO and K_{ATP} channels were not involved in the relaxation (Ventura *et al*., 2000). In our study, the non-selective β-adrenoceptor agonist, isoprenaline, did not affect the configuration of the spontaneous slow waves recorded in the guinea-pig prostate (Figure 5). In contrast, previous studies have shown that isoprenaline readily inhibits the phenylephrine-induced and nerve-mediated contractions in the rat (Kalodimos and Ventura, 2001) and guinea-pig (Haynes and Hill, 1997). These conflicting results highlight, perhaps that the properties of the nerve or agonist-induced contractions of the prostate gland and the spontaneous activity of the prostate gland are completely different.

We have previously shown that slow wave activity recorded in prostatic smooth muscle cells are likely to contribute to the smooth muscle tone. This study has demonstrated that the frequency of slow wave discharge is modulated by BK_{Ca} and 4-AP-sensitive K^+ channels but not SK_{Ca} and K_{ATP} channels. However, K_{ATP} channels do play a role in the CGRP-induced inhibition of slow wave activity, while the SNP-induced inhibition arises from the opening of 4-AP-sensitive K^+ channels, BK_{Ca} and K_{ATP} channels. The lack of membrane hyperpolarization associated with the SNP and CGRP-induced inhibition of the slow waves perhaps suggests that the channels involved in this action are located on PIC.

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Conflict of interest

The authors state no conflict of interest.

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