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α_1-adrenoceptor modulation of spontaneous electrical waveforms in the guinea-pig prostate ☆

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ABSTRACT

The aim of this study was to investigate the effects of phenylephrine on the spontaneous slow wave and pacemaker activity in the guinea-pig prostate. Membrane potential recordings were made using intracellular microelectrodes. Guinea-pig prostatic cells either displayed 'slow wave' activity or 'pacemaker' potentials. In the presence of nifedipine, none of the parameters measured were significantly different between both waveforms. Phenylephrine (1 μM) or histamine (5 μM) increased the frequency of slow waves and pacemaker potentials in the absence or presence of nifedipine (1 μM). In the presence of nifedipine (1 μM), the addition of either cyclopiazonic acid (CPA, 10 μM) or carbonyl cyanide 3-chlorophenylhydrazone (CCCP, 1–10 μM) caused an initial transient increase in frequency of the spontaneous electrical activity that was associated with a membrane depolarisation of 3–7 mV before abolishing activity. Following the cessation of electrical activity, phenylephrine (1 μM) was unable to restore the spontaneous electrical events. Although niflumic acid (10–100 μM) (in the presence of 1 μM nifedipine) similarly abolished all activity, electrical activity was promptly initiated upon the addition of phenylephrine (1 μM). These results demonstrate that in the presence of nifedipine, there are no differences between the slow waves and pacemaker potentials suggesting that both activities arise from cells that are well coupled. Both phenylephrine and histamine increased the frequency of pacemaker activity which is likely to; at least, partly explain the increase in slow wave discharge also evoked by these agents. In the presence of nifedipine, the effects observed upon α_1-adrenoceptor activation were dependent on Ca^{2+} cycling by both the intracellular Ca^{2+} stores and mitochondria.

1. Introduction

Benign prostatic hyperplasia is becoming an increasingly common condition with today's aging population. Although not life threatening, the lower urinary tract symptoms associated with this condition severely affect the quality of life for patients. Due to the invasive nature of surgery, many patients prefer pharmacotherapies to treat their symptoms and in the last 2 decades, non-surgical procedures have become increasingly popular (Emberton and Martorana, 2006). One class of drugs currently available is the α_1-adrenoceptor antagonists. The effectiveness of these agents relies on their ability to attenuate the increased muscular tone of the prostate, one of the major contributing factors responsible for the lower urinary tract symptoms associated with benign prostatic hyperplasia.

In the guinea-pig prostate, contractions from electrical field stimulation (EFS) or exogenously-applied noradrenaline have been shown to be attenuated by α_1-adrenoceptor antagonists (Haynes and Hill, 1997; Pennefather et al., 1999) while, α_1-adrenoceptor activation by phenylephrine readily evokes contractions (Haynes and Hill, 1997; Mikuma et al., 1997). While most experiments have focused on these large contractions, the guinea-pig prostate has also been shown to contract spontaneously (Exintaris et al., 2002). These contractions are markedly smaller than those evoked by EFS studies and have also been observed in the dog prostate (Shafik et al., 2006) and human cultured prostatic smooth muscle cells (Boesch et al., 2000).

We have previously established that spontaneous slow wave activity recorded from the guinea-pig prostate arises from typical spindle-shaped smooth muscle cells and is likely to underlie the spontaneous contractions which contribute to the smooth muscle tone of the prostate (Exintaris et al., 2002). We have also recorded 'pacemaker' activity which arises from a specialised group of c-Kit immunoreactive prostatic interstitial cells that lie between the glandular epithelium and smooth muscle stroma (Exintaris et al., 2002). Prostatic interstitial cells are likely to provide the depolarising pulse to neighbouring smooth muscle cells thereby initiating

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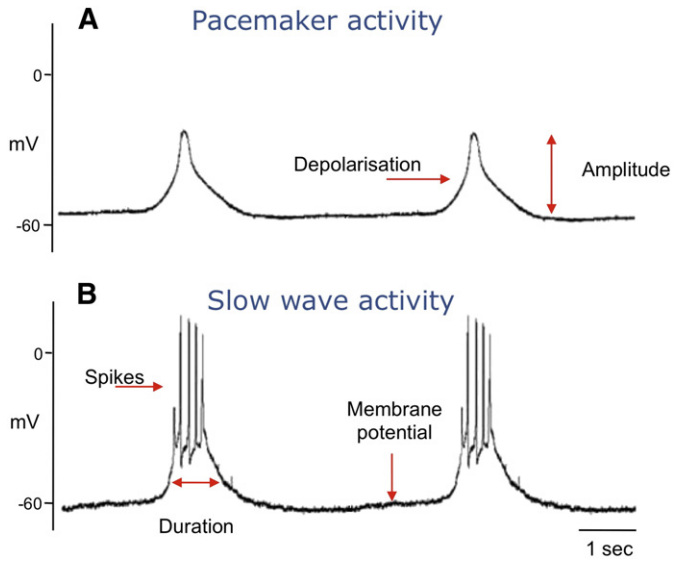

Fig. 1. Various parameters of the spontaneous activity were measured: the membrane potential 1000 ms before the onset of each slow wave/pacemaker potential, the frequency and overall amplitude of spontaneous electrical activity, and half amplitude duration of the depolarisation.

slow wave activity and ensuing contractility in the guinea-pig prostate. However, little is known about the spontaneous electrical and contractile activities in the prostate. Moreover, we have previously demonstrated that phenylephrine, histamine and a raised K^+ saline increase the frequency of the spontaneous slow wave activity in the guinea-pig prostate (Exintaris et al., 2006). However, we have yet to establish the effects of these agents on pacemaker potentials or to identify the mechanisms involved in the modulation of the spontaneous electrical activity in the guinea-pig prostate upon α_1-adrenoceptor activation. This is especially relevant as an increased smooth muscle tone is observed in benign prostatic hyperplasia and changes in the density of α_1-adrenoceptors have already been linked to aging (Yono et al., 2006), further accounting for the effective use of α_1-adrenoceptor antagonists to clinically treat lower urinary tract symptoms in patients with this condition.

In this report, we have confirmed that the spontaneous electrical activity in the guinea-pig prostate mainly consists of slow waves and pacemaker potentials. We have extended previous findings by investigating the effects of nifedipine and contractile agents; phenylephrine and histamine on both slow waves and pacemaker potentials. In addition we have investigated the contribution of internal Ca^{2+} stores, mitochondria and Ca^{2+} activated Cl^- (Cl_{Ca}) channels to the phenylephrine-induced changes in spontaneous slow wave and pacemaker activity.

2. Materials and methods

Guinea-pigs (300–500 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 Monash University Animal Experimentation Ethics Committee in accordance with the National Health and Medical Research Council of Australia. 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 saline solution (PSS) at 3–4 ml/min (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 digitised and stored using a TL1 DMA analog-to-digital interface (Axon Instruments), pClamp software (Axon Instruments) and a personal computer (Exintaris et al., 2002).

2.1. Solutions and drugs 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.

The following drugs were used: carbonyl cyanide 3-chlorophenylhydrazone (CCCP), cyclopiazonic acid (CPA), flufenamic acid, histamine, nifedipine, niflumic acid, phenylephrine (all from Sigma-Aldrich, U.S.A). The concentration of all stock solutions ranged between 0.1 mM and 10 mM. Most drugs were dissolved in DMSO diluted with PSS to their final concentrations as indicated. Histamine and phenylephrine were dissolved in filtered distilled water. Nifedipine

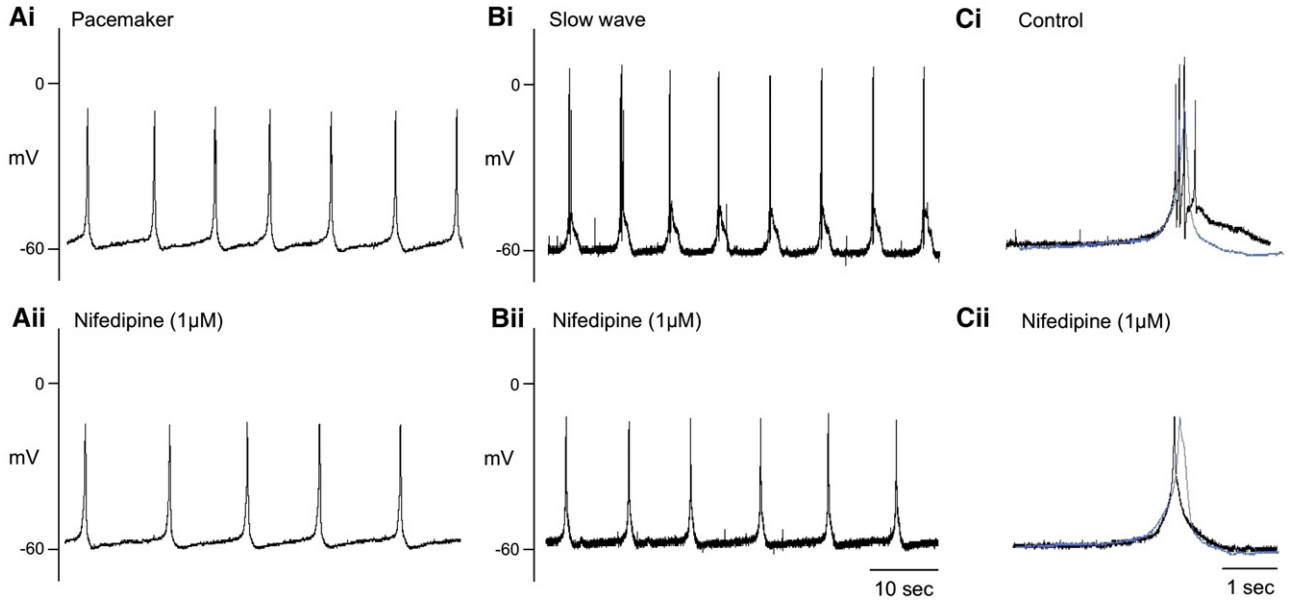

Fig. 2. Two main types of spontaneous electrical activity were recorded from the guinea-pig prostate; pacemaker potentials (A) and slow waves (B). Nifedipine (1 μM) had little effect on the pacemaker potentials (Aii), however it abolished the superimposed spike potentials of the slow wave (Bii). When the parameters of both waveforms were compared in the presence of nifedipine(C), pacemaker potentials (blue) and slow waves were similar (Cii).