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Electrical properties of purinergic transmission in smooth muscle of the guinea-pig prostate

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ABSTRACT

Prostatic smooth muscle develops spontaneous myogenic tone which is modulated by autonomic neuromuscular transmission. This study aimed to investigate the role of purinergic transmission in regulating electrical activity of prostate smooth muscle and whether its contribution may be altered with age.

Intracellular recordings were simultaneously made with isometric tension recordings in smooth muscle preparations of the guinea-pig prostate. Immunostaining for P2X1 receptors on whole mount preparations was also performed.

In prostate preparations which generated spontaneous slow waves, electrical field stimulation (EFS)-evoked excitatory junction potentials (EJPs) which were abolished by guanethidine (10 μM), α-β-methylene ATP (10 μM) or pyridoxal phosphate-6-azophenyl-2,4-disulfonic acid (PPADS, 10 μM) but not phentolamine (1 μM). Consistently, immunostaining revealed the expression of P2X1 receptors on prostatic smooth muscle. EJPs themselves did not cause contractions, but EJPs could sum to trigger a slow wave and associated contraction. Yohimbine (1 μM) and 3,7-dimethyl-1-propargylxanthine (DMPX, 10 μM) but not propranolol (1 μM) potentiated EJPs. Although properties of EJPs were not different between young and aging guinea-pig prostates, ectoATPase inhibitor ARL 67156 (100 μM) augmented EJP amplitudes by 64.2 ± 29.6% in aging animals, compared to 22.1 ± 19.9% in young animals.

These results suggest that ATP released from sympathetic nerves acts on P2X1 purinoceptors located on prostate smooth muscle to evoke EJPs, while pre-junctional α_2-adrenergic and adenosine A_2 receptors may play a role in preventing excessive transmitter release. Age-related up-regulation of enzymatic ATP breakdown may be a compensatory mechanism for the enhanced purinergic transmission which would cause hypercontractility arising from increased ATP release in older animals.

1. Introduction

Benign prostatic hyperplasia (BPH) in elderly men is often associated with urinary symptoms that result from a combination of enhanced prostate bulk and contractility. An increased dynamic prostate smooth muscle tone results from the age-related changes in neurogenic and myogenic spontaneous contractions.

Neurogenic contractions in the prostate stem from the activation of sympathetic nerves that predominantly act on post-synaptic α_1-adrenoceptors, however, prostate innervation appears to be species-dependent. The blockade of nerve-evoked contractions with α_1-adrenoceptor antagonists is partial in the guinea-pig (Ohkawa, 1983), rabbit (Seki et al., 1988), mouse (White et al., 2011) and human prostate (Yu et al., 1994). Early studies examining evoked EJPs in the rabbit prostate capsule (Seki and Suzuki, 1989) demonstrated that α_1-adrenoceptor blockade failed to suppress EJP generation, although bath-applied noradrenaline caused slow depolarization. In the guinea pig prostate, stimulating intramural nerves is known to activate α_1-adrenoceptors (Najbar-Kaszkiel et al., 1997; Ohkawa, 1983) but a secondary transmission is thought to be purinergic.

Although ATP modulates the release of noradrenaline via adenosine A_2 receptors in the rat prostate (Burnstock, 2014; Morikawa et al., 2007), little is known about the electrical properties involved in neuromuscular transmission in the guinea-pig prostate. P2Y and P2X receptors are present in the guinea-pig prostate. In the guinea-pig intestine, P2Y1 receptors coupled to slow K^+ (SK) channels, are implicated in the generation of inhibitory junction potentials (Grasa et al., 2009) which are events that do not occur in the guinea-pig prostate since SK channel inhibitor, apamin, has no effect on spontaneous electrical activity as well as the membrane potential (Nguyen et al., 2009). On the other hand, P2X1 receptors are known to elicit contractions in the guinea-pig prostate (Buljubasich and Ventura, 2004) but how it contributes to nerve-induced contractions has not been elucidated. Therefore this study examines the properties of EJPs elicited during nerve stimulation to elucidate the electrical mechanisms underlying nerve-evoked contractions.

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Additionally, the age-related changes in human prostate innervation have been well documented including the up-regulation of α_1-adrenoceptor activity (Yamada et al., 1987) which influences smooth muscle tone. The aging mouse prostate also reportedly develops contractions sensitive to purinergic blockade (White et al., 2015); however, few studies have examined the changes in purinergic innervation in the aging guinea-pig prostate. Consequently we have also investigated potential age-related changes in the generation of EJPs.

2. Methods

Male guinea-pigs at about 3 weeks old (250 to 300 g) were classified as young animals while guinea-pigs more than 1 year old (900 g to 1150 g) were considered older animals. The majority of experiments were conducted using young animals throughout this study except for section 3.4 as indicated. Guinea-pigs were killed by exsanguination under sevoflurane anesthesia according to procedures approved by the Nagoya City University Animal Experimentation Ethics Committee. An acinus was removed from the prostate and opened up to form a sheet. The tissue was firmly secured mucosal side up on a Sylgard-lined organ bath and was superfused with physiological salt solution (PSS) that was bubbled with 95% O_2: 5% CO_2 at 37 °C to maintain physiological conditions.

2.1. Intracellular recording

After 1 h of equilibration, conventional intracellular recording techniques were used as described previously (Shigemasa et al., 2014). Once a cell was impaled and spontaneous slow wave activity was recorded, EFS was applied between a pair of platinum stimulating electrodes using single impulses with 50 μs pulse width and 10 V intensity delivered every 10 s to induce single EJPs. Repetitive stimuli were applied at 10 Hz to evoke summed EJPs. Once EJP generation stabilized, drugs at predetermined concentrations were perfused through the preparation.

2.2. Tension recording

Opened acini lobes were pinned to the organ bath along one side of the preparation, while the other side was attached to an isometric force transducer by a thread. 1 g tension was placed on the tissue and the preparation left to equilibrate for 1 h. Muscle contractions were recorded simultaneously with changes in the membrane potential using isometric tension recording and intracellular recording techniques.

2.3. Nerve-induced contractions

After an hour of equilibration stable spontaneous contractions were observed, EFS-induced contractions were elicited over a frequency range of 1–20 Hz before and after the addition of drugs. Tetrodotoxin (1 μM) was applied at the end of the experiments to ensure contractions were not due to direct muscle stimulation.

2.4. Immunohistochemistry

Whole mount preparations were prepared by removing the mucosal layer of opened lobes by blunt dissection under a microscope and immersed in a fixative containing 2% formaldehyde and 15% saturated picric acid in 0.1 M phosphate buffer (PBS, pH 7.4).

Preparations were then immersed in PBS containing 0.3% Triton X-100 for 10 min, immersed in Block Ace for 20 min and incubated with rabbit anti-P2X1 receptor antibody (1:1000, Dako Glostrup, Denmark) for 4 days at 4 °C. Tissues were washed in PBS and incubated first with biotinylated swine anti-rabbit IgG antibody (1:300, Dako, Glostrup, Denmark) for 30 min at room temperature followed by Cy-3-conjugated streptavidin (4.5 μg/mL, Jackson ImmunoResearch, PA, USA) for 2 h. To produce a negative control, the antibody was incubated with P2X1 receptor antigen (Alomone, Jerusalem, Israel) 1:1000 for 1 h prior to the primary antibody incubation for 4 days. Specimens were then examined using a confocal laser scanning microscope (LSM 5 PASCAL, Zeiss).

Both negative and P2X1 receptor positive preparations were treated simultaneously and micrographs were taken with the same parameters of the confocal microscope.

2.5. Analysis

2.5.1. Electrical recordings

In a typical trace of EJP activity, parameters such as the resting membrane potentials (RMP, mV), the peak amplitude of the EJP from the RMP (mV), the half-amplitude duration (half-width, ms), decay tau (ms) and voltage integral (mV ms) were measured. The parameters of 10 consecutive electrical events were analyzed in the absence and presence of drugs.

In unstimulated tissue, the parameters of five spontaneous slow waves included amplitude (mV), half-amplitude duration (half-width, ms), frequency (min^{-1}) and RMP (mV).

2.5.2. Nerve-induced contractions

The amplitude of contractions elicited by EFS were measured from the baseline to the peak amplitude and calculated as a percentage of the control contraction induced by a high concentration of K^+ (20 mM). After plotting the log frequency of contractions, the frequency producing a maximal effect (E_{max}) was compared to control.

All values are expressed as mean ± standard deviation and n denotes the number of cells impaled. In this study, 'n' also denotes the number of animals used since each data set recorded was taken from separate individual animals. A Student's t-test for paired data was performed for most tests of significance and differences were considered significant at $P < 0.05$. Bonferroni post-hoc tests were conducted for nerve-induced contractions to compare contractions in control and drug at each frequency. Additional two-way ANOVA tests were used to compare EJP parameters between young and older animals. All statistical analyses were performed using Clampfit (Axon Instruments, CA, USA) and GraphPad Prism (GraphPad Software, CA, USA) while graphics were designed using GraphPad Prism and OriginPro 7.5 (OriginLab Co., MA, USA).

2.6. Drugs used

Phentolamine, 3,7-dimethyl-1-propargylxanthine (DMPX), yohimbine hydrochloride, tetrodotoxin (TTX) and α-β-methylene ATP were purchased from Sigma-Aldrich (MO, USA), ARL 67156 and MRS 2179 was from Tocris Bioscience (MI, USA), guanethidine was from Tokyo Chemical Industries (Tokyo, Japan) while pyridoxal phosphate-6-azophenyl-2,4-disulfonic acid (PPADS) was from Cayman Chemicals (MI, USA). All drugs were dissolved in distilled water to stock concentrations of 10 mM prior to use.

3. Results

3.1. General observations

In young guinea-pigs, the resting membrane potential of prostatic smooth muscle cells ranged between −72.6 mV and −39.5 mV (mean = −56.5 ± 9.1 mV, n = 29, Table 1). Single impulses induced EJPs that had an amplitude of 4.7 ± 2.9 mV and lasted for 260 ± 80 ms with a decay tau of 495.2 ± 39 ms. Repetitive stimuli evoked summed EJPs that triggered the generation of a slow wave with superimposed action potentials (Fig. 1Aa) and corresponding contraction (Fig. 1Ab). The EJPs themselves did not cause detectable