Voltage Dependence of Slow Wave Frequency in the Guinea Pig Prostate

Yusuke Shigemasa, Michelle Lam, Retsu Mitsui and Hikaru Hashitani*

Department of Cell Physiology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

Abbreviations and Acronyms

α-SMA = α-smooth muscle actin
AMP = peak amplitude
ATP = adenosine triphosphate
ATPase = adenosine triphosphatase
ICC = interstitial cell of Cajal
$[K^+]_o$ = extracellular K^+
K_{ATP} = ATP sensitive K^+
PIC = prostate interstitial cell
PSS = physiological salt solution
RMP = resting membrane potential
SW = slow wave

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* Correspondence: Department of Cell Physiology, Nagoya City University Graduate School of Medical Sciences, Nagoya 467-8601, Japan (telephone: 81-52-8538131; FAX: 81-52-8421538; e-mail: hasitani@med.nagoya-cu.ac.jp).

Purpose: Spontaneous phasic contractions of the guinea pig prostate stroma result from the generation of slow waves that appear to primarily rely on spontaneous Ca^{2+} release from the endoplasmic/sarcoplasmic reticulum and subsequent opening of Ca^{2+} activated chloride channels. We investigated voltage dependent mechanisms in the regulation of slow wave frequency.

Materials and Methods: Changes in membrane potential were recorded using conventional intracellular recording techniques while simultaneously measuring the isometric tension of guinea pig prostate lobes. Fluorescence immunohistochemistry was done to determine the cellular composition of the prostate stroma.

Results: Depolarization induced by high K^+ solution, K^+ free solution or outward current injection was associated with increased slow wave frequency. In contrast, hyperpolarization induced by the re-addition of K^+, adenosine triphosphate sensitive K^+ channel openers or inward current injection prevented slow wave generation. K^+ channel openers induced hyperpolarization and the cessation of slow waves was reversed by glibenclamide (10 μM). Nifedipine (1 to 10 μM) shortened the duration of slow waves and pacemaker potentials but often failed to prevent their generation and associated contractions. Subsequently Ni^{2+} (100 μM) or mibefradil (1 μM) largely suppressed slow waves and abolished residual contractions. Immunohistochemistry revealed small interconnected smooth muscle bundles as well as vimentin positive interstitial cells but failed to show a network of Kit positive interstitial cells.

Conclusions: Prostate slow wave frequency is voltage dependent due to the significant contribution of L-type and T-type Ca^{2+} channels. Prostate slow waves may arise from cooperation between spontaneous Ca^{2+} release from internal stores and plasmalemmal voltage dependent Ca^{2+} channels.

Key Words: prostate; prostatic hyperplasia; muscle, smooth; calcium channels; interstitial cells of Cajal

Lower urinary tract symptoms associated with benign prostatic hyperplasia can be attributable to dynamic and static mechanisms. The dynamic component is mediated by increased smooth muscle contractility while static obstruction arises from bulk enlargement due to proliferation of smooth muscle, fibroblasts and epithelium.[1] Increased prostate smooth muscle tone partly results from enhanced α_1-adrenergic contractions which current pharmacological therapies target to relieve benign prostatic hyperplasia/lower urinary tract symptoms[1] but myogenic contractions may also contribute to dynamic obstruction.

In many smooth muscle organs spontaneous myogenic contractions

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arise from the generation of electrical SWs initiated by specialized pacemaker cells, eg ICCs in the gastrointestinal tract.[2] PICs immunoreactive to antibodies against the ICC marker Kit receptor were identified in guinea pigs[3] and humans,[4] and proposed as pacemaker cells for SW generation. However, growing evidence indicates that Kit negative interstitial cells in the mouse renal pelvis[5] or bladder suburothelium[6] are also spontaneously active.

Prostate SW generation presumably begins with spontaneous Ca^{2+} release from the endoplasmic reticulum, which triggers the opening of Ca^{2+} activated Cl^- channels.[7,8] This leads to membrane depolarization, which propagates to neighboring smooth muscle cells in which the opening of L-type Ca^{2+} channels results in spontaneous contractions.[9] Isolated PICs generate spontaneous Ca^{2+} transients but are noncontractile while prostate myocytes are quiescent and vigorously contract upon application of high K^+ solution.[10] Thus, PICs may act as pacemaker cells to drive prostate smooth muscle contractions. Prostate SWs are relatively less sensitive to nifedipine than action potentials[3,7] but our recent study of isolated PICs revealed otherwise.[10] Unlike ICC[2] or interstitial cells in the urinary tract[6,11,12] spontaneous Ca^{2+} transients in PICs are readily abolished by nifedipine. Thus, PICs do not conform to the conventional criteria used to describe smooth muscle pacemaker cells[13] and they may not represent an exclusive origin for SW generation.

We addressed these controversies by characterizing the voltage dependence of prostate SW generation, particularly focusing on the role of L-type and T-type Ca^{2+} channels. Cellular composition of the prostate stroma was also reevaluated by immunohistochemical analysis.

METHODS

Tissue Preparation

Male guinea pigs weighing 250 to 300 gm were sacrificed by exsanguination under sevoflurane anesthesia according to procedures approved by the Nagoya City University animal experimentation ethics committee. The prostate gland was extracted and the individual lobes were cut open to form a sheet. The sheet was stretched and pinned mucosal side up to the bottom of a 1 ml recording chamber coated with Sylgard®. Preparations were superfused with oxygenated PSS warmed to 35C at a constant flow rate of about 2 ml per minute.

Intracellular and Tension Recording

Prostate preparations were impaled by glass capillary microelectrodes filled with 1 M KCl (tip resistance 150 to 250 MΩ). Membrane potential changes were recorded with an Axoclamp™ 2B high impedance amplifier. After low pass filtering at 1 kHz membrane potential changes were digitized and stored on a personal computer for later analysis. In some experiments the preparation was impaled by 2 independent microelectrodes. One electrode was used to deliver current and the other was used to record changes in membrane potential. In a separate series of experiments opened lobes were pinned along one side of the preparation while the other side was attached to an isometric force transducer by a thread. Isometric tension was recorded simultaneously with intracellular recording.

Immunohistochemistry

For α-SMA or vimentin staining prostate whole mount preparations were immersed in fixative containing 2% formaldehyde and 15% saturated picric acid in 0.1 M phosphate buffer (pH 7.4). Preparations used for Kit immunostaining were fixed with acetone.

A whole mount preparation was immersed in phosphate buffered saline containing 0.3% Triton™ X-100 for 10 minutes, immersed in Block Ace (Bio-Rad®) for 20 minutes and incubated with primary antibodies for 4 days at 4C. The primary antibodies used were mouse monoclonal anti-α-SMA antibody (1:200, clone1A4, Sigma-Aldrich®), mouse anti-vimentin antibody (1:50, Dako, Glostrup, Denmark) and rat anti-Kit antibody (1:200, Affymetrix®). Tissues were washed in phosphate buffered saline and incubated with Cy-3 conjugated goat anti-mouse IgG (2.5 μg/ml, Millipore®) or Alexa 488 conjugated goat anti-rat IgG antibody (1:200, Molecular Probes®) for 2 hours. Specimens were examined using a LSM 5 Pascal confocal laser scanning microscope (Carl Zeiss, Jena, Germany).

Whole mount preparations of guinea pig stomach smooth muscle layers served as positive controls for Kit immunostaining. Prostate and stomach preparations were treated simultaneously and micrographs were taken with a confocal microscope using the same parameters.

Drugs and Solutions

PSS was composed of 137.4 mM Na^+, 5.9 mM K^+, 2.5 mM Ca^{2+}, 1.2 mM Mg^{2+}, 15.5 mM HCO_3^-, 1.2 mM $H_2PO_4^-$, 134 mM Cl^- and 11.5 mM glucose. PSS pH was 7.2 when bubbled with 95% O_2 and 5% CO_2, and the measured pH of the recording bath was approximately 7.4. Solutions containing high $[K^+]_o$ were prepared by replacing NaCl with an equimolar amount of KCl. The drugs used were nifedipine, glibenclamide, mibefradil (Sigma-Aldrich), levcromakalim, Y-26763 (Tocris Bioscience, Bristol, United Kingdom) and nickel chloride (Katayama Chemicals, Osaka, Japan). Glibenclamide, levcromakalim and Y-26763 were dissolved in dimethyl sulfoxide and nifedipine was dissolved in absolute ethanol. The final concentration of these solvents in PSS did not exceed 1:1,000.

Analysis

Measured values are shown as the mean ± SD. Statistical significance was tested using the paired t-test and considered significant at $p < 0.05$. Since AMP (SW) could not readily be defined, we measured the parameters of 5 consecutive SW complexes, including RMP, AMP (RMP to the peak of the SW complex) and half-width (time between assumed 50% AMP [SW] on the depolarizing and repolarizing phases) (fig. 1, *A*). Frequency was calculated as an average during 3 to 5 minutes of recording. Mean