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Effects of K^+ channel openers on spontaneous action potentials in detrusor smooth muscle of the guinea-pig urinary bladder

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ABSTRACT

The modulation of spontaneous excitability in detrusor smooth muscle (DSM) upon the pharmacological activation of different populations of K^+ channels was investigated. Effects of distinct K^+ channel openers on spontaneous action potentials in DSM of the guinea-pig bladder were examined using intracellular microelectrode techniques. NS1619 (10 μM), a large conductance Ca^{2+}-activated K^+ (BK) channel opener, transiently increased action potential frequency and then prevented their generation without hyperpolarizing the membrane in a manner sensitive to iberiotoxin (IbTX, 100 nM). A higher concentration of NS1619 (30 μM) hyperpolarized the membrane and abolished action potential firing. NS309 (10 μM) and SKA31 (100 μM), small conductance Ca^{2+}-activated K^+ (SK) channel openers, dramatically increased the duration of the after-hyperpolarization and then abolished action potential firing in an apamin (100 nM)-sensitive manner. Flupirtine (10 μM), a Kv7 channel opener, inhibited action potential firing without hyperpolarizing the membrane in a manner sensitive to XE991 (10 μM), a Kv7 channel blocker. BRL37344 (10 μM), a β_3-adrenceptor agonist, or rolipram (10 nM), a phosphodiesterase 4 inhibitor, also inhibited action potential firing. A higher concentration of rolipram (100 nM) hyperpolarized the DSM and abolished the action potentials. IbTX (100 nM) prevented the rolipram-induced blockade of action potentials but not the hyperpolarization. BK and Kv7 channels appear to predominantly contribute to the stabilization of DSM excitability. Spare SK channels could be pharmacologically activated to suppress DSM excitability. BK channels appear to be involved in the cyclic AMP-induced inhibition of action potentials but not the membrane hyperpolarization.

1. Introduction

During filling phases, the bladder appears spontaneously contractile to maintain a shape that minimizes its surface area (Brading, 2006). In human bladder *in vivo*, 'micromotions' develop with little rises in intravesical pressure (Coolsaet et al., 1993; Drake et al., 2005; Robertson, 1999). Corresponding activity is seen as autonomous activity in the whole bladder (Drake et al., 2003; Parsons et al., 2012) or as spontaneous phasic contractions in detrusor smooth muscle (DSM) strips. Increases in spontaneous activity are observed in human overactive bladders or in detrusor overactivity in animal models (Brading, 1997; Vahabi and Drake, 2015). In addition, spontaneous contractions of DSM have an important influence on afferent nerve activity (Heppner et al., 2016). Therefore, the manipulation of the spontaneous excitability in DSM would be an ideal strategy for the treatment of the overactive bladder.

Spontaneous phasic contractions of DSM result from the firing of action potentials (Hashitani et al., 2004). Large conductance Ca^{2+}-activated K^+ (BK) channels contribute to action potential repolarization as well as their after-hyperpolarization (Hashitani and Brading, 2003a, 2003b; Heppner et al., 1997) and play a predominant role in stabilizing DSM excitability. Deletion of pore-forming subunits of BK channels results in overactive bladder phenotype (Meredith et al., 2004). Voltage-dependent and small conductance Ca^{2+}-activated K^+ (SK) channels appear to be involved in maintaining the resting membrane potential (Herrera and Nelson, 2002; Thorneloe and Nelson, 2003) as well as determining the pattern of action potential firing (Hashitani and Brading, 2003a, 2003b). Suppressing SK3 channel expression in mouse bladder is associated with an increase in DSM spontaneous contractions and bladder overactivity (Herrera et al., 2003). Essential role of SK2 channel to regulate DSM contractility has also been demonstrated using mice lacking SK2 gene expression (Thorneloe et al., 2008).

K^+ channels openers have been optimistically considered for the treatment of the overactive bladder, although their clinical application has not yet succeeded (Fabiyi et al., 2003). Previous studies investigated the actions of K^+ channel openers on DSM excitability/contractility mostly employed patch-clamp techniques

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using isolated DSM cells and/or contractile studies using DSM strips (Bonev and Nelson, 1993; Herrera et al., 2003; Thorneloe et al., 2008). Thus, only limited information is available for K^+ channels openers-induced suppression of DSM action potential firing (Hashitani et al., 2004; Layne et al., 2010; Petkov et al., 2001). Therefore, a re-evaluation of the action of different K^+ channel openers on DSM spontaneous electrical activity is required to fill the gap between single cell studies and contractile studies.

β_3 adrenoceptor agonists and PDE4 inhibitors that increase intracellular cAMP levels are reported to hyperpolarize the membrane by opening BK channels to relax DSM (Hristov et al., 2008; Xin et al., 2014). Other studies reported that β_2 adrenoceptor agonist relax DSM by activating K_{ATP} channels (Hudman et al., 2000) and that isoproterenol, a non-selective β adrenoceptor agonist hyperpolarizes DSM membrane by stimulating the sodium pump (Nakahira et al., 2001). Thus, the contribution of BK channel-induced hyperpolarization to cAMP-dependent relaxation of DSM is also of interest.

2. Material and methods

2.1. Animals

Male guinea-pigs weighing 230–310 g were housed in plastic cages in a special temperature-controlled room (23.5 ± 2 °C, 50 ± 10% humidity) on a 12/12 h light/dark cycle with free access to food and water. The procedures described have been approved by the animal experimentation ethics committee at Nagoya City University Graduate School of Medical Sciences.

2.2. General procedure

Animals were anesthetized with sevoflurane (Maruishi Pharmaceutical, Osaka, Japan) and exsanguinated by decapitation. The urinary bladder was removed and its ventral wall was opened longitudinally from the top of the dome to the bladder neck. The mucosal layer and several 'inner' smooth muscle layers were then removed leaving underlying single smooth muscle bundles attached to the outer connective tissue sheet. A connective tissue sheet, which contained one or a few single bundles of smooth muscle, 2 – 3 mm long and 0.2 – 0.7 mm wide was then prepared. This preparation was pinned out on a silicone plate (Silpot 184, Dow Corning Toray, Tokyo, Japan) at the bottom of the recording chamber (volume, approximately 1 ml) that was mounted on a stage of an inverted microscope. The preparations were superfused with warmed (35 °C) PSS at a constant flow rate (2 ml/min).

2.3. Intracellular recording

Individual DSM cells in single muscle bundles were impaled with a glass capillary microelectrode, filled with 1 M KCl (tip resistance, 120 – 210 MΩ). Membrane potential changes were recorded using a high input impedance amplifier (Axoclamp-2B, Molecular Devices, Sunnyvale CA 94,089, USA), and displayed on a cathode-ray oscilloscope (SS-7602, Iwatsu, Tokyo, Japan). After low-pass filtering (cut-off frequency, 1 kHz), membrane potential changes were digitized using Digidata 1440A interface (Molecular Devices, Sunnyvale CA 94,089, USA) and stored on a personal computer for later analysis.

2.4. Solutions and drugs

The composition of PSS was (in mM): Na^+, 137.5; K^+, 5.9; Ca^{2+}, 2.5; Mg^{2+}, 1.2; HCO_3^-, 15.5; $H_2PO_4^-$, 1.2; Cl^-, 134 and glucose, 11.5. The pH of PSS was 7.2 when bubbled with 95% O_2 and 5% CO_2, and the measured pH of the organ bath solution was approximately 7.4.

Drugs used were apamin, iberiotoxin (IbTX) (from Peptide Institute, Osaka, Japan), BRL37344 ((R*, R*)-[4-[2-[[2(3-chlorophenyl)-2-hydroxyethyl] amino]propyl]phenoxy] acetic acid sodium hydrate), isoproterenol, NS1619 (1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2 h-benzimidazol-2-one) and rolipram (from Sigma-Aldrich Corp. St. Louis, MO, USA), NS309 (3-oxime-6,7-dichloro-1H-indole-2,3-dione), SKA-31 (naphtho[1,2-d]thiazol-2-amine), flupirtine and XE991 (from Tocris Bioscience, Bristol, UK). Apamin, BRL37344, IbTX, isoproterenol were dissolved in deionized water. Other drugs were dissolved in DMSO. The final concentration of these solvents in PSS did not exceed 0.1% that is reported to have no relaxing effect on DSM contractility (Shiga et al., 2007).

2.5. Calculations and statistics

The following parameters of action potentials were measured: resting membrane potential; peak amplitude, measured as the value from the resting membrane potential to the action potential peak; maximum rate of voltage change over time (Max *dV*/*dt*), measured as the maximum *dV*/*dt* on the rising phase; half-width, measured as the time between 50% peak amplitude on the rising and falling phases. The amplitude of membrane after-hyperpolarizations was measured as the value from the resting membrane potential to the peak of the after-hyperpolarizations. The half-width of these after-hyperpolarization was measured as the time between 50% peak amplitude on the falling and rising phases. For preparations in which K^+ channel openers abolished action potentials, 8–10 action potentials before complete cessation were used for analysis.

Measured values were expressed as mean ± standard deviation (n = number of preparations as well as animals). Statistical significance was tested using paired *t*-test, and probabilities of less than 5% different from the control were considered significant.

3. Results

3.1. General observations

All DSM preparations (n = 46) exhibited spontaneous action potentials and had resting membrane potentials ranging between −47.6 and −36.5 mV (mean −42.9 ± 2.9 mV). Action potentials were generated at frequencies ranging between 3.8 and 38.5 min^{-1} (mean 15.9 ± 8.7 min^{-1}) and had peak amplitudes ranging between 35.5 and 66.5 mV (mean 51.2 ± 6.3 mV), half-width ranging between 4.4 and 13.7 ms (mean 6.7 ± 1.8 ms) and max d*V*/d*t* ranging between 1.8 and 22.4 mV ms^{-1} (mean 11.0 ± 3.8 mV ms^{-1}). After-hyperpolarizations had amplitudes ranging between −19.6 and −1.6 mV (mean −11.3 ± 3.5 mV). These values were not noticeably different from those previously reported in the same tissue (Hashitani and Brading, 2003a; Hashitani et al., 2004).

3.2. Effects of BK channel openers on spontaneous action potentials

First, the effects of an opener of BK channels that play a predominant role in stabilizing DSM excitability were examined.

NS1619 (10 μM) unexpectedly caused a transient increase in the frequency of spontaneous action potentials associated with a reduction in their after-hyperpolarization amplitudes (Fig. 1A, C). NS1619 then abolished (n = 8) or slowed (n = 1) action potential firing without hyperpolarizing the membrane. NS1619 (10 μM) also significantly increased the half-width and decreased the max d*V*/d*t* of action potentials. These results are summarized in Table 1. Action potential generation was restored by iberiotoxin (100 nM),

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