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Characterization of stretch-activated calcium permeable cation channels in freshly isolated myocytes of the cricket (*Gryllus bimaculatus*) lateral oviduct

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Abstract

Stretch-activated channels (SACs) were investigated in myocytes isolated from the lateral oviduct in cricket *Gryllus bimaculatus* using the cell-attached or excised inside-out patch clamp technique. Application of both negative and positive pressure (10–100 cm H_2O) into the patch pipettes induced the unitary channel current openings. The open probability (NPo) of the channel increased when negative pressure applied into the patch pipettes increased. The single channel conductance for this channel was approximately 20 pS with 140 mM Na^+, K^+, or Cs^+ in the patch pipettes and was approximately 13 pS with 100 mM Ca^{2+} or Ba^{2+} in the patch pipettes. External application of Gd^{3+}, La^{3+}, Cd^{2+} and Zn^{2+} inhibited the channel with the IC_{50} values of 14, 15, 28, and 18 μM respectively. Interestingly external application of TEA, a specific blocker of K^+ channel, also inhibited this channel with IC_{50} value of 8.8 mM. These results show for the first time the presence of stretch activated Ca^{2+}-permeable nonselective cation channel in myocytes isolated from the cricket lateral oviduct. The physiological significance of this channel in oviposition behavior is discussed.

Keywords: Stretch-activated channel; Isolated myocytes; *Gryllus bimaculatus*; Lateral oviduct; Patch clamp

1. Introduction

It has been reported that stretch of cell membrane can be an important physiological stimulus and that stretching muscle membrane can cause depolarization of the cell membrane, an increased frequency of action potentials, and subsequent muscle contraction (Burbring, 1955; Bulbring and Kuriyama, 1963; Johansson and Mellander, 1975; Harder, 1984; Harder et al., 1985; Harder et al., 1987; Wellner and Isenberg, 1993, 1994). The membrane stretch-activated channels (SACs) have been described in many kinds of muscle cells including skeletal (Guharay and Sachs, 1984), visceral (Kirber et al., 1988; Wellner and Isenberg, 1993, 1994) and vascular (Davis et al., 1992; Setoguchi et al., 1997) smooth muscle cells. Most of the SACs so far examined have been shown to be Ca^{2+}-permeable, nonselective cation channels, and the activation of which introduces some Ca^{2+} into the cell. This Ca^{2+} influx via SA channels has been shown to activate phospholipase C and stored Ca^{2+} is released by inositol 1,4,5-triphosphate (Matsumoto et al., 1995). Thus, a number of electrophysiological studies have now directed greater attention towards vertebrate SACs. However, little information exists on invertebrate SACs despite their importance in various cellular function although the presence of stretch activated K^+ channels has been reported in insect (Zagotta et al., 1988; Vais and Usherwood, 1995; Gu et al., 2001) and in mollusca (Sigurdson et al., 1987).

Recently, Mutoh and Yoshino (2004) have suggested the possible existence of SACs in insect visceral muscle cells isolated from the cricket lateral oviduct with the use of whole-cell patch clamp technique. However, at present no single channel analysis of the SAC has been

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done in these cells. In the present study, SACs were first identified in the cricket lateral oviduct myocytes and their electrophysiological properties directly examined at the single channel level in cell-attached or excised inside-out patches.

2. Materials and methods

2.1. Animals

Experiments were carried out on female crickets, *G. bimaculatus* which were maintained in a colony in the Department of Biology at 25–30 °C with a relative humidity 65–85% under a 16:8 h light-dark photoperiod. Crickets were fed on an artificial insect diet (Oriental Yeast) and supplied with water.

2.2. Dissection of lateral oviduct

Before dissection, animals were anesthetized using CO_2. An animal was fixed dorsal side up and the lateral oviduct exposed in normal saline containing (in mM) 140 NaCl, 10 KCl, 1.6 $CaCl_2$, 2 $MgCl_2$, 44 glucose, 2 HEPES, buffered to pH 7.4 with Tris (hydroxymethyl) aminomethane.

2.3. Cell isolation

Single muscle cells of the lateral oviduct were isolated enzymatically using a pronase dispersion method. Briefly, dissected lateral oviduct was placed in a plastic 35 mm dish filled with Ca^{2+}-free normal saline: 140 NaCl, 10 KCl, 3.6 $MgCl_2$, 44 glucose, 2 HEPES, pH 7.4 (Tris) and cut into 0.5×0.5-mm pieces in this solution, and the pieces were incubated for 30 min. They were then transferred into Ca^{2+}-free normal saline containing 0.3% pronase and incubated for 25 min at 37 °C, and were mechanically dissociated with the use of acrobat stirrer. Dissociated single myocytes of lateral oviduct were then superfused with a fresh normal saline. Viability of enzyme dissociated myocytes was checked by the two critera: (1) a smooth, bright plasma membrane with a clear striation and (2) an ability of spontaneouse free contraction or an ability of contraction in response to the excitatory neurotransmitter, L-glutamate (100 μM). We have mainly used the first criteria for electrophysiological recordings and each experiment was done within 1 h after isolation of myocytes.

2.4. Patch clamp recording and analysis

Patch pipettes were pulled from capillary tubes (Hematocrits capillary, Drummond Scientific Co.) with a two stage pipette puller (PC-10, Narishige Co., LTD) and had a tip resistance of approximately 5 MΩ when filled with a solution for each experimental purpose. Freshly dispersed cells were allowed to settle on the flat-glass bottom of a silicone chamber mounted on the stage of an inverted microscope ($I \times 70$ OLYMPUS) and the patch electrode positioned on the cell surface with a three-dimensional hydraulic micromanipulator (MHW-3, Narishige Co., LTD). Single channel currents were recorded in cell-attached or inside-out patch configuration, as described in detail by Hamill et al. (1981) through a patch clamp amplifier (Axopatch 200 B) and A/D converter (Axon Instruments). Current signals sampled at 5 kHz and were low-pass filtered at 1 kHz (four-pole Bessell). Recorded currents were digitized and stored on a computer system. Data acquisition and analysis were performed with pClamp 8.0 software (Axon Instruments). Membrane potentials and reversal potentials are defined as the potential at the cytosolic face of membrane with respect to the potential at external face of the membrane. Averaged data are expressed as the mean $\pm$S.E.M. where n equals the number of patches (cells). All experiments were performed at room temperature (20–25 °C).

2.5. Mechanical stretch in the patch membrane

Mechanical stretch in the patch pipette was made by applying negative or positive pressure in the patch pipettes. Pressure in the patch pipettes was changed as follows: One end of a 1-m-high manometer filled with distilled water was connected to the pipette holder, and the other end was connected to a calibrated syringe. Changes in pipettes pressure could be achieved rapidly and accurately to desired levels with an automated calibrated syringe. Pressure levels were measured as millimeters of H_2O.

2.6. Solutions and chemicals

The standard pipette solutions used for the cell-attached patch clamp recordings contained (in mM): 100 $BaCl_2$, 1.6 $CaCl_2$, 2 $MgCl_2$, 2 HEPES, pH 7.4 (Tris). In some experiments, extracellular Ba^{2+} (100 mM) was replaced with Ca^{2+} (100 mM), KCl (140 mM), NaCl (140 mM), and CsCl (140 mM). In excised inside-out patch recordings, pipette solution contained (in mM): 100 $BaCl_2$, 2 $MgCl_2$, 2 HEPES, pH 7.4 (Tris) and the bath solution contained (in mM): 140 KCl, 2 HEPES, pH 7.4 (Tris). The free Ca^{2+} concentration in the bath solution was determined by adjusting with EGTA on the basis of calculations using the computer program EqCal (Biosoft) according to the stability constant from Owen (1976).

2.7. Backfill procedure

Effects of Gd^{3+}, La^{3+}, Cd^{2+}, and Zn^{2+} on the SAC were examined by the backfill procedure. Briefly, tips of