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Involvement of plasma membrane Ca^{2+} channels, IP_3 receptors, and ryanodine receptors in the generation of spontaneous rhythmic contractions of the cricket lateral oviduct

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ABSTRACT

In the present study, the isolated cricket (*Gryllus bimaculatus*) lateral oviduct exhibited spontaneous rhythmic contractions (SRCs) with a frequency of 0.29 ± 0.009 Hz ($n = 43$) and an amplitude of 14.6 ± 1.25 mg ($n = 29$). SRCs completely disappeared following removal of extracellular Ca^{2+} using a solution containing 5 mM EGTA. Application of the non-specific Ca^{2+} channel blockers Co^{2+}, Ni^{2+}, and Cd^{2+} also decreased both the frequency and amplitude of SRCs in dose-dependent manners, suggesting that Ca^{2+} entry through plasma membrane Ca^{2+} channels is essential for the generation of SRCs. Application of ryanodine (30 μM), which depletes intracellular Ca^{2+} by locking ryanodine receptor (RyR)-Ca^{2+} channels in an open state, gradually reduced the frequency and amplitude of SRCs. A RyR antagonist, tetracaine, reduced both the frequency and amplitude of SRCs, whereas a RyR activator, caffeine, increased the frequency of SRCs with a subsequent increase in basal tonus, indicating that RyRs are essential for generating SRCs. To further investigate the involvement of phospholipase C (PLC) and inositol 1,4,5-trisphosphate receptors (IP_3Rs) in SRCs, we examined the effect of a PLC inhibitor, U73122, and an IP_3R antagonist, 2-aminoethoxydiphenyl borate (2-APB), on SRCs. Separately, U73122 (10 μM) and 2-APB (30–50 μM) both significantly reduced the amplitude of SRCs with little effect on their frequency, further indicating that the PLC/IP_3R signaling pathway is fundamental to the modulation of the amplitude of SRCs. A hypotonic-induced increase in the frequency and amplitude of SRCs and a hypertonic-induced decrease in the frequency and amplitude of SRCs indicated that mechanical stretch of the lateral oviduct is involved in the generation of SRCs. The sarcoplasmic reticulum Ca^{2+}-pump ATPase inhibitors thapsigargin and cyclopiazonic acid impaired or suppressed the relaxation phase of SRCs. Taken together, the present results indicate that Ca^{2+} influx through plasma membrane Ca^{2+} channels and Ca^{2+} release from RyRs play an essential role in pacing SRCs and that Ca^{2+} release from IP_3Rs may play a role in modulating the amplitude of SRCs, probably via activation of PLC.

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1. Introduction

Myogenic spontaneous rhythmic contractions (SRCs) are basic physiological processes considered to be fundamental to muscle physiology (McHale et al., 2006; Martin-Cano et al., 2009; Park et al., 2010; Xu and Wen-Xie, 2010; Komari et al., 2013). Many studies have been performed in insects to uncover the properties of myogenic activity in visceral muscles. The oviducts of the African migratory locust *Locusta migratoria* have been a valuable model preparation for investigating myogenic contraction and its neural and hormonal regulation (Orchard and Lange, 1985, 1986; Kalogianni and Theophilidis, 1995; Noronha and Lange, 1997; Nykamp and Lange, 1998; Donini et al., 2001; Donini and Lange, 2002, 2004; Lange, 2002, 2004). Ca^{2+} entry from the extracellular space is crucial to the generation of basal tonus and SRCs of the oviduct muscle (Wang et al., 1995; Wilcox and Lange, 1995; Noronha and Lange, 1997). Evidence has also been presented for the involvement of the adenylate cyclase/cAMP signaling pathway in octopamine action on locust oviduct (Lange and Nykamp, 1996; Nykamp and Lange, 2000) and for the involvement of the phospholipase C (PLC)/inositol 1,4,5-trisphosphate (IP_3) signaling pathway in proctolin action on locust oviduct (Lange, 1988; Lange and Nykamp, 1996) and foregut muscle (Hinton and Osborne, 1995; Hinton et al., 1998). However, there is little evidence of a primary mechanism underlying the myogenic responses related to the intrinsic properties of the lateral oviduct. Moreover, the relative importance

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of the contributions of the different Ca^{2+} influx mechanisms, of intracellular Ca^{2+} mobilization via IP_3 or ryanodine receptors (IP_3Rs or RyRs, respectively), of Ca^{2+}-uptake into the sarcoplasmic reticulum (SR), and of mechanical stretching to the generation of SRCs remains to be assessed.

Thus, the aim of the present study was to evaluate the contribution of Ca^{2+} released via RyRs and IP_3Rs located in intracellular Ca^{2+} stores as well as Ca^{2+} influx via plasma membrane Ca^{2+} channels in the generation of SRCs in isolated lateral oviduct of the cricket *Gryllus bimaculatus*. In addition, the contribution of mechanical stretch and SR Ca^{2+} uptake to SRCs were investigated. The present results indicated that cyclical Ca^{2+} release from RyRs, as well as Ca^{2+} influx through plasma membrane Ca^{2+} channels, plays an essential role in pacing and oscillatory rhythm and that IP_3Rs play a role in modulation of the amplitude of SRCs, probably via activation of PLC due to mechanical stretch of the lateral oviduct.

2. Materials and methods

2.1. Animals

Experiments were performed on adult female *G. bimaculatus* crickets that were maintained in a colony at our institution's Department of Biology at 25–30 °C with a relative humidity of 65%–85% in a 12 h:12 h light:dark photoperiod. Crickets were fed on an artificial insect diet (Oriental Yeast) and supplied with water.

2.2. Dissection of the lateral oviduct

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

2.3. Recording of isometric contractions

The upper part of the lateral oviduct was attached horizontally to a mechano-electrical force transducer (FD Pickup SB-1T; Nihon Kohden) using a fine thread while the anterior end of the ovaries was attached to the base of the trough in a Sylgard-coated dish (containing 400 μl saline) for measurement of isometric tension. The output signal of the force transducer was amplified by a carrier amplifier and further amplified by a high-gain DC amplifier (AVH; Nihon Kohden). Muscle contractions were recorded by using a chart recorder (Omniace, NEC Sanei) and also stored by using a computer-based field recording unit (es8, TEAC Corporation, Japan) for later analysis. The amplitude of SRCs was measured from average 10–15 contractions. The frequency of the SRCs was measured from the time interval between successive contractions of lateral oviduct. The measurements of the amplitude and frequency of SRCs in the presence of drug were done between 15 s after addition of drug and 2 min after addition of drug. We expressed the action of drug as an average relative value to the control response (assigned a value of 1.0) recorded in the absence of drug to reduce tissue-to-tissue variability.

2.4. Solution

The Ca^{2+}-free EGTA solution contained (in mM): 140 NaCl, 10 KCl, 3.6 $MgCl_2$, 44 glucose, 2 HEPES, and 4 EGTA, buffered to pH 7.4 with Tris–Cl. To examine the effect of reduced or increased in extracellular osmolarity on SRCs, we used three solutions differing in tonicity. The isotonic solution contained (in mM) 100 NaCl, 10 KCl, 1.6 $CaCl_2$, 2 $MgCl_2$, 44 glucose, 80 mannitol, and 2 HEPES, buffered to pH 7.4 with Tris. The ionic composition of the hypotonic solution was the same as that of the isotonic solution, but the osmolarity was reduced by removing mannitol. The ionic composition of the hypertonic solution was the same as that of the isotonic solution but the osmolarity was increased by adding 40 mM mannitol.

2.5. Drugs

2-Aminoethoxydiphenyl borate (2-APB) (Wako), tetracaine (Wako), ryanodine (Wako), U-73122, cyclopiazonic acid (CPA; Wako), and thapsigargin (TG; Wako) were prepared as a stock in dimethyl sulfoxide (DMSO); the final concentration of DMSO in the bath was less than 1:1000. All drug solutions were freshly prepared before use and were continuously perfused at a rate of approximately 5 ml/min.

2.6. Statistical analysis

Statistics were performed on the data once they were expressed relative to controls. Values are given as the means ± SEM, with *N* representing the number of preparations. For comparisons between two groups, a Student's *t* test, paired or unpaired, was used for statistical analysis, with *P* values <0.05 considered statistically significant.

3. Results

3.1. Effect of external Ca^{2+} on SRCs

Isolated cricket lateral oviduct exhibited SRCs with a frequency of 0.29 ± 0.009 Hz ($n = 43$) and an amplitude of 14.6 ± 1.25 mg ($n = 29$). To investigate the dependency of the frequency and amplitude of SRCs on external Ca^{2+} concentration, we examined the effect of varying concentrations of extracellular Ca^{2+} on SRCs. A reduction in extracellular Ca^{2+} from the normal value of 1.6 mM to nominal Ca^{2+} free conditions immediately decreased the frequency and amplitude of SRCs (Fig. 1A_1). However, even in nominal Ca^{2+} free solution, slower and lower amplitude contractions were evident (Fig. 1A_1). The relative amplitude and frequency of SRCs reduced to 0.41 ± 0.04 ($n = 7$) and 0.32 ± 0.06 ($n = 7$) from 1.0 (Control, NR), respectively (Fig. 1B_1 and B_2). Following washout with normal saline, SRCs rapidly reappeared (Fig. 1A_1). To determine whether this remaining contraction was also extracellular Ca^{2+} dependent, we examined the effect of chelating extracellular Ca^{2+} with EGTA (5 mM) on SRCs. This treatment completely abolished SRCs (Fig. 1A_2, B_1 and B_2), indicating that SRCs are fully dependent on extracellular Ca^{2+}.

3.2. Effect of Ca^{2+} channel blockers on SRCs

The sensitivity of the frequency and amplitude of SRCs to the external Ca^{2+} concentration would suggest that Ca^{2+} influx is essential for the maintenance of normal SRCs. To test whether plasma membrane Ca^{2+} channels are involved in the generation of SRCs, we investigated the effect of the non-specific Ca^{2+} channel blockers Co^{2+}, Ni^{2+}, and Cd^{2+} on SRCs. Application of Co^{2+} reduced both the frequency and amplitude of SRCs (Fig. 2A_1 and A_2). This effect was found to be dose-dependent between 0.1 mM and 5 mM (Fig. 2B_1 and B_2). Similarly, Ni^{2+} and Cd^{2+} also reduced both the frequency and amplitude of SRCs in dose-dependent manners (Fig. 2C_1, C_2, D_1, and D_2). The highest doses for Co^{2+} (5 mM), Ni^{2+} (5 mM), and Cd^{2+} (1 mM) completely abolished SRCs. Thus, Ca^{2+} influx via plasma membrane Ca^{2+} channels is necessary for

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