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Modulation of the Ca^{2+} signaling pathway by celangulin I in the central neurons of *Spodoptera exigua*

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ABSTRACT

Celangulin I is an insecticidal component isolated from Chinese bittersweet *Celastrus angulatus*. The present study explored the possible effects of celangulin I on the calcium signaling pathway, especially on the L-type Ca^{2+} channel and the calcium channels in the endoplasmic reticulum in the central neurons isolated from the third instar larvae of *Spodoptera exigua* using whole-cell patch-clamp and calcium imaging technique. The results showed that celangulin I could activate the high voltage-gated calcium channel at the concentration of 150 μM. The peak currents were increased by 17% of the initial value at the end of the 10-min recording after treated with celangulin I. The rises of intracellular calcium ion concentration ($[Ca^{2+}]_i$) in neurons treated by celangulin I showed that the effects of celangulin I were concentration-dependent. Activation of the RyRs by ryanodine decreased the calcium release induced by celangulin I, indicating that celangulin I exerts effect on insect RyRs. Furthermore, we also provided evidence for the first time that celangulin I activates inositol 1,4,5-trisphosphate (IP_3) sensitive intracellular calcium release channels in the endoplasmic reticulum third instar larvae neurons of *S. exigua*. Plausibly, these experimental results can explain the characteristic symptoms of anesthesia and paralysis in celangulin I treated insects.

1. Introduction

Pesticides have offered means towards meeting the challenge of growing global populations. However, massive use of chemical pesticides, has brought some adverse impact on environment [1]. Imidacloprid, the largest-selling insecticide worldwide, has been implicated as the cause of honeybee death and the Colony Collapse Disorder (CCD) problem [2]. Recently, a resistant strain of *Plutella xylostella* (L.) with 9.09-fold resistance to chlorantraniliprole (an activator of insect ryanodine receptors) was also deemed by leaf-dipping method treated for 10 generations [3,4].

Botanical insecticides [5], such as azadirachtin, pyrethrins and rotenone are biodegradable [6] and they also serve as lead compounds and pharmacological probes to help us better understand biochemical and physiological mechanisms [7]. Celangulins, a series of bioactive compounds isolated from *Celastrus angulatus* (Celastraceae), were reported to possess antifeedant, narcotic and insecticidal properties against several pests including *Mythimna separata* etc. [8]. Celangulin I (Fig. 1), one of the insecticidal ingredients from the *C. angulatus*, had antifeedant activity [9]. In recent years, it has been discovered that this "antifeedant action" was associated with flaccid paralysis induced by Celangulin I [8]. Hannig [10] reported that the speed of action of chlorantraniliprole against target pest species, based on time for feeding cessation and reduction in feeding damage, is significantly greater than that of most recently developed insecticides and comparable only with that of the fast-acting carbamates and pyrethroids. Based on this information, it can be conjectured that celangulin I may modulate the calcium signaling pathway and lead to the loss of calcium homeostasis.

Calcium is a common second messenger that regulates many processes in cells (e.g., contraction, secretion, synaptic transmission, fertilization, nuclear pore regulation, transcription) [11]. Ryanodine receptors (RyRs) and inositol 1,4,5-triphosphate receptors (IP_3Rs) mediate the release of calcium from intracellular stores of the sarcoplasmic/endoplasmic reticulum (SR/ER) [12]. Unlike mammals, insects possess a single form of the RyRs with only 47% similarity to that of mammalian receptors at the amino acid level [13]. Insect RyRs appear to be an almost completely ignored field of research.

Previously research suggested that celangulin holds great promise to fit in the pest-management strategies [8], but the details of the mechanism of action of celangulin I are largely unknown. The targets in insects, especially calcium channel in the central neurons, unlike general human

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targets, are very complex and are not fully understood yet [14]. In order to provide useful information for designing novel insecticides based botanical insecticides and to explore the effects of celangulin I on calcium channel function, the whole-cell patch-clamp and calcium imaging technique were used simultaneously to investigate the effects on calcium signaling pathway in the central neurons of *S. exigua*.

2. Materials and methods

2.1. Isolation of neural cells

S. exigua were initially obtained from shallot fields in Tianjin, China and reared indoors in climatic chambers on an agar-based semisynthetic diet at 27 ± 1°C, 75 ± 5% relative humidity, and a LD 16:8 h photocycle [15]. The insects were reared for two generations prior to the experiment. Third instar larvae of *S. exigua* were first anesthetized with 70% ethanol and their thoracic and abdomen ganglia were removed and placed in saline. The thoracic and abdomen ganglia were transferred to a solution containing 0.3% trypsin for 6 min at 28°C, plated into a 35 mm culture dish containing 1 mL of improved L-15 Leibovitz culture medium supplemented with fetal calf serum (15%, v:v) [16] and then mechanically dissociated using a fire-polished Pasteur pipette. The cultures were maintained at 28°C for 2 h to allow the cell to adhere to the dish. All procedures were carried out under sterile conditions.

2.2. Electrophysiological reagents

The extracellular solution contained (mM) NaCl 100, CsCl 4, $BaCl_2$ 3, $MgCl_2$ 2, HEPES 10, Glucose 10, TEA-Cl 30, TTX 0.001, and the intracellular solution contained (mM) CsCl 120, $MgCl_2$ 2, Na_2-ATP 5, EGTA 11, HEPES 5, were prepared in house separately. Both pH were adjusted to 7.0 with 1 mM CsOH. CsCl, CsOH and HEPES were purchased from Gibco. Celangulin I was generously provided by Prof. Wenjun Wu (Institute of Pesticide Science, Key Laboratory of Applied Entomology, Northwest A&F University). Chlorantraniliprole was synthesized as reported previously [17].

2.3. Electrophysiological recordings

Single neurons with diameter of 20–25 μm were measured under the microscope. Currents of calcium (Ica) were recorded using the whole-cell patch-clamp technique at room temperature [18,19]. Micropipettes (diameter 1–2 μm) made from borosilicated glass capillary tubing were pulled in a two-step vertical puller (Narishige, PP-830, Japan) and fire polished. The resistance of the micropipettes was 2–3 MΩ after being filled with intracellular solution. Neurons were clamped using a patch-clamp amplifier (EPC-10, HEKA Electronik, Lambrecht, Germany). Capacitive current was compensated by a certain cancelation routine. A series of resistance was compensated electronically by 70–85%. The voltage clamp protocols were generated on a computer using Pulse software (version 8.52; HEKA Electronic).

After a giga-seal, the membrane was ruptured by applying a slight negative suction. The current traces were elicited with 90 msec depolarizing voltage steps from −40 mV up to 60 mV in 10 mV increments from a holding potential of −70 mV. The calcium current trace became relatively stable 4–10 min after patch rupture (20 cells tested). We recorded the current of calcium at 5 min as the control group, and then chlorantraniliprole was injected in the surrounding of the cell.

2.4. Calcium imaging

Calibration of the fluorescence signal was achieved by using the method of Takahashi et al. with modifications [20]. Briefly, the attached neurons were rinsed in standard physiological saline [(mM): NaCl 150, KCl 4, $MgCl_2$ 2, $CaCl_2$ 2, and HEPES 10, buffered to pH 7.0] and then incubated in the dark for 45 min at 28°C in standard external saline containing the dye fluo-3 AM (10 μM). After dye loading, cells were again rinsed in physiological saline twice. Calcium free extracellular fluid has the following composition (mM): NaCl 150, KCl 4, $MgCl_2$ 2, EGTA 2, and HEPES 10, buffered to pH 7.0. For full depletion of thapsigargin-sensitive stores, neurons were incubated with 3 μM thapsigargin 10 min. After dye loading, neurons were again rinsed in standard physiological saline twice. Calcium ratio imaging studies were conducted using the imaging system coupled to an inverted fluorescence microscope with a Fluor 40× oil immersion objective (Olympus IX71). Cells were excited at 488 nm and the 530 nm fluorescence emission acquired using a CCD (Image Pro-6.0).

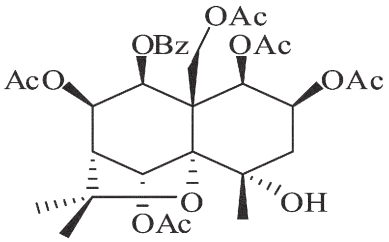

Fig. 1. Chemical structure of Celangulin I.

2.5. Data analysis

Each experiment was repeated at least six times. The data were analyzed using Spss Inc., version 17.0 and Microcal Origin, version 8.0 (Origin Lab Corp., Northampton, MA). Results were expressed as mean ± SD (n = number of cells).

Fluorescence values were expressed as F/F0, F0 being the resting (or baseline) fluorescence, and F the change in fluorescence from baseline after the drug application.

3. Results and discussion

Calcium is a common second messenger that regulates many processes in cells [21]. At resting levels, cells are typically bathed in solutions containing a relatively high Ca^{2+} concentration (millimolar level), while the cytoplasm of most cells contains much lower resting Ca^{2+} concentration (100 nM) [22]. Intracellular Ca^{2+} increases through

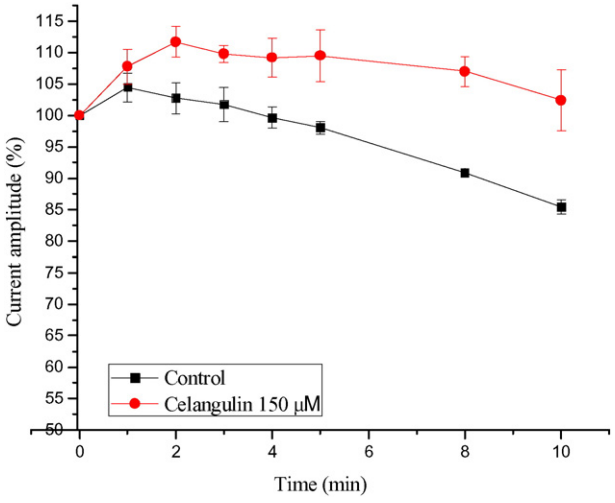

Fig. 2. The change rates of peak current amplitude versus recording time in the celangulin I-treated and control neurons.