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Modulations by CTX of the L-type Ca^{2+} channels in the central neurons of the cyhalothrin-resistant and cyhalothrin-susceptible cotton bollworm, *Helicoverpa armigera*

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Abstract: Cholera toxin (CTX) activates the α-subunit of stimulatory heterotrimeric G-proteins ($G\alpha_s$) and stimulates voltage-gated L-type ($Ca^{2+}_{(L)}$) channels, which may be primary targets of pyrethroids. To investigate the potential mechanisms underlying the resistance to pyrethroids in agriculturally important insect pests, we examined the modulations by CTX of $Ca^{2+}_{(L)}$ channels in the central neurons of the cyhalothrin-resistant (Cy-R) and cyhalothrin-susceptible (Cy-S) cotton bollworms (*Helicoverpa armigera*). Neurons were isolated from the 3rd – 4th instar larvae of the Cy-R and Cy-S cotton bollworms, respectively. The isolated neurons from each group were cultured for 12 – 16 h in an improved L15 insect culture medium with or without CTX (700 ng/mL). Barium currents (I_{Ba}) through $Ca^{2+}_{(L)}$ channels were recorded by the whole-cell patch-clamp technique. The results showed that CTX increased the I_{Ba} peak current density by 36.1 % and caused a hyperpolarizing shift by 5 mV in the I-V curve in the Cy-S neurons, but had no such effect in the Cy-R neurons. Moreover, CTX exerted little effects on other parameters such as the activation potential, reverse potential, activation and inactivation curves in either Cy-S or Cy-R neurons. No significant differences of the parameters mentioned above in the $Ca^{2+}_{(L)}$ channels were detected between the Cy-S and Cy-R neurons cultured without CTX. The results suggest that the G_s-adenylyl cyclase (AC)-cAMP-protein kinase A (PKA)-$Ca^{2+}_{(L)}$ channel signal transduction pathway may exist in cotton bollworm neurons, and the reduced sensitivity of $Ca^{2+}_{(L)}$ channels to the CTX modulation in the Cy-R neurons (but not in the Cy-S neurons) may account for the reduced nerve sensitivity in the pyrethroids-resistant insects.

Key words: *Helicoverpa armigera*; resistance; cyhalothrin; cholera toxin; L-type voltage-gated calcium channels; patch-clamp technique; neurons

1 INTRODUCTION

Many studies focus on the mechanisms underlying knockdown resistance (*kdr*) to pyrethroid insecticides in agriculturally important insect pests. The related voltage-gated sodium channels (VGSCs), also called the *para*-homologous sodium channels in insects, are the most important target sites of pyrethroids (Wu and Zhao, 2004). It is commonly believed that changes in VGSC properties might account for the reduced nerve sensitivity in the resistant insects (Head *et al*., 1998). The voltage-gated calcium channels (VGCCs) have close phylogenetic and structural relationships with VGSCs. They both belong to the same superfamily of voltage-gated cation channels. Furthermore, VGCCs have been proposed to be another primary target of pyrethroids (Narahashi, 1992, 1996). These suggest that VGCCs may also play an important role in the insect resistance to pyrethroid insecticides.

L-type calcium ($Ca^{2+}_{(L)}$) channels are modulated by G proteins and G protein-coupled phosphorylation (Kaumann and Molenarr, 1997; Heubach *et al*., 2001). Cholera toxin (CTX) consists of an A subunit (27 kD) and five B subunits. Its hydrophobic A subunit catalyzes ADP-ribosylation of the α-subunit of stimulatory heterotrimeric G-proteins ($G\alpha_s$), which reduces GTPase activity and activates the α-subunit of $G\alpha_s$ (Kaslow *et al*., 1981; Abood *et al*., 1982). The activation of $G\alpha_s$ leads to an increase of adenylyl cyclase

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(AC) activity, an elevation of cyclic AMP (cAMP), and stimulation of protein kinase A (PKA), which results in phosphorylation of effector proteins (Kaumann and Molenarr, 1997). $Ca^{2+}_{(L)}$ channels were documented as targets for cAMP-dependent PKA-mediated phosphorylation (McDonald *et al*., 1994; Sun and Zhu, 2004).

A cyhalothrin selected cyhalothrin-resistant (Cy-R) strain of the cotton bollworm, *Helicoverpa armigera* (Hübner), was reported to have high relative resistance ratio compared with the non-selected cyhalothrin-susceptible (Cy-S) strain (Meng *et al*., 1998). However, the differences of the G_s-AC-cAMP-PKA-$Ca^{2+}_{(L)}$ channel signal transduction pathway and the other related signal transduction pathways between the Cy-R and Cy-S neurons of the cotton bollworm have not been well studied by using the whole-cell patch-clamp technique. To determine whether there are any variations in the signal transduction pathways between the Cy-R and Cy-S cotton bollworms and further investigate the pyrethroid-resistant mechanisms in agriculturally important insect pests, we compared the modulations by CTX of the $Ca^{2+}_{(L)}$ channels in the Cy-R and Cy-S neurons of the cotton bollworm.

2 MATERIALS AND METHODS

2.1 Sources of tested insects

A field population of *H. armigera* was initially obtained from cotton fields in Hebei Province of China in 1992.

After 2 generations of rearing, this population was divided into two strains. One strain without exposure to any insecticides was the Cy-S strain with LD_{50} of 0.00419 μg/larva for cyhalothrin (Meng *et al*., 1998) and has been maintained at Institute of Plant Protection, Chinese Academy of Agricultural Sciences (CAAS) for the past years.

The other strain selected by cyhalothrin (at Institute of Plant Protection, CAAS), was the Cy-R strain with LD_{50} of 0.4903 μg/larva and relative resistance ratio of 117.02 for cyhalothrin (Meng *et al*., 1998). Briefly, 40 generations were routinely selected by cyhalothrin and the average mortality in every generation was 60% – 80%. Besides, four additional rounds of a single-pair selection were intermittently carried out, which was a process in which single pairs of cyhalothrin-resistant adult moths were reared alone and the offspring from two to three pairs with higher resistance to cyhalothrin were chosen for reproduction. The Cy-R strain had been reared at Institute of Plant Protection, CAAS for the past years and reselected several times in the recent years. Larvae in all instars were reared indoors at (27 ± 1) ℃, with 70% – 80% of relative humidity and 14 h of light.

2.2 Isolation and culture of neurons

Neurons were acutely dissociated and maintained in a primary culture method (Hayashi and Levine, 1992; He *et al*., 2001). Firstly, thoracic and abdominal ganglia were removed from the 3rd – 4th instar larvae, which were anaesthetized and sterilized with 75% ethanol. Secondly, after having been incubated in insect normal saline at 4 ℃ for 5 min, the ganglia were desheathed and transferred to the solution containing 0.3% trypsin Ⅲ for 10 min at 27 ℃. Thirdly, cells were mechanically dissociated by repeated triturations using a fire-polished Pasteur pipette, and plated on poly-L-lysine coated coverslips. Cells were incubated in improved L15 Leibovitz culture medium (GIBCO, Grand Island, NY) supplemented with fetal calf serum (15%, v/v) with or without 700 ng/mL CTX for 12 – 16 h at 27 ℃ before being used for patch clamp experiments. The neurons were divided into the following four groups based different treatments: the Cy-S group (neurons from the Cy-S larvae, cultured without CTX), the Cy-R group (neurons from the Cy-R larvae, cultured without CTX), the Cy-S-CTX group (neurons from the Cy-S larvae, cultured with CTX) and the Cy-R-CTX group (neurons from the Cy-R larvae, cultured with CTX). All the procedures were performed under sterile conditions.

2.3 Solutions

The internal pipette solution contained (in mmol/L): 140 CsCl, 10 HEPES, 1.1 EGTA, 0.1 $CaCl_2$, 5 Mg-ATP and 2 Na_2GTP; its pH was adjusted to 6.6 with 1 mol/L CsOH. The bath solution contained (in mmol/L): 117 NaCl, 5 $BaCl_2$, 4 CsCl, 2 $MgCl_2$, 10 HEPES, 10 Glucose, 20 TEA-Cl, 14-AP and 0.0005 tetrodotoxin (TTX); its pH was adjusted to 6.8 with 1 mol/L NaOH. CTX, Mg-ATP, Na_2GTP, EGTA, HEPES, TEA-Cl, CsCl, EGTA, 4-AP and TTX were all purchased from Sigma Chemical Company (St. Louis, MO). The other reagents were of analytical grade and purchased in China.

2.4 Electrophysiological recording

$Ca^{2+}_{(L)}$ currents were recorded from single neurons with diameter of 15 – 25 μm using the whole-cell patch-clamp technique (Hamill *et al*., 1981) at room temperature (20 – 23 ℃). Recording pipettes were constructed from borosilicate glass (Institute of Zoology, Chinese Academy of Sciences, Beijing) and pulled with a two-step vertical micropipette puller (Narishige, PP-830, Japan), with resistances of 1.5 – 2.5 MΩ after being fire-polished and filled with the pipette solution. Neurons were clamped using a patch-clamp amplifier (EPC-10, HEKA Electronik, Lambrecht, Germany). Capacitive currents were compensated by a certain cancellation routine. Series

resistances were compensated electronically by 75% – 85%. The voltage clamp protocols were generated on a computer using Pulse software (version 8.52; HEKA Electronik). Whole-cell currents were leak-subtracted (P/4), low-pass filtered (2.9 kHz, four-pole Bessel), digitized (20 kHz), and stored on magnetic medium for off-line analysis. The holding potential (V_{hold}) was – 40 mV.

2.5 Data analysis and statistics

The data were analyzed using Igor 4.04. The figures and fittings were performed with the Microcal Origin (version 7.0; Origin Lab Corp., Northampton, MA). Results are expressed as mean ± *SD* (n = number of cells). Statistical significance was determined by Student's paired or unpaired two-tailed t tests and differences were considered significant when $P < 0.05$, which were shown as the single asterisk.

3 RESULTS

3.1 The representative Ba^{2+} current traces of $Ca^{2+}_{(L)}$ channels in the central neurons of cotton bollworms

Ba^{2+} currents through Ca^{2+} channels were measured in the whole-cell voltage-clamp configuration. Fig. 1 shows representative inward Ba^{2+} current traces of $Ca^{2+}_{(L)}$ channels in the central neurons of cotton bollworms. The traces were elicited by 150 msec depolarizing steps from – 35 mV up to 65 mV in + 5 mV increments at 3 sec stimulus intervals from a V_{hold} of – 40 mV. After reaching their peak amplitudes, the currents decayed within the pulse durations. As recorded by He *et al*. (2002), the currents were identified as I_{Ba} of $Ca^{2+}_{(L)}$ channels because they were almost entirely inhibited by $CdCl_2$ (100 μmol/L) or nifedipine (50 μmol/L).

To avoid the Ca^{2+}-induced run-down phenomenon, which was inevitable because of the lose of some plasma components and the degradation of energy substances, we used 5 mmol/L Ba^{2+} as the charge carrier, added some energy substances, such as Mg-ATP and Na_2GTP, into pipette solution and appropriately adjusted the osmotic pressure of pipette and bath solutions. Because of I_{Ba} currents being relatively stable during 5 – 20 min after the whole-cell patch-clamp configuration having been established, we recorded the I_{Ba} within 5 – 10 min. In addition, we only compared the data from different treatment groups and did not depict the time courses, so the errors caused by the Ca^{2+}-induced run-down phenomenon were systematic and exerted little effect on our data accuracy.

To eliminate contaminations from other voltage-gated cation channels and the other calcium channel subtype, we used some specific blockers, such as intracellular Cs^+, extracellular Cs^+, TTX, TEA-Cl and 4-AP.

3.2 Effects of CTX (700 ng/mL) on the current-voltage (I-V) curves of I_{Ba} currents of $Ca^{2+}_{(L)}$ channels in the Cy-S and Cy-R neurons

The current-voltage (I-V) relationships of Ba^{2+} currents are shown in Fig. 2. The activation thresholds in the four treatment groups were all at about – 30 – – 35 mV. The currents reversed from inward to outward at around 53 mV under present ionic conditions. The active voltages of peak inward currents were at about 0 mV in the Cy-S, Cy-R and Cy-R-CTX neurons, but at – 5 mV in the Cy-S-CTX neurons. The voltage dependence of activation in the Cy-S-CTX neurons was shifted 5 mV toward hyperpolarization direction. The current density (pA/pF) is expressed as the recorded current value (pA) divided by cell capacitance value (pF) of the corresponding neuron. CTX significantly enhanced the I_{Ba} peak density of the Cy-S neurons by 36.1 % ($P < 0.05$). The differences of I_{Ba} peak density between the Cy-S and the Cy-R neurons and between the Cy-R and the Cy-R-CTX neurons were not significant.

3.3 Effects of CTX (700 ng/mL) on activation curves of I_{Ba} currents of $Ca^{2+}_{(L)}$ channels in the Cy-S and Cy-R neurons

Ba^{2+} current traces of $Ca^{2+}_{(L)}$ channels were elicited by 500 msec depolarizing steps from – 35 mV up to 5 mV in + 5 mV increments from a V_{hold} of – 40 mV at 3

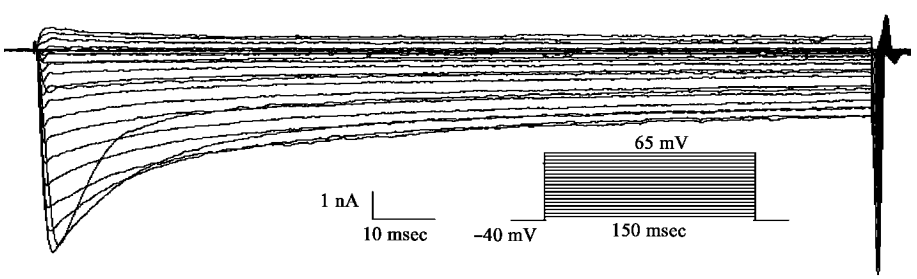

Fig. 1 Representative Ba^{2+} current traces of $Ca^{2+}_{(L)}$ channels in the central neurons of cotton bollworms
The traces were elicited by 150 msec depolarizing steps from – 35 mV up to 65 mV in + 5 mV increments at 3 sec stimulus intervals from a V_{hold} of – 40 mV.

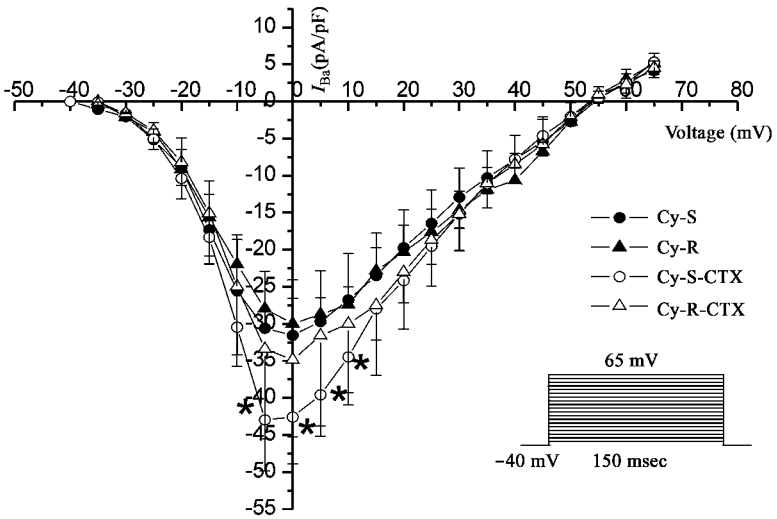

Fig. 2 Effects of CTX (700 ng/mL) on the current-voltage (I-V) curves of I_{Ba} currents of $Ca^{2+}_{(L)}$ channels in the Cy-S and Cy-R neurons

The current density (pA/pF) is the recorded current value (pA) divided by cell surface, which is the slow capacitance value (pF) of the corresponding neuron. ●: Cy-S neurons, $n = 6$; ▲: Cy-R neurons, $n = 5$; ○: Cy-S-CTX neurons, $n = 5$; △: Cy-R-CTX neurons, $n = 7$. Single asterisk * denotes that the I_{Ba} amplitudes of the Cy-S-CTX neurons at the corresponding testing potentials (voltage) were significantly different from those of the Cy-R-CTX neurons and Cy-S neurons at $P < 0.05$

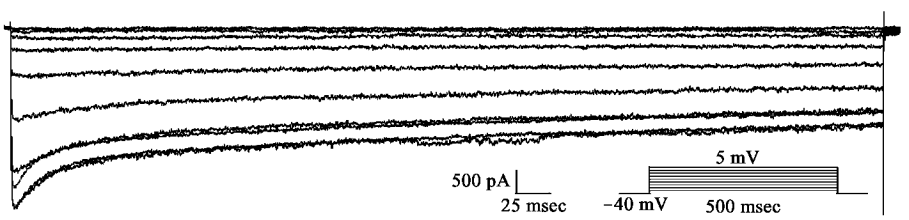

Fig. 3 Representative Ba^{2+} current traces of $Ca^{2+}_{(L)}$ channels elicited by 500 msec depolarizing steps from −35 mV up to 5 mV in +5 mV increments from a V_{hold} of −40 mV at 3 sec stimulus intervals

sec stimulus intervals. Fig. 3 showed the representative current traces.

The activation curves were fitted by the Boltzmann equation: $G/G_{max} = 1/\{1 + \exp[-(V_{test} - V_{0.5act})/k]\}$, where at each test potential (V_{test}) [$G = I_{Ba}/(V_{test} - V_{rev})$], I_{Ba} is the peak current and V_{rev} is the reversal potential. The three free parameters are $V_{0.5act}$ (the potential at which the probability of a single gate opening is one-half), k (the slope factor, which corresponds to a change in potential that produces an e-fold change in conductance), and G_{max} (the maximal Ba^{2+} conductance).

There was no significant difference between the $V_{0.5act}$ of the Cy-R-CTX neurons and Cy-S-CTX neurons. The activation curves are shown in Fig. 4. The $V_{0.5act}$ and the slope factor (k) for the four treatment groups are listed in Table 1.

3.4 Effects of CTX (700 ng/ml) on the inactivation curves of I_{Ba} currents of $Ca^{2+}_{(L)}$ channels in the Cy-S and Cy-R neurons

Using a standard two-pulse protocol, we quantified the changes of the voltage dependence of the $Ca^{2+}_{(L)}$ inactivation produced by CTX (700 ng/mL). The traces were elicited by a prepulse of 2 sec depolarizing steps from −40 mV up to 5 mV in +5 mV increments from a V_{hold} of −40 mV and recorded by a test pulse of 150 msec with a potential of 0 mV at 5 sec stimulus intervals. Fig. 5 showed the representative current traces.

The inactivation curves were fitted by the Boltzmann equation: $I/I_{max} = 1/\{1 + \exp[(V_{prepulse} - V_{0.5inact})/k]\}$, where $V_{prepulse}$ is the prepulse potential, $V_{0.5inact}$ is the voltage at which the probability of a single gate closing is one-half, k is the slope factor which corresponds to a change in potential that produces an e-fold change in conductance, I is the recorded Ba^{2+} current value and I_{max} is the recorded maximal Ba^{2+} current value.

CTX (700 ng/mL) hardly shift $V_{0.5inact}$ and the slope factor k of the Cy-S neurons and Cy-R neurons. The inactivation curves are shown in Fig. 6. The $V_{0.5inact}$ and the slope factor k for the four groups are listed in Table 1.

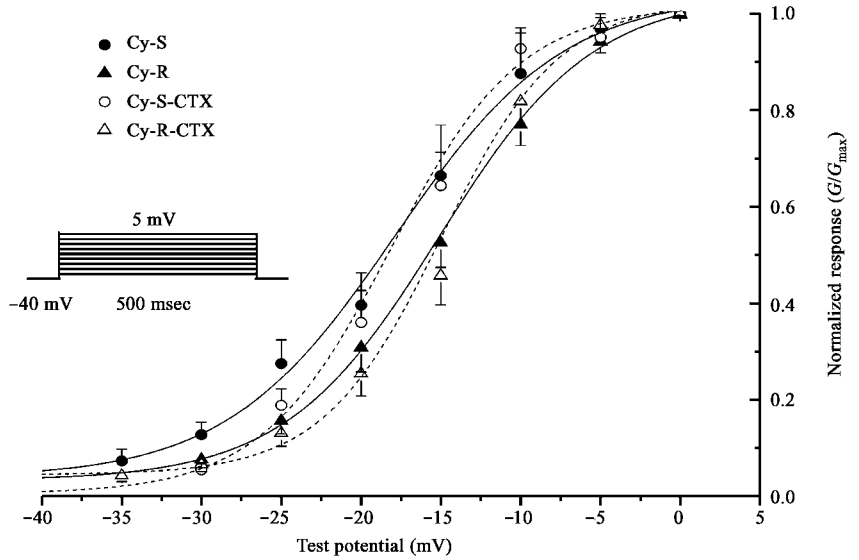

Fig. 4 Effects of CTX (700 ng/mL) on activation curves of I_{Ba} currents of $Ca^{2+}_{(L)}$ channels in the Cy-S and Cy-R neurons

The curves were fitted by the Boltzmann equation: $G/G_{max} = 1/\{1 + \exp[-(V_{test} - V_{0.5act})/k]\}$, where at each membrane potential (V_{test}) [$G = I_{Ba}/(V_{test} - V_{rev})$], I_{Ba} is the peak current and V_{rev} is the reversal potential. The three free parameters are $V_{0.5act}$ (the potential at which the probability of a single gate opening is one-half), k (the slope factor, which corresponds to a change in potential that produces an e-fold change in conductance), and G_{max} (the maximal Ba^{2+} conductance). There were no significant differences in $V_{0.5act}$ among the four treatment groups. ●: Cy-S neurons, $n = 5$; ▲: Cy-R neurons, $n = 7$; ○: Cy-S-CTX neurons, $n = 7$; △: Cy-R-CTX neurons, $n = 6$.

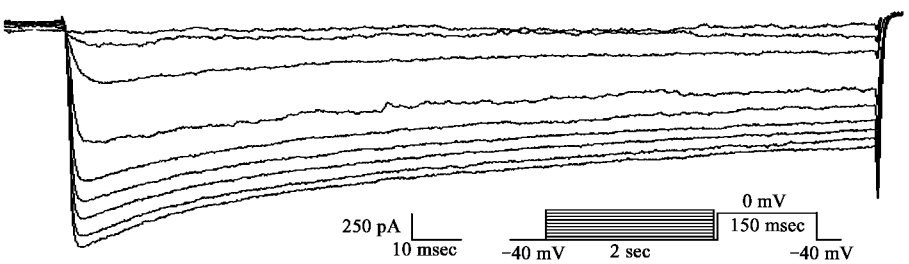

Fig. 5 Representative Ba^{2+} current traces of $Ca^{2+(L)}$ channels evoked by a standard two-pulse protocol

The traces were elicited by a conditioning pulse of 2 sec depolarizing steps from − 40 mV up to 5 mV in + 5 mV increments from a V_{hold} of − 40 mV and recorded by a test pulse of 150 msec with a potential of 0 mV at 5 sec stimulus intervals.

Table 1 Effects of CTX on the activation and inactivation curves of I_{Ba} currents through $Ca^{2+}_{(L)}$ channels in the Cy-S and Cy-R neurons

Parameters	Cy-S	Cy-S-CTX	Cy-R	Cy-R-CTX
$V_{0.5act}$	− 17.89 ± 1.35 ($n = 5$)	− 8.13 ± 1.02* ($n = 7$)	− 15.14 ± 0.71 ($n = 5$)	− 14.99 ± 0.66* ($n = 6$)
Slope factor (k) of activation curves	5.27 ± 1.26	4.06 ± 0.95*	4.83 ± 0.72	3.72 ± 0.43*
$V_{0.5inact}$	− 18.63 ± 1.06 ($n = 5$)	− 20.54 ± 0.82* ($n = 6$)	− 19.62 ± 1.01 ($n = 5$)	− 20.36 ± 0.99* ($n = 8$)
Slope factor (k) of inactivation curves	5.57 ± 1.10	5.46 ± 0.68*	5.98 ± 0.80	5.64 ± 0.69*

*: No significant effects on $V_{0.5}$ and slope factor were caused by CTX in either group ($P > 0.05$) compared with the non-CTX-treated group.

4 DISCUSSION

Previous experiments in our laboratory showed that 250 ng/mL CTX exerted little effects on the I_{Ba} activation potential, peak potential and the voltage-dependent of $Ca^{2+}_{(L)}$ channels, but decreased the Ca^{2+}-induced rundown and slightly increased peak current in the Cy-S neurons (Li, 2004). These results suggest that 250 ng/mL CTX relatively enhances I_{Ba} amplitudes of Cy-S neurons. In addition, CTX was added to culture medium and pipette solution, and only the Cy-S neurons cultured for 12 − 16 h were studied in her experiments. Based on our aforementioned findings, the

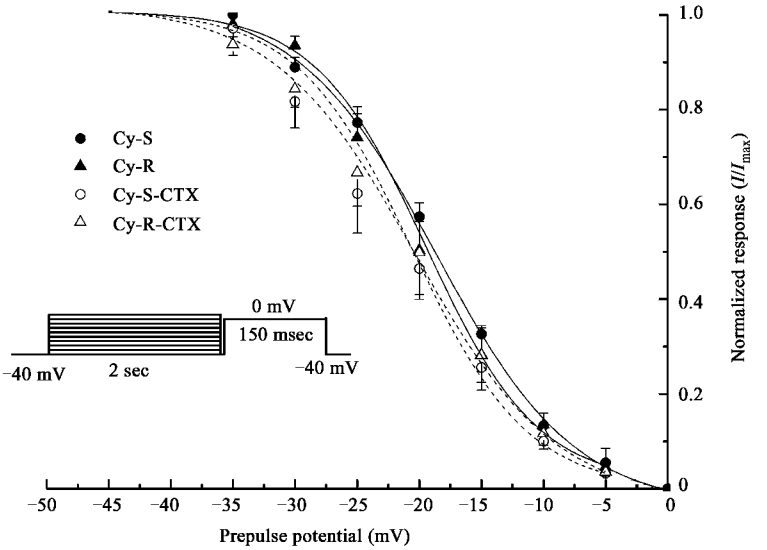

Fig. 6 Effect of CTX (700 ng/mL) on inactivation curves of I_{Ba} currents of $Ca^{2+}_{(L)}$ channels in the Cy-S and Cy-R neurons
The curves were fitted by the Boltzmann equation: $I/I_{max} = 1/\{1 + \exp[(V_{prepulse} - V_{0.5inact})/k]\}$, where $V_{prepulse}$ is the prepulse potential, $V_{0.5inact}$ is the potential at which the probability of a single gate closing is one-half, k is the slope factor which corresponds to a change in potential that produces an e-fold change in conductance, I is the recorded Ba^{2+} current value and I_{max} is the recorded maximal Ba^{2+} current value. There were no significantly differences in $V_{0.5act}$ among the four groups. ●, Cy-S neurons, $n = 5$; ▲, Cy-R neurons, $n = 5$; ○, Cy-S-CTX neurons, $n = 6$; △, Cy-R-CTX neurons, $n = 8$.

knowledge of the main functional α-subunit of CTX being hydrophobic, and the other researchers' excellent work (Bubien *et al*., 1994; Ma *et al*., 1994), we chose 700 ng/mL CTX as an appropriate concentration and incubated both the Cy-S neurons and Cy-R neurons in the CTX-supplemented medium.

At the same time, we studied the differences of excitability between the Cy-S neurons and Cy-R neurons using the current-clamp technique and found Cy-R neurons with a decreased nerve sensitivity (data not shown), which is similar to that reported by Lee *et al*. (1999), who used the adult moths of a permethrin-resistant *H. virescens* strain. With the same Cy-R strain for cyhalothrin as we used in the present work, our previous electrophysiological studies showed that *kdr* ratio is 10.7-fold to cyhalothrin by recording the spontaneous miniature excitatory junctional potentials (Ru *et al*., 1998).

Our present results showed that 700 ng/mL CTX significantly increased the I_{Ba} peak current density and caused a significant hyperpolarizing shift by 5 mV in the voltage-dependence in the Cy-S neurons, but not in the Cy-R neurons. These results indicate that $Ca^{2+}_{(L)}$ channels of the Cy-S neurons are easier to be modulated by CTX than that of the Cy-R neurons, and the single $Ca^{2+}_{(L)}$ channels in the Cy-S neurons get an relatively enhanced open probability and prolong open duration, same as reported by Yue *et al*. (1990). The phenomenon may be attributed to the reduced nerve sensitivity of pyrethroids-resistant insects. No significant differences were observed in the $Ca^{2+}_{(L)}$ channel parameters between the Cy-S and Cy-R neurons. Further experiments are needed to test $Ca^{2+}_{(L)}$ channel kinetics in order to further elucidate the relationships between $Ca^{2+}_{(L)}$ channel kinetics of the Cy-R neurons and pyrethroids resistance. CTX does not shift the membrane reverse potential of $Ca^{2+}_{(L)}$ channels in the Cy-R and Cy-S neurons, suggesting that CTX does not change the ion selectivity of $Ca^{2+}_{(L)}$ channels on the Cy-R and Cy-S neurons.

$Ca^{2+}_{(L)}$ channels are involved in excitation-contraction coupling, hormone secretion, calcium signaling, synaptic transmission and gene regulation (Sugiura and Ko, 1997; Catterall, 2000; Dolmetsch *et al*., 2001). VGCCs mediate Ca^{2+} entry into neurons, and it is very important to maintain interior calcium homeostasis of neurons. When neurons are not excited, cytoplasm Ca^{2+} concentration is often in a narrow range (20 – 100 nmol/L) in their physiological condition (Sun *et al*., 2001). More and more reports support the opinion that VGCCs are one primary target of pyrethroids (Soderlund *et al*., 2002). Using the whole-cell calcium current recordings, Guo *et al*. (2000) discovered deltamethrin increases the current amplitude of $Ca^{2+}_{(L)}$ channels of rat embryotic MN9D cell lines by 20.64% in 1 min and 15.48% in 5 min. It was demonstrated that Ca^{2+} channels in rat brain neurons are activated by fenvalerate and tetramethrin at low dose and inhibited at high dose (He *et al*., 1997). Narahashi *et al*. (1992) and Hildebrand *et al*. (2004) reported pyrethrin, fenvalerate and allethrin inhibited $Ca^{2+}_{(L)}$ channels. He *et al*. (2002) showed the

I-V relationships for $Ca^{2+}_{(L)}$ channels in the cotton bollworm Cy-S and Cy-R neurons were leftward shifted by 10 – 20 mV after the action of cyhalothrin, indicating that cyhalothrin also affects the gating behavior of $Ca^{2+}_{(L)}$ channels. In summary, pyrythroids may affect $Ca^{2+}_{(L)}$ channels, either inhibit or activate channel activities, which may be related to the subunit structures of $Ca^{2+}_{(L)}$ channels, tissue specificities, and different analogue or concentration of pyrethroids examined.

It had been reported that CTX activates the G_s in retinal rod outer segments (Abood *et al.*, 1982). Using cardiac ventricle membrane patches, Yatani *et al.* (1988) revealed activation of the purified G_s by CTX increases both AC stimulatory and Ca^{2+} channel stimulatory effects. CTX enhances stimulation of adenylyl cyclases activity in cerebellar neurons of rat pups (postnatal day 5 – 8) in combination with depolarizing agents (Reddy *et al.*, 1995). Liu *et al.* (1992) demonstrated the stimulation of the dopamine-D_1 receptor on rat pituitary GH_4-hD_1 cells induces a marked increase in cAMP accumulation and potentiates activation of $Ca^{2+}_{(L)}$ channels in a cAMP-dependent manner. All these studies suggest the existence of the G_s-AC-cAMP-PKA-$Ca^{2+}_{(L)}$ channel signal transduction pathway in mammal cells which can be activated by CTX. Our results suggest the existence of the same signal transduction pathway in the cotton bollworm neurons. However, the second messenger cAMP is a key component, which can exert modulating action not only *via* activation of cAMP-PKA signal transduction pathway (Yatani *et al.*, 1988), but *via* the other ways, such as cyclic nucleotide-gated (CNG) cationic channels in some sorts of cells (Kawai and Miyachi, 2001). The multiple effects of cAMP can not be completely excluded. Indeed, there had been reports showing various effects of cAMP on calcium channels in different preparations, such as suppressing (Solntseva and Borisova, 1997) or no effect on calcium channels (David and Pitman, 1996). Therefore, the reduced sensitivity of the Cy-R neurons to the regulation by CTX may involve various steps/levels of the signal transduction cascade, such as direct membrane-limited G-protein-ion channel interactions (Wickman and Clapham, 1995; Sun *et al.*, 2001).

It had been well shown that point mutations in the *para*-homologous sodium channel (or VGSC) gene are associated with *kdr* to pyrethroids in *Drosophila melanogaster* (Knipple *et al.*, 1994). Coding sequences from the intracellular segment linking repeat domains Ⅲ and Ⅳ of the VGSC gene reveals that mutations from aspartic acid to valine and a glutamic acid to glycine may account for the resistance of the cotton bollworm to pyrethroids (Head *et al.*, 1998).

The present work has revealed a close correlation between reduced sensitivity of VGCC to CTX regulation and pyrethroid-resistance in cotton bollworms. Further experiments are needed to determine whether mutations in the VGCC gene may also exist in pyrethroids-resistant insects.

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霍乱毒素对三氟氯氰菊酯抗性及敏感棉铃虫神经细胞 L 型钙通道的调节作用

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摘要：霍乱毒素(CTX)可激活兴奋性异三聚体 G 蛋白($G\alpha_s$)的 α-亚基和刺激电压门控 L-型钙通道,而昆虫的 L-型钙通道可能是拟除虫菊酯类杀虫剂的作用靶点。为进一步探讨农业害虫对拟除虫菊酯类杀虫剂产生抗药性的作用机理,我们检测了 CTX 对三氟氯氰菊酯抗性及敏感棉铃虫 *Helicoverpa armigera* 中枢神经细胞电压门控 L-型钙通道的调节作用。分别急性分离三氟氯氰菊酯抗性及敏感的 3 ~ 4 龄棉铃虫幼虫胸腹神经节细胞,并在改良的 L15 培养基(加入或未加入 700 ng/mL 的 CTX)中培养 12 ~ 16 h。钡离子为载流子,应用全细胞膜片钳技术记录电压门控 L-型钙通道电流。结果显示,CTX 可使敏感组棉铃虫神经细胞 L-型钙通道的峰值电流密度增大 36%、峰值电压左移 5 mV,但对抗性组棉铃虫神经细胞 L-型钙通道无上述作用。并且,CTX 对敏感组及抗性组棉铃虫神经细胞 L-型钙通道的激活电位、翻转电位、激活曲线和失活曲线等其他一些参数的影响也不明显。在无 CTX 作用时,所检测到的抗性组与敏感组棉铃虫神经细胞 L-型钙通道的上述参数值间差异不显著。结果提示,棉铃虫神经细胞内存在 G_s-腺苷酸环化酶(AC)-cAMP-蛋白激酶 A (PKA)-L-型钙通道信号调节系统；与敏感棉铃虫神经细胞 L-型钙通道相比,三氟氯氰菊酯抗性棉铃虫神经细胞 L-型钙通道的活性相对不易受到 CTX 调节,这可能与昆虫对拟除虫菊酯产生抗药性的机理有关。

关键词：棉铃虫；抗药性；三氟氯氰菊酯；霍乱毒素；L-型钙通道；膜片钳技术；神经细胞

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