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Inhibition effects of cyhalothrin on the delayed rectifier potassium current in the central neurons of *Helicoverpa armigera*

WANG Yong, HE Bing-Jun*, WU Chun-Lin, LIU An-Xi

(College of Life Sciences, Nankai University, Tianjin 300071, China)

Abstract: The effects of cyhalothrin on the delayed rectifier potassium current in the central neurons of *Helicoverpa armigera* were studied using the patch clamp technique for the first time. The results showed that before application of cyhalothrin, 81% and 39% cells were activated at – 30 mV and – 40 mV (n = 21), respectively. In 15 min after cyhalothrin (10^{-5} mmol/L) application, 63% and 38% cells were activated at – 40 mV and – 50 mV (n = 8), respectively. The amplitude of the current decreased significantly after application of cyhalothrin and the inhibition percentage reached 37.7% in 1 min (n = 19). After the application, activation curve shifted to the negative direction and the value of V_h changed significantly, but the k value did not change remarkably. In conclusion, the results suggest that under the action of cyhalothrin, the potassium channels can be activated more easily and current amplitude can be inhibited significantly, this is related to the nervous insensitivity, and potassium channels of *Helicoverpa armigera* central neurons are the action targets of pyrethroid insecticides.

Key words: *Helicoverpa armigera*; potassium channel; cyhalothrin; patch clamp; neurons; nerve sensitivity

1 INTRODUCTION

Helicoverpa armigera is an important pest and the decrease of nerve sensitivity is one of common mechanisms conferring resistance to pyrethroid insecticides (Zhao *et al*., 1996). Nervous sensitivity is related closely to the function of sodium, potassium, and calcium channels. Sodium channels play a crucial role in the upstroke of action potential, which is a regenerative wave of Na^+ permeability increase. Calcium channels can supply a maintained inward current for longer depolarizing responses, and they serve as the link to transduce depolarization into all the nonelectrical activities that are controlled by excitation. Potassium channels are broadly diversified to help set the resting potential, repolarize and hyperpolarize the cell, so they play significant roles in shaping the excitability and firing patterns of cells (Hille, 1992).

Cyhalothrin (Cyh) is a pyrethroid insecticide and has the properties of high knockdown activity, so it is used widely in China. *H. armigera* has developed resistance at different levels, and in particular, the resistance increased in recent years (He *et al*., 2002). Sodium channels in insect nerve cells are the main targets of the insecticides (Narahashi, 1992). Recently the patch-clamp electrophysiological research of *H. armigera* central neurons focused on the sodium and calcium channels (He *et al*., 2002; He *et al*., 2003; Li *et al*., 2004; Li *et al*., 2005). He *et al*. (2001) reported that *H. armigera* central neurons expressed the tetraethylammonium (TEA)-sensitive delayed rectifier potassium current (I_k) and the 4-aminopyridine (4-AP)-sensitive transient outward potassium current (I_A). But we have not found any reports about the effects of insecticides on the potassium current of *H. armigera* central neurons. So it has great significance to study whether and how Cyh influence potassium current. In our study, the whole cell patch clamp technique was used to study the effects of Cyh on I_k for the first time. This work was expected to obtain new clues to explain the mechanism of resistance formation of *H. armigera*, and provide theoretical foundations for identifying potential new targets for novel and safe insecticides.

2 MATERIALS AND METHODS

2.1 Isolation of neural cells

The *H. armigera* moths used in this experiment were initially obtained from cotton fields in Hebei Province and reared indoors at 27℃, with 70% – 80% of relative humidity and 14 h of light. The thoracic and abdomen ganglia were removed from 3rd to 5th instars larvae, which were anaesthetized by 70% ethanol. The tissues were incubated in insect saline at 4℃ (Hayashi

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作者简介: 王勇, 男, 1980 年生, 硕士研究生, 研究方向为动物生理生化

* 通讯作者 Author for correspondence, Tel.: + 86-22-23509094; E-mail: hebj@eyou.com

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et al., 1992; He *et al*., 2001) for 5 min and then were lacerated. The lacerated ganglia were transferred to solution which contained 0.3% trypsinase for 10 min at (27 ± 1)℃. After trituration with a polished pipette, cells were placed into plastic culture dishes (diameter, 35 mm) and kept in 1.5 mL culture medium per dish. The cells were allowed to settle and adhere to the surface of the dishes for 2 to 4 h. All the procedures were carried out under sterile conditions.

2.2 Electrophysiological recording

Neurons with diameter of (20 ± 1) μm were used in this experiment. Micropipettes made from borosilicated glass capillary tubing were pulled in a two-step vertical puller (Narishige, PP-830, Japan) and polished. The resistance of micropipettes was 2 – 4 MΩ. Liquid junction potential between the pipette and bath solution was always corrected before seal formation. When the high sutured resistance of the electrode tip and the membrane exceeded 1 GΩ, the membrane was disrupted by negative pressure to form the whole cell configuration. EPC-10 (HEKA, Germany) amplifier was used and the series resistance was compensated by about 40% – 80%. Current recordings were filtered at 10 kHz and 2.9 kHz. Leak currents were subtracted using P/4 procedure. All the experiments were carried out at room temperature 20 – 25℃.

In all the experiments, the neurons were held at – 80 mV and test voltage depolarized from – 60 mV to + 50 mV for 60 ms in 10 mV steps, with the frequency being 0.5 Hz. We found that the peak current reached steady state in 5 min. In the following experiments, we recorded the current at 5 min as the control group, and then Cyh was injected in the surroundings of the cell.

2.3 Solutions

Solutions to record whole-cell current contained (mmol/L): intracellular solution: NaCl 10, KCl 100, $CaCl_2$ 2, $MgCl_2$ 2, Hepes 10, pH 6.8 adjusted with KOH; extracellular solution: NaCl 100, KCl 4, $CaCl_2$ 2, $MgCl_2$ 2, Hepes 10, glucose 10, pH 6.8 adjusted with NaOH.

Solutions to record whole-cell potassium current contained (mmol/L): intracellular solution: KF 140, $MgCl_2$ 2, EGTA 10, Hepes 10, pH 6.6 adjusted with KOH; extracellular solution: NaCl 100, KCl 4, $CaCl_2$ 2, $MgCl_2$ 2, Hepes 10, glucose 10, pH 6.6 adjusted with NaOH. Tetraethylammonium Chloride (TEA-Cl), 4-AP, tetrodotoxin (TTX) and $CdCl_2$ were added to the bath solution before the experiments.

EGTA and Hepes were purchased from Gibco. CsOH, TEA-Cl, 4-AP and TTX were purchased from Sigma. Cyh was purchased from Britain. The concentration of Cyh used in the experiment was 10^{-5} mmol/L.

2.4 Data analysis

Statistical comparisons between control and drug treated cells were analyzed using student's unpaired *t*-test. Data were expressed as mean ± *SD*. A value of $P < 0.05$ was considered significant.

3 RESULT

3.1 The effect of Cyh on whole-cell current

By using the solutions for recording whole-cell current, Cyh was injected to the surroundings of the cell when the current reached steady state. The distance between the drug tube tip and the cell was about 100

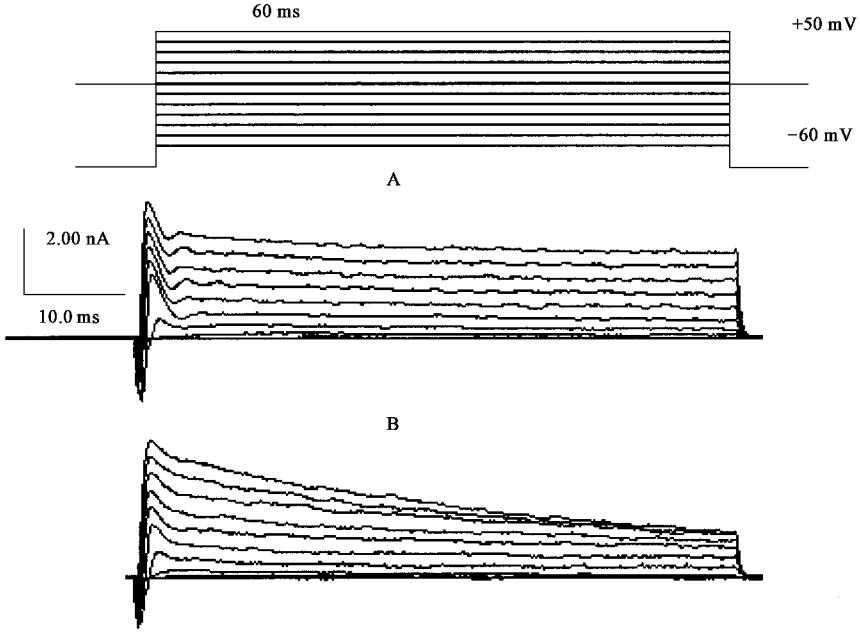

Fig. 1 The change of the whole-cell current before (A) and during the application of Cyh (10^{-5} mmol/L) (B)

Current traces obtained by 60 ms depolarizing pulses from – 60 mV to + 50 mV in 10 mV steps.

μm. It was found that before and during the application of Cyh, the delayed rectifier current decreased most obviously (Fig. 1：A, B). So the I_k was chosen as the study object in this experiment.

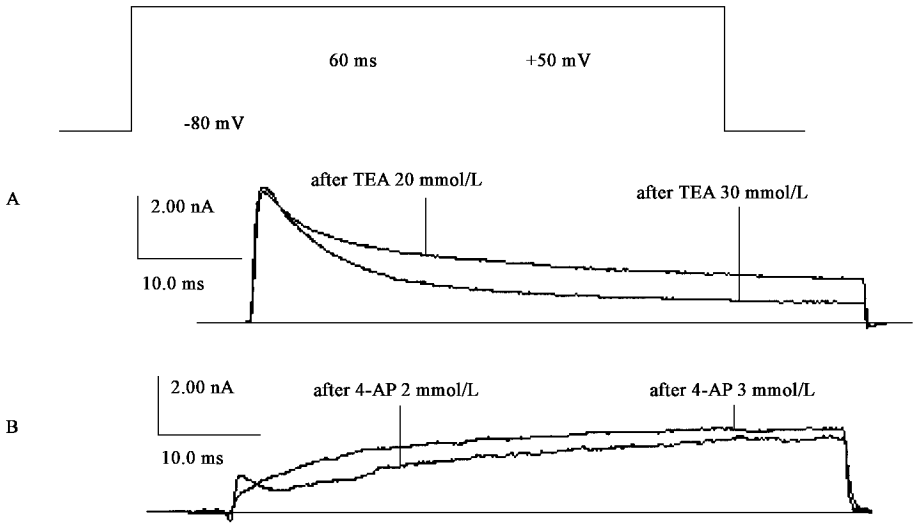

Fig. 2 The effects of TEA (A) and 4-AP (B) on potassium current

Currents shown are at + 50 mV from different cells.

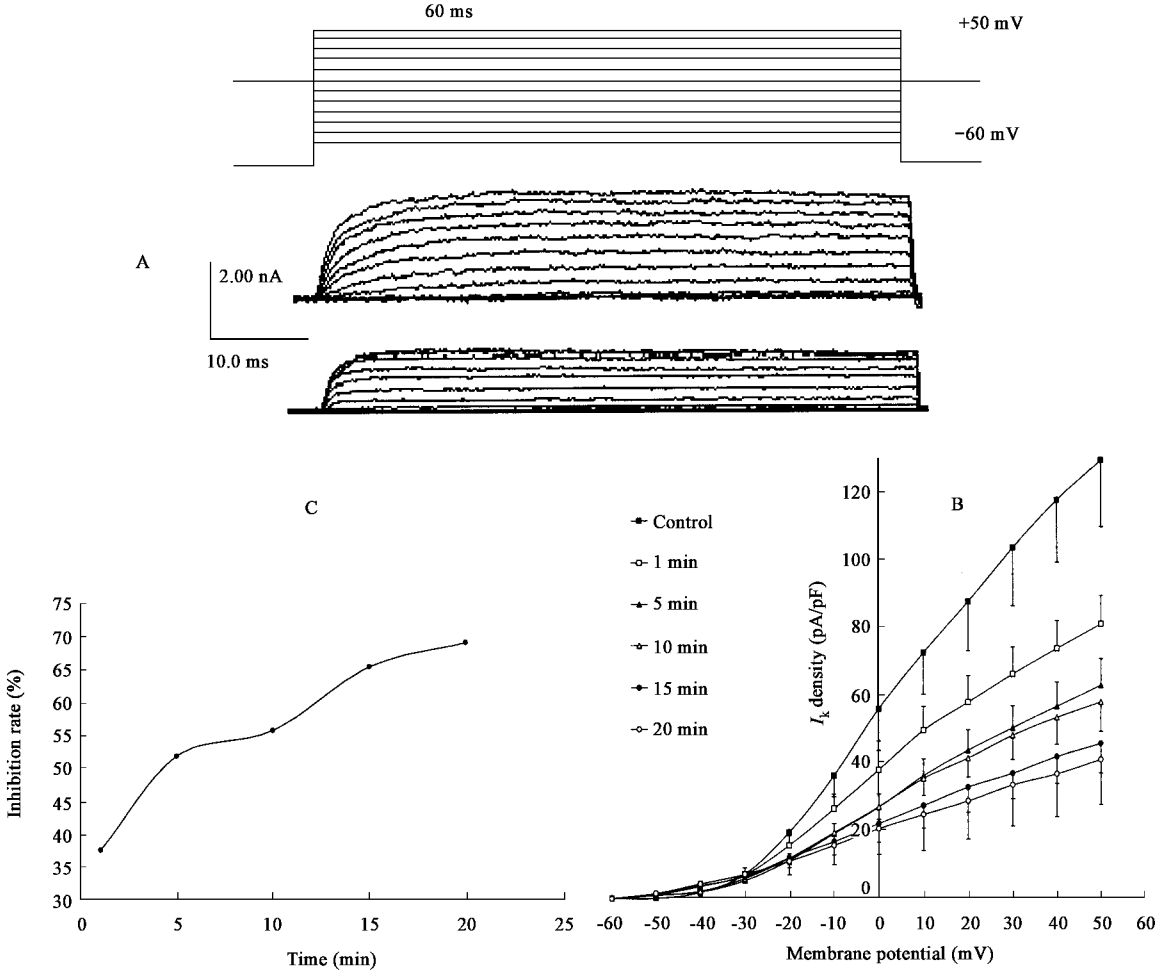

Fig. 3 A：The effects of Cyh (10^{-5} mmol/L) on I_k. Current traces obtained by 60 ms depolarizing pulses from − 60 mV to + 50 mV in 10 mV steps. B：*I-V* curves of I_k before and after the application of Cyh (10^{-5} mmol/L) at different times. C：Variation percentage of peak I_k at different times after the application of Cyh (10^{-5} mmol/L).

3.2 The effects of TEA and 4-AP on potassium current

TEA or 4-AP was added to the bath solution before the experiment, then the current was recorded after the application of the blockers. TEA of 20 mmol/L and 30 mmol/L decreased the I_K differently (Fig. 2∶A); 3 mmol/L 4-AP blocked the I_A completely (Fig. 2∶B).

3.3 The effect of Cyh on I_k

4-AP was added to the bath solution before the experiment and the ultimate concentration was 3 mmol/L. Utilizing the protocol above, we obtained the current before and after the application of Cyh (Fig. 3∶A). The current recorded at 5 min was considered as the control group, then, the drug was added to the surroundings of the cells. The mean current-voltage (*I-V*) relationship demonstrated that I_k densities decreased obviously after adding Cyh (Fig. 3∶B). In control group, 81% and 39% cells were activated at −30 mV and −40 mV ($n = 21$), respectively. In 15 min after adding Cyh, 75%, 63% and 38% cells were activated at −30 mV, −40 mV and −50 mV ($n = 8$), respectively. The results showed that more channels were activated more easily at lower potentials. Fig. 3 (C) shows the inhibition rate of Cyh to the current. The amplitude of I_k at 1 min and 15 min after drug application reduced by 37.7% and 65.1%. The decreases by using *t*-test vs. control group, $P = 0.0016 < 0.05$ ($n = 19$) and $P = 0.0003 < 0.05$ ($n = 8$), respectively. So we concluded that Cyh (10^{-5} mmol/L) could inhibit the delayed rectifier potassium current significantly.

3.4 The effect of Cyh on kinetics of steady-state activation of I_k

The same pulse protocol as described above was used to investigate its steady-state activation kinetics. Activation plots were fitted to the Boltzmamm equation: $G/G_{max} = 1/\{1 + \exp[(V\text{-}V_h)]/k\}$, where G/G_{max} is the normalized conductance; V is the conditioning potential; V_h is the voltage at half-maximal activation and the k is the slope factor. The activation curve shifted to left direction obviously 10 min after the application of Cyh (Fig. 4). The V_h before and 10 min after drug application was 11.4 ± 2.3 and 2.5 ± 0.9 ($n = 13$, $P < 0.05$), respectively. The k before and 10 min after drug application was 20.5 ± 2.2 and 24.9 ± 1.2 ($n = 13$, $P > 0.05$), respectively. So we concluded that Cyh (10^{-5} mmol/L) could significantly change the V_h of activation curve, but could not change the k value significantly.

4 DISCUSSION

The term "knockdown resistance" (designated *kdr*) is used to describe cases of resistance to

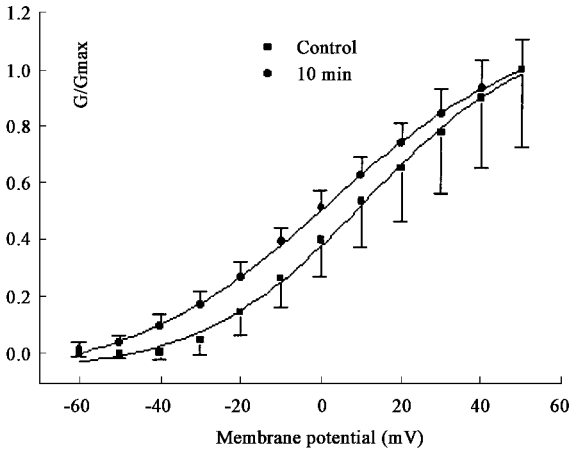

Fig. 4 Effects of Cyh (10^{-5} mmol/L) on the kinetics of steady-state activation of I_k

diphenylethane and pyrethroid insecticides in insects and other arthropods with reduced sensitivity of the nerve system. Till recently, most researches employed house fly Musca domestica L. as a model system (Soderlund and Knipple, 2003). The target of pyrethroid action is the voltage-sensitive sodium channel. This has been shown by electrophysiological studies on different types of neuronal cells or tissues (Vijverberg *et al*., 1982; Taylor *et al*., 1993; Dong and Scott, 1994; Lee *et al*., 1999). But what are the effects of pyrethroid insecticides on potassium channels of insects has barely been studied. It is well known that potassium channels play an important role in maintaining neuronal excitability. Accordingly, alternations of neuronal potassium channels may lead to profound changes in excitability and subsequent cell apoptosis or death (Zhang *et al*., 2004).

The action of cypermethrin enantiomers was studied on isolated giant axons of the cockroach *Periplaneta fuliginosa* (Serville) by using oil-gap and voltage-clamp recording techniques. The results showed that drug blocked potassium channels and decreased the amplitude of I_k (Liu *et al*., 1990), but whether the drug had effects on I_A was not reported. Salgado (1992) described studies leading to a proposed neurotoxic mechanism of RH-5849. The injection of RH-5849 into cockroaches and other insects at 50 μg/g caused a rapid hyperactivity, leading to prostration and constant movement of all appendages within a few minutes. I_K in voltage-clamped ventral longitudinal muscles was reduced 70% by 100 μM RH-5849, but the I_A and Ca^{2+}-dependent maintained K^+ current were not affected by this treatment (Salgado, 1992). Kraliz's research demonstrated that tacrine (THA) 10 μmol/L inhibited I_K selectively (Kraliz and Singh, 1997). The effects of Cyh on central neurons of *H. armigera* was studied by He *et al*. (2002), who found

that Cyh could change the activation potential, peak potential and current amplitude of sodium and calcium channels. The results of our experiment suggested that I_K channels were also the targets of Cyh.

The steady-state activation curve of I_K shifted towards more negative potentials after the application of Cyh, which indicates earlier activation and normally, but not always, results in an increase in current amplitude (Pan *et al*., 2003). However, a rapid decrease in I_K was clearly observed in the present study, suggesting that Cyh may interact directly with the channels ' subunits from the extracellular side or Cyh can penetrate the membrane rapidly. Therefore, the inhibition effect induced by Cyh is much stronger than the channel activation resulting from the negative shift of the activation curve. This observation had also been noted in Pan 's studies on hippocampal neurons (Pan *et al*., 2003). I_K is the main component of the repolarization period, and it can shorten the duration and influence the figuration of action potential. I_K could be inhibited in a short time, so more I_K channels were closed and the channels in open state were inhibited after Cyh application, which delayed the recovery from depolarized state to polarized state. This may cause the prolonged course of action potential and the decreased sensitivity. So I_K channels may play a role in the resistance formation of *H. armigera* and may be the new targets for designing novel insecticides. Further studies are needed to investigate the precise mechanism of the effects of Cyh on action potentials by using current-clamp configuration and to know what kind of I_K channel subunit is involved in such effect, as well as what is the precise mechanism of the inhibition effect.

References

Hille B, 1992. Ionic Channels of Excitable Membranes. 2nd ed. Sunderland, MA, Sinauer Associates. 59 – 140.

Dong K, Scott JG, 1994. Linkage of *kdr*-type resistance and the para-homologous sodium channel gene in German cockroaches (*Blattela germanica*). *Insect Biochemistry and Molecular Biology*, 24: 647 – 654.

Hayashi JH, Levine RB, 1992. Calcium and potassium currents in leg motoneurons during postembryonic development in the hawkmoth *Manduca sexta*. *J. Exp. Biol.*, 171: 15 – 24.

He BJ, Liu AX, Chen JT, Sun JS, Rui CH, Meng XQ, 2001. Acute isolation and culture of nerve cell from cotton bollworm and patch-clamp study on the voltage-gated ion channels in the cultured neurons. *Acta Entomologica Sinica*, 44(4): 422 – 427. [贺秉军,刘安西,陈家童,孙金生,芮昌辉,孟香清, 2001. 棉铃虫幼虫神经细胞的急性分离培养及其电压门控通道的膜片钳研究. 昆虫学报,44(4): 422 – 427]

He BJ, Liu AX, Chen JT, Sun JS, Rui CH, 2002. Effects of cyhalothrin on the sodium and calcium channels in central neurons of *Helicoverpa armigera*. *Acta Biophysica Sinica*, 18(2): 201 – 205. [贺秉军,刘安西,陈家童,孙金生,芮昌辉, 2002. 三氟氯氰菊酯对棉铃虫神经细胞钠及钙通道作用机理研究. 生物物理学报, 18(2): 201 – 205]

He BJ, Liu DB, Chen Q, Liu AX, Wang QM, Huang RQ, Rui CH, 2003. Effect of optically-active pyrethroid on sodium channels in the central neurons of *Helicoverpa armigera*. *Acta Entomologica Sinica*, 46(2): 156 – 160. [贺秉军,刘东波,陈强,刘安西,汪清明,黄润秋,芮昌辉, 2003. 光学活性拟除虫菊酯对棉铃虫神经细胞钠通道电流的影响. 昆虫学报, 46(2): 156 – 160]

Kraliz D, Singh S, 1997. Selective blockade of the delayed rectifier potassium current by tacrine in *Drosophila*. *J. Neurobiol.*, 32: 1 – 10.

Lee D, Park Y, Brown TM, Adams ME, 1999. Altered properties of neuronal sodium channels associated with genetic resistance to pyrethroids. *Mol. Pharmacol.*, 55(3): 584 – 593.

Li J, He BJ, Liu AX, 2004. Modulation of the voltage-gated sodium and calcium channels by pertussis toxin sensitive Gi protein in neurons isolated from cotton bollworm (*Helicoverpa armigera*). *Acta Biophysica Sinica*, 12(1): 26 – 30. [李杰,贺秉军,刘安西,2004. 百日咳毒素对棉铃虫神经细胞电压门控钠、钙通道的调节作用. 生物物理学报, 12 (1): 26 – 30]

Li J, Tong F, He BJ, Hu ZN, Liu AX, 2005. Modulations of the high-voltage sensitive Ca^{2+} channels by GTP and GDP analogues in the central neurons of cotton bollworm (*Helicoverpa armigera*). *Acta Entomologica Sinica*, 48(1): 31 – 35. [李杰,佟帆,贺秉军,胡兆农,刘安西,2005. GTP 和 GDP 类似物对棉铃虫神经细胞高电压敏感钙通道的调节作用. 昆虫学报, 48(1): 31 – 35]

Liu AX, Ning QJ, Chen XL, Huang RQ, Chen ST, 1990. Modified action of cypermethrin enantiomers on axonal sodium and potassium channels of *Periplaneta fuliginosa* (Serville). *Acta Entomologica Sinica*, 33(1): 1 – 6. [刘安西,宁黔冀,陈叙龙,黄润秋,陈守同,1990. 氯氰菊酯异构体对黑胸大蠊神经钠钾通道的调制作用. 昆虫学报, 33 (1): 1 – 6]

Narahashi T, 1992. Nerve membrane Na^+ channels as targets of insecticides. *Trends Pharmacol. Sci.*, 13: 236 – 241.

Pan YP, Xu XH, Wang XL, 2003. Rivastigmine blocks voltage-activated K^+ currents in dissociated rat hippocampal neurons. *British Journal of Pharmacology*, 140: 907 – 912.

Salgado VL, 1992. The neurotoxic insecticidal mechanism of the nonsteroidal ecdysone agonist RH-5849: K^+ channel block in nerve and muscle. *Pestic. Biochem. Physiol.*, 43: 1 – 13.

Soderlund DM, Knipple DC, 2003. The molecular biology of knockdown resistance to pyrethroid insecticides. *Insect Biochemistry and Molecular Biology*, 33: 563 – 577.

Taylor MFJ, Heckel DG, Brown TM, Kreitman ME, 1993. Linkage of pyrethroid insecticides resistance to a sodium channel locus in the tobacco budworm. *Insect Biochemistry and Molecular Biology*, 23: 763 – 775.

Vijverberg HPM, van der Zalm JM, van den Bercken J, 1982. Similar mode of action of pyrethroids and DDT on sodium channel gating in myelinated nerves. *Nature*, 295: 601 – 603.

Zhang W, Jin HW, Wang XL, 2004. Effects of presenilins and β-amyloid precursor protein on delayed rectifier potassium channels in cultured rat hippocampal neurons. *Acta Pharmacol. Sin.*, 25(2): 181 – 185.

Zhao Y, Liu AX, Ru LJ, Fan XL, Wei C, 1996. Decreased nerve sensitivity is an important pyrethroid resistance mechanism of cotton bollworm. *Acta Entomologica Sinica*, 39(4): 347 – 353. [赵勇,刘安西,茹李军,范贤林,魏岑,1996. 神经敏感性的降低是棉铃虫对拟除虫菊酯抗药性的重要机制. 昆虫学报, 39(4): 347 – 353]

三氟氯氰菊酯对棉铃虫神经细胞延迟整流钾电流的抑制作用

王　勇,贺秉军*,伍春林,刘安西

(南开大学生命科学学院,天津　300071)

摘要: **用膜片钳技术首次研究了三氟氯氰菊酯对离体培养的棉铃虫中枢神经细胞延迟整流钾通道电流的影响。结果表明,药物作用前有** 81% **和** 39% **的细胞的通道分别在** −30 mV **和** −40 mV **激活(**n = 21**)。三氟氯氰菊酯(**10^{-5} mmol/L**)作用** 15 min **后,有** 63% **和** 38% **细胞的通道分别在** −40 mV **和** −50 mV **激活(**n = 8**);作用** 1 min **后电流幅值明显降低,抑制率达到了** 37.7% **(**n = 19**)加药后激活曲线明显左移且** V_h **值变化显著,但** k **值没有明显变化。实验结果说明,三氟氯氰菊酯作用后,通道更容易激活,但显著抑制电流峰值,导致神经敏感性降低,棉铃虫中枢神经细胞钾通道也是拟除虫菊酯类药物的作用靶标之一。**

关键词: **棉铃虫;钾通道;三氟氯氰菊酯;膜片钳;神经细胞;神经敏感性**

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