Effect of alpha-cypermethrin and theta-cypermethrin on delayed rectifier potassium currents in rat hippocampal neurons

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A B S T R A C T
Cypermethrin is a photosstable synthetic pyrethroid and the most widely used Type II pyrethroid pesticide. The effects of two different stereoisomers of cypermethrin insecticides, alpha-cypermethrin and theta-cypermethrin, on the delayed rectifier potassium current (I K) in hippocampal neurons of rat, were studied using whole-cell patch clamp technique. Alpha-cypermethrin and theta-cypermethrin decreased the amplitude value of I K and shifted the steady state activation curve of I K towards negative potential at any concentrations (10 - 8 M, 10 - 7 M). Furthermore, at higher concentration, alpha-cypermethrin (10 - 7 M) and theta-cypermethrin (10 - 8 M, 10 - 7 M) had observable effects of the steady state inactivation of I K. The results suggest that I K is the target of alpha-cypermethrin and theta-cypermethrin, which may explain the mechanism of toxic effects of both stereoisomers of cypermethrin on mammalian neurons. Cypermethrin-altered properties of voltage gated delayed rectifier K+ channels may contribute to neurotoxicity by eliciting abnormal electrical discharges in hippocampal CA3 neurons.

1. Introduction
Insecticides have become an integral part of our environment. The biological effects due to the repeated exposure to low concentrations of these substances have to be taken into consideration both in human and in animal health. In this sense, cypermethrin presents a particularly interesting compound, because it is contained in a variety of insecticides used at home and in agriculture.

Cypermethrin, [(R, S) a-cyano-3-phenoxybenzyl (1R, S)-cis-trans-3-(2, 2-dimethylcyclopropane carboxylate), is a photosstable synthetic pyrethroid (Yousef et al., 2003). It is the most widely used Type II pyrethroid pesticide. The insecticide cypermethrin belongs to the group of pyrethroids classified by the World Health Organization as moderately harmful, class II (WHO, 1995). And, if these compounds reach the nervous system of mammals, including man in sufficient concentrations, they can cause adverse neurotoxic effects (Eriksson and Fredrickson, 1991; Narahashi, 1992; Malaviya et al., 1993). Several references concerning the epileptogenic neurotoxic effect of cypermethrin have been found (Condes-Lara et al., 1999). Moreover, there were many reports indicated existing neurobehavioral toxicology of pyrethroid insecticides in adult animals (Wolansky and Harrill, 2008). Cybermethrin is highly hydrophobic compounds and this suggests that their action in biological membranes might be related to association with integral proteins, such as some important ion channels (Michelangeli et al., 1990). There is evidence that cypermethrin delays the closing of sodium voltage-sensitive membrane channels in the neurons, and also inhibit GABAA receptors (Bloomquist, 1996). So cypermethrin could change the properties of the synaptic membrane potential (Eells et al., 1992, 1993).

Under physiological conditions, voltage-gated K+ currents are especially important for the regulation of neuronal excitability, because they repolarize neurons in response to depolarizing events and help to stabilize the membrane potential below firing threshold (Yost, 1999). In addition, studies also demonstrated that voltage-dependent transient outward K+ currents (I A) and delayed rectifier K+ currents (I K) play a major role in regulating neuronal excitability (Hablitz and Johnson, 1981; Wong and Traub, 1983; Klee et al., 1995). Changes in the number of transient outward and delayed rectifier K+ channels or changes in their kinetic properties can alter neuronal electrical function (LeMasson et al., 1993). Although most researches have focused on the interaction between cypermethrin and sodium channels, we have recently demonstrated that cypermethrin can alter the function of transient outward K+ channels in rat hippocampal neurons in a voltage-dependent manner (Tian et al., 2008). However, there

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were few references concerning the neurotoxic effect of cypermethrin on delayed rectifier potassium channel and, therefore, in the present study, we sought to investigate the toxic effects of cypermethrin in rat hippocampal neurons by using whole-cell patch clamp recording.

2. Materials and methods

2.1. Slice preparation

Male Wistar rats on postnatal days 14–18 were provided from Experimental Animal Center, Chinese Academy of Medical Sciences. The experiments were conducted in accordance with the guidelines of the Medical Experimental Animal Administrative Committee of Nation. Hippocampal neurons were prepared as previously described (Liu et al., 2007) with some modifications. Horizontal slices that included the entire hippocampus and subiculum (350 μm in thickness) were prepared with a vibratome (VT1000S, Leica, Germany) and incubated with artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl 125, NaHCO3 25, KCl 1.25, NaH2PO4 1.25, MgCl2 2.0, CaCl2 2.0, D-glucose 10, pH 7.4.

2.2. Electrophysiological recordings

For whole-cell recording, slices were transferred to a recording chamber placed on the stage of a modified upright infrared DIC microscope (BX51WI, Olympus, Japan). Hippocampal CA3 neurons were visualized on a television monitor connected to a low light sensitive CCD camera (710 M, DVC, USA). A micropipette puller (P-97, HEKA, Germany) was used to pull the electrodes. Patch pipettes had a tip resistance of 4–6 MΩ when filled with pipette solution containing (in mM): KCl 130, HEPES 10, EGTA 10, MgCl2 2.0, CaCl2 2.0, p-glucose 10, pH 7.4. Slices were maintained in aCSF for at least 1 h before moving them into the recording chamber. During recordings, the slices were kept in submerged a chamber perfused with aCSF. In the experiments, the aCSF was saturated with 95% O2–5% CO2. All experiments were performed at room temperature (22–24°C).

2.3. Drug application

Cypermethrin (alpha-cypermethrin and theta-cypermethrin) was provided by the college of chemistry of Nankai University (PR China). Cypermethrin was prepared as stock solutions in dimethylsulfoxide (DMSO) and diluted directly in artificial cerebrospinal fluid (aCSF), the concentrations of cypermethrin used in the experiment were 10−9 M, 10−8 M, and 10−7 M, and the final DMSO concentration was <0.1%, respectively. DMSO (less than 0.1% in the final dilution) had no observed effects on the voltage dependent K+ (IK) channel currents. All other drugs were diluted in oxygenated aCSF immediately prior to use, and were applied via the perfusion system.

2.4. Data analysis

All data were analyzed by Igor Pro 5.04 and Origin 7.0. For activation, currents at each test potential were converted to conductance (G) using the following formula G = I/(V − Vh), where Vh is reversal potential. The peak conductance value for each test potential was normalized to Gmax and plotted against the test potential to produce a voltage-conductance relationship curves, which were fitted using Boltzmann functions G/Gmax = 1/ ({1 + exp[−(V − Vh)/k]}), where Vh is the voltage at which conductance being half-maximal, and k is slope factor. Steady-state inactivation curves were fitted with the Boltzmann equations: I/Imax = 1/{1 + exp[−(V − V0)/k]}, where I/Imax is normalized current, V0 the potential for half-maximal inactivation, and k is the slope factor.

Data are presented as mean ± S.E.M. Statistical significance was assessed using a one-way ANOVA followed Tukey’s multiple comparison, and P < 0.05 was considered significant. All data analyses were performed using the software SPSS 11.5.

3. Results

3.1. Dose-dependent effects of alpha-cypermethrin and theta-cypermethrin on IK

In the present study, measurements for data analyses were made only at steady-state amplitudes of IK. Holding potential was −50 mV, IK was elicited by applying a single depolarizing voltage pulse to +70 mV following a 120-ms prepulse at −110 mV with a 50-ms interval at −50 mV to inactivate IK. Fig. 1A gave the example...
current traces before and after the application of alpha-cypermethrin. The application of different concentration (10⁻⁹ M, 10⁻⁸ M, and 10⁻⁷ M) alpha-cypermethrin and theta-cypermethrin produced a dose dependent effect on $I_K$ amplitude (Fig. 1B and C). The peak amplitudes of $I_K$ were decreased about 26.53 ± 5.39%, 34.48 ± 5.60%, and 40.80 ± 4.50% (Fig. 1B), respectively, by alpha-cypermethrin. And the amplitudes of $I_K$ were decreased about 5.21 ± 5.58%, 27.22 ± 4.47%, and 53.28 ± 5.67% by theta-cypermethrin (Fig. 1C).

3.2. Effects of alpha-cypermethrin and theta-cypermethrin on $I_K$ I–V relationship

$I_K$ was elicited with depolarizing voltage pulse from −60 to +80 mV in increments of 10 mV following a prepulse at −110 mV with a 50-ms interval at −50 mV to inactivate $I_K$. Fig. 2A showed the example current traces before and after the application of alpha-cypermethrin. Upon the application of alpha-cypermethrin and theta-cypermethrin, the $I_K$ current amplitudes were reduced in a different way at different membrane potential (Fig. 2B and C). And, these effects were in a dose-dependent manner.

3.3. Effects of cypermethrin on the steady-state $I_K$ activation curve

$I_K$ was elicited with depolarizing voltage pulse from −60 to +80 mV in increments of 10 mV following a prepulse at −110 mV with a 50-ms interval at −50 mV to inactivate $I_K$ (Fig. 2A). The steady-state activation curves for $I_K$ under control and after exposure to cypermethrin are shown in Fig. 3. It can be seen that any level of concentration (10⁻⁹ M, 10⁻⁸ M, or 10⁻⁷ M), both alpha-cypermethrin (Fig. 3A) and theta-cypermethrin (Fig. 3B) can significantly shifted the V₅₀ of $I_K$ activation curve to the negative potential. Furthermore, the slope factor was markedly decreased at 10⁻⁸ M, 10⁻⁷ M of alpha-cypermethrin and 10⁻⁷ M of theta-cypermethrin, respectively. The effects of cypermethrin on the activation parameters of $I_K$ were summarized in Table 1.

3.4. Effects of cypermethrin on the steady-state inactivation properties of $I_K$

To investigate the effects of cypermethrin on the steady-state inactivation properties of $I_K$ (Fig. 4), 1000 ms hyperpolarizing prepulses between −130 mV and −30 mV from a holding potential of 0 mV were followed by a 6000-ms test command pulse to 0 mV. Fig. 4A showed the example steady-state inactivation current traces in normal state. The stimulus interval was set at 25 s. Since neurons were held at 0 mV $I_K$ inactivated in 24–30 s. Upon returning to 0 mV from hyperpolarizing voltage steps, $I_K$ reappeared, and its entire time course of decay could be followed. In the present study, to avoid contaminations by $I_A$, current values at 100 ms after returning from the hyperpolarizing potentials were as the $I_K$ amplitudes.
Cypermethrin is the most widely used Type II pyrethroid pesticide. It is a composite synthetic pyrethroid, a broad spectrum, biodegradable insecticide, and a fast-acting neurotoxin with good bioactivities; therefore, there may be some difference in modulating the activation and inactivation of I_K. These results shown that it causes free radical-mediated tissue damage in brain, liver (Gray et al., 2001).

It has reported that cypermethrin can affect mammals’ membrane depolarization (Eells et al., 1992) and GABA-gated chloride channels at low concentration (at least 10^{-7} M) (Lawrence et al., 1985). Thus, the concentrations of cypermethrin, employed in the present experiments, were in order to investigate whether the low concentrations of cypermethrin could change the delayed rectifier potassium channels function and kinetics characters, and we also want to investigate whether the different stereoisomers of cypermethrin have dissimilar characters. Both activation and inactivation properties of I_K can be affected by either alpha-cypermethrin or theta-cypermethrin.

At low concentration alpha-cypermethrin was more potent on activation of I_K whereas at high concentration it seemingly appeared much less potent compared to that of thetha-cypermethrin. It was only at higher concentration that the alpha-cypermethrin (10^{-8} M) significantly shifted the V_h of I_K inactivation curve to the negative potential, without a significant change in the slope factor (Fig. 4B). Meanwhile, the theta-cypermethrin can shift the inactivation curve to the negative potential at 10^{-7} M and 10^{-6} M (Fig. 4C). Moreover, there was no change of slope factor k at any concentration alpha-cypermethrin and theta-cypermethrin.

### 4. Discussion and conclusion

Cypermethrin is the most widely used Type II pyrethroid pesticide. It is a composite synthetic pyrethroid, a broad spectrum, biodegradable insecticide, and a fast-acting neurotoxin with good contact and stomach action. Such a compound is used to control many pests, including moths, pests of cotton, fruit, and vegetable crops. Consistent with its lipophilic nature, cypermethrin has been found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, and brain. In vitro and in vivo studies have also found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, and brain. In vitro and in vivo studies have also found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, and brain. In vitro and in vivo studies have also found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, and brain. In vitro and in vivo studies have also found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, and brain. In vitro and in vivo studies have also found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, and brain. In vitro and in vivo studies have also found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, and brain.

![Table 1](image1)

<table>
<thead>
<tr>
<th>Cypermethrin (n = 8)</th>
<th>V_h (mV)</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.19 ± 8.68</td>
<td>24.56 ± 2.85</td>
</tr>
<tr>
<td>10^{-8} M cypermethrin</td>
<td>-8.90 ± 4.97**</td>
<td>21.37 ± 1.85</td>
</tr>
<tr>
<td>10^{-7} M cypermethrin</td>
<td>-9.12 ± 8.16</td>
<td>20.62 ± 3.39</td>
</tr>
<tr>
<td>10^{-8} M cypermethrin</td>
<td>-8.94 ± 7.51</td>
<td>20.81 ± 1.05</td>
</tr>
</tbody>
</table>

**Note:** V_h: the membrane potential at half-activation; k: slope factor.

![Table 2](image2)

<table>
<thead>
<tr>
<th>Cypermethrin (n = 6)</th>
<th>V_h (mV)</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-56.21 ± 7.21</td>
<td>25.38 ± 6.05</td>
</tr>
<tr>
<td>10^{-8} M cypermethrin</td>
<td>-58.31 ± 6.70</td>
<td>25.07 ± 2.27</td>
</tr>
<tr>
<td>10^{-7} M cypermethrin</td>
<td>-61.06 ± 7.11</td>
<td>23.82 ± 3.49</td>
</tr>
<tr>
<td>10^{-8} M cypermethrin</td>
<td>-74.59 ± 12.90**</td>
<td>23.83 ± 5.85</td>
</tr>
</tbody>
</table>

**Note:** V_h: the membrane potential at half-activation; k: slope factor.

"P < 0.05 vs control."
suggest that alpha-cypermethrin and theta-cypermethrin exhibit different effects on the delayed rectifier potassium channels.

In the previous study, we showed that cypermethrin effects on \( I_k \) in acutely dissociated rat hippocampal neurons (Tian et al., 2008). Our present results also provided the evidence that cypermethrin was a potent inhibitor of delayed rectifier potassium current (\( I_k \)) in rat hippocampal neurons. It is well known that changes in the function of the \( K^+ \) channels will directly affect the neuronal activities including setting cell's resting potential, repolarizing the cell after an action potential (AP), and controlling the shape and firing of AP (Nestler et al., 2001). \( I_h \) and \( I_K \) contribute to AP repolarization and repetitive firing (Fisher et al., 1998; Rudy, 1988; Saito and Isa, 2000; Xu et al., 2003). Thus, block of delayed rectifier \( K^+ \) channels will be likely to broaden or widen the duration of AP, and our present and previous studies showed that cypermethrin changed the properties of \( I_k \) and \( I_h \) in a concentration-dependent manner, and higher concentrations of cypermethrin (10 -7 M) could change the kinetic properties of the two currents. Moreover, these kinetic properties' changes of \( I_k \) and \( I_h \) would make neurons maintain a continuance of depolarization, prolong the time to AP, broaden the duration of action potentials and increase the rate of repetitive firing. It seems reasonable to assume that cypermethrin induced \( K^+ \) currents changes in the brain may contribute to its neurotoxicity by eliciting abnormal neuronal discharge.

As delayed rectifier \( K^+ \) channels widely distribute in the rat hippocampal neurons and are responsible for the neuronal firing pattern and influence neuronal excitability as well as synaptic transmission. The present study raises that the relative low concentrations of cypermethrin is related to the electrophysiological activities of rat hippocampal CA3 neurons by effects voltage gated delayed rectifier \( K^+ \) channels. The cypermethrin induced change of \( K^+ \) currents in the hippocampal CA3 neurons may contribute to its neurotoxicity by eliciting abnormal neuronal discharge.

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References