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Original Article

Effects of Fenvalerate on the Central and Peripheral Nervous Systems of the American Cockroach, *Periplaneta americana* (L.)*

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The differences in the effects of the (*R*,*S*)-*S* isomer of fenvalerate, (*R*,*S*)-alpha-cyano-3-phenoxybenzyl (*S*)-2-(4-chlorophenyl)-isovalerate on the electrophysiological activity of the central and peripheral nervous systems have been studied in the American cockroach, *Periplaneta americana* (L.). The (*R*,*S*)-*S* isomer of fenvalerate produced an increase followed by a decrease in impulse frequency of both nervous systems. In the central nervous system, the frequency level fluctuated among the each nerve impulse isolated from external recording following application of the (*R*,*S*)-*S* isomer of fenvalerate. The maximum frequency of repetitive firing in each nerve was not significantly different. In the peripheral nervous system, the process of intoxication was nearly identical to that of the central nervous system except that a change of frequency was not observed. The time required for the inhibition of spontaneous discharge following the application of the (*R*,*S*)-*S* isomer of fenvalerate was longer in the central nervous system as compared to the peripheral nervous system. There was no significant difference with regard to the times to cessation spontaneous nerve activity or the maximum frequency of repetitive firing induced by fenvalerate between central and peripheral nervous system activities. These results indicate that one major difference between the peripheral and the central nervous systems regarding the effect of the (*R*,*S*)-*S* isomer of fenvalerate on each nervous system is the onset time of the toxic effect after application.

INTRODUCTION

Many studies have shown that pyrethroids alter the activity of both central and peripheral nervous systems.[1,2] One major interest has been about the mode of action of pyrethroids and DDT type insecticides, especially in regard to which nervous system is the actual site of toxic action at the organismal level.[3] Although many studies have demonstrated that the peripheral nervous system is more sensitive to pyrethroids than the central nervous system,[4-9] why the peripheral nervous system is more sensitive than the central nervous system is still unknown. The purpose of the present study was to clarify the differences in the effect of pyrethroid, fenvalerate, between the central and peripheral nervous systems of the American cockroach.

The central nervous system was represented by the ventral nerve cord while the cerci was used as a model for the peripheral nervous system. Fenvalerate has two asymmetric carbon atoms, therefore four optical isomers exist. We have used a pair of diastereomers

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of fenvalerate, (*R,S*)-*S* isomer of fenvalerate, that is more toxic than the (*R,S*)-*R* isomer.[10-13]

MATERIALS AND METHODS

1. Insects

Two- to 4-week adults of male American cockroaches were taken from stock culture reared with water and rat diet (CE-2, Clea Japan Inc., Tokyo, Japan) at 25°C and 16L–8D condition.

2. Insecticides

The (*R,S*)-*S* isomer of fenvalerate was provided by Sumitomo Chemical Co. (Osaka, Japan). Stock solutions of fenvalerate were made in dimethyl sulfoxide (DMSO) at concentration of 1×10^{-2} M, 1×10^{-3} M and 1×10^{-4} M.

3. Physiological Saline Solution

Physiological saline solution contained 200 mM of NaCl, 3.1 mM of KCl, 5.0 mM of $CaCl_2\cdot H_2O$, and was buffered at pH 7.2 with 1/15 M of NaH_2PO_4 and Na_2HPO_4.

4. Insecticide Application

Stock solutions of fenvalerate were diluted with the physiological saline solution. The concentration of DMSO in the solution did not exceed 1%. This level of concentration caused no adverse effect on nerve activity. The test solution was applied directly to the nerve preparations at room temperature.

5. Central Nervous System Preparation

After being anesthetized with CO_2, the legs and head of the cockroaches were removed. A large opening was made in the cuticle of the abdomen to remove the gut and associated tissues. The tracheae associated with the nervous system was also partially dissected out. The neural sheaths of ganglia were removed using microscissors and fine forceps. Small nerves emerging from each ganglion were crushed with forceps to eliminate sensory discharges. The abdominal cavity was filled with physiological solution and silver/silver chloride electrode (0.05 mm in diameter) was placed under the ventral nerve cord between 3rd and 4th ganglia.

6. Peripheral Nervous System Preparation

The dissection was identical to that described for the central nervous system preparations. In this case, however, the recording electrode was placed under the nerve cord between the 6th ganglion and cercus to record the extracellular sensory discharges.

7. Recording

The external recording was performed using the air-gap method. A small part of the nerve cord was above the level of the bath solution. Stainless wire was used for the reference electrode which was placed in bath solution. All recorded electrical signals were preamplified by a microelectrode amplifier (MZE-8201) and a biophysical preamplifier (AVB-2B), and were displayed on an oscilloscope (VB-8) to which a recording camera (PC-2B) was attached (Nihon Kohden Co., Tokyo, Japan). All experiments were started 10 to 15 min following the isolation of the nerves. This time length was sufficient for the preparation to recover to its normal level of spontaneous activity. The subsequent nerve activity of each preparation was recorded for periods of up to 4 to 6 hr.

8. Calculation of the Spike Frequency

The activity of individual nerve fibers was identified by the amplitude of their extracellularly recorded action potential and was analyzed separately. The frequency of impulse was calculated as number of spikes per min (mean$\pm$S.D.).

9. Statistical Analysis

Data were analyzed by a two samples *t*-test and a one-way analysis of variance using a micro computer.

RESULTS

1. Central Nervous System

In the central nervous system, spontaneous activity increased followed by repetitive firing occurred at 5.83$\pm$4.0 min after the application of 1×10^{-6} M of the (*R,S*)-*S* isomer of fenvalerate (Table 1). A conduction block of the spontaneous discharge occurred with a mean time of 43.6$\pm$18.5 min in the external recording (Table 1). Each fiber contributing to the external recording was identified by its

Table 1 The time (in min) for onset of intoxication and cessation of action potential or repetitive firing following application of the (*R,S*)-*S* isomer of fenvalerate to the central and peripheral nervous systems of the American cockroach, *Periplaneta americana* (L.) and the time (in min) for duration of intoxication.

	Onset of toxicity	Duration	Cessation
Central			
External	5.83±4.0 (6)a		43.6±18.5 (7)x
Identified	19.7±9.6 (5)b	8.48±2.8 (4)1	23.8±12.2 (4)y
Peripheral	0.43±0.23 (7)c	11.6±5.3 (6)1	12.5±5.2 (6)z

Data were given in mean±S. D. with the number of experiments in parentheses. The same letters are not significantly different (*t*-test; $p>0.05$). Data were obtained from 7 preparations for external, 5 preparations for identified and 7 preparations for peripheral. Some data were excluded when the unsuitable experimental conditions occurred for measurement including unclear time course of intoxication.

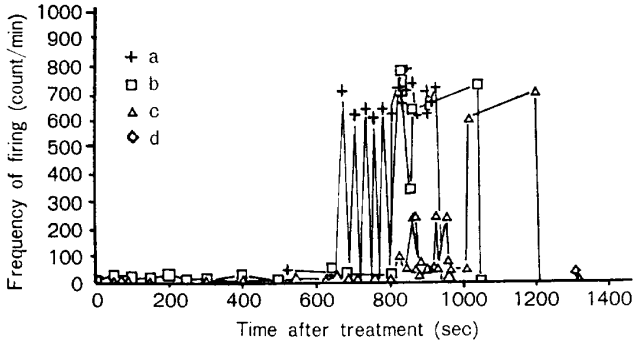

Fig. 1 The frequency of impulse of the central nervous system in the American cockroach after application of the (*R,S*)-*S* isomers of fenvalerate.

Letter shows each identified nerve.

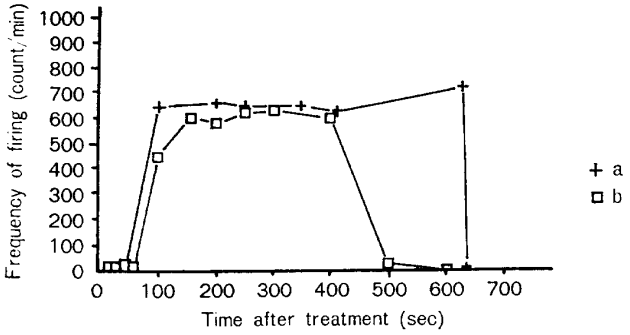

Fig. 2 The frequency of impulse of the peripheral nervous system in the American cockroach after application of the (*R,S*)-*S* isomers of fenvalerate.

Letter shows each identified nerve.

spike amplitudes, revealing differences in the time course of toxicity (Fig. 1). In one fiber (a), the spontaneous frequency was fluctuated during the intoxication process. In another (c), the frequency of repetitive firing was sustained longer and then finally stopped. In another (d), the frequency of the control firing activity was monitored after application and then inhibited. The onset of the spontaneous activity increase in each identified nerve fiber was averaged 19.7±9.58 min (Table 1). The average period of time between the onset of repetitive firing and cessation was 8.48±2.8 min (Table 1).

2. *Peripheral Nervous System*

In the peripheral nervous system, the effects of the (*R,S*)-*S* isomer were almost identical to those observed in the central nervous system. In the peripheral nervous system, the frequency of repetitive firing was more steady, within 1 min after application of the (*R,S*)-*S* isomer of fenvalerate, spontaneous activity increased (0.43±0.23 min). This level was kept until cessation occurred (Fig. 2a, b). The mean duration of the fenvalerate effect was 11.6±5.3 min (Table 1). There was no difference in duration between the central and peripheral nervous systems (*t*-test; $p>0.05$). The time of cessation of repetitive firing after application of 1×10^{-6} M the (*R,S*)-*S* isomer averaged 12.5±5.2 min (Table 1).

3. *Frequency of Repetitive Firing*

Table 2 shows the frequency of repetitive firing (frequency per min) recorded during the application of the 1×10^{-6} M of (*R, S*)-*S* isomer of fenvalerate. In the central nervous system

Table 2 Frequency of repetitive firing during application of the (*R*,*S*)-*S* isomer of fenvalerate to the central and peripheral nervous systems of the American cockroach.

Central	Peripheral
642±133 (9)	611±183 (6)

Data were count per min and given in mean± S.D. with the number of experiments in parentheses. Fifteen cockroaches were used for these experiments.
There was no significantly different between two values (*t*-test, $p>0.05$).

the maximum frequency of repetitive firing was 642±133 spike per min compared to the peripheral nervous system frequency of 611± 133 spike per min. There was no significant difference between the central and peripheral values (*t*-test; $p>0.05$).

DISCUSSION

Many studies have attempted to resolve whether pyrethroids and DDT should be considered as centrally or peripherally acting toxins. Clements & May[1] reported that alpha-cyano type pyrethroid had potent stimulatory effects on the peripheral nervous of the desert locust, *Schistocerca gregaria* (L.), and that the repetitive discharge originated at the cell rather than the axon. On the basis of these results, they concluded that the primary site of pyrethroid action was the peripheral nervous system. Adams & Miller[5,6] investigated the effect of tetramethrin on the motor nerve activity of the housefly, *Musca domestica* (L.). An extremely low dose of this compound produced a repetitive discharge in the central nervous system but not in the peripheral nervous system. In the present study, the repetitive discharge occurred both in the central and the peripheral nervous systems after applying 1×10^{-6} M of the (*R*,*S*)-*S* isomer of fenvalerate. The two systems differ in the time needed for the repetitive firing to cease following insecticide application (Table 1). In this aspect, the peripheral nervous system is more sensitive than the central nervous system to the (*R*,*S*)-*S* isomer of fenvalerate. We examined identified nerve activity making up the external recording in the central nervous system. The time required for the cessation of repetitive firing is averaged 8.48±2.8 min. This value was not significantly different from that of the peripheral nervous system (11.6±5.3 min). The central nervous system is surrounded by connective tissues such as the nerve sheath that impedes some compounds from reaching the site of action.[14,15] This barrier might have produced the delayed appearance of the toxic effects observed in the central nervous system. In contrast, the peripheral nervous system lacks such barrier, therefore the insecticide easily reaches the nerve membrane sodium channels, *i.e.*, the presumed site of action.

The frequency of repetitive discharge was highly stable (Table 2). In our recent experiment, the threshold of transition from resting state to spontaneous repetitive firing state caused by the external Na^+ concentration was changed by the pyrethroids (Nagata *et al.*, unpublished data). Although the threshold Na^+ concentration was shifted lower value in the preparatorily applied fenvalerate than in the control preparation, the frequency of spontaneous repetitive firing in high external Na^+ concentration was not significantly different in the presence or absence of pyrethroid. This suggests that the appearance of repetitive discharge is an intrinsic response of the nervous system. Repetitive activity was observed in many circumstances such as the application of intense stimulation, changing in ionic circumstances, high temperature, *etc.* Pyrethroids and DDT type insecticides may change the intrinsic response of the nervous system to changes in the surrounding conditions. There may be no difference in the action of pyrethroid on Na channels in the central and peripheral nervous systems, therefore an organismal difference might be the cause of the differential sensitivity to pyrethroid in each nervous system. The mechanistic process relating the disruption of the nervous function to insect death has not been established, so that the importance of identifying the organismal action site is not clear. Further studies establishing the relationship between toxicity and altered nervous activity are needed to clearly identify the mode of

action of fenvalerate.

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要　　約

ワモンゴキブリ中枢神経系および末梢神経系への fenvalerate の作用

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ワモンゴキブリ中枢神経系および末梢神経系への fenvalerate (*R*, *S*)-*S* 体の作用の違いを電気生理学的手法を用いて調べた．Fenvalerate (*R*, *S*)-*S* 体はどちらの神経系に対しても反復興奮を発生し，伝導阻害を起こした．中枢神経系では反復興奮の周波数は変動したが末梢神経系では変動は観察されなかった．末梢神経系のほうが伝導阻害を起こすまでの時間は中枢神経系に比較して短く，fenvalerate (*R*, *S*)-*S* 体に対する感受性に違いがあることが示された．中枢神経系の細胞外記録から個々の神経を同定してそれぞれの中毒症状を示すまでの時間，中毒の持続時間，伝導阻害を起こすまでの時間を調べ末梢神経系と比較したところ中毒症状の現れるまでの時間に大きな違いが示された．反復興奮の周波数については両神経系間で違いは示されなかった．以上のことから中枢神経系と末梢神経系間での fenvalerate (*R*, *S*)-*S* 体の作用の違いは中毒症状を示すまでの時間に違いがあり，そのことが両神経系間での薬剤に対する感受性の違いの一つの主要な原因になっていると考えられる．