原　著

Studies on the Insecticidal Action of Nereistoxin, 4-*N*, *N*-dimethylamino-1, 2-dithiolane. V. Blocking Action on the Cockroach Ganglion. Michihiko Sakai (Kyoto Herbal Garden, Research and Development Division, Takeda Chemical Industries, Ltd. Ichijoji, Sakyo-ku, Kyoto) Received March 7, 1967. *Botyu-Kagaku*, 32, 21, 1967).

3. イソメ毒 (Nereistoxin, 4-*N*, *N*-dimethylamino-1, 2-dithiolane) の殺虫作用に関する研究 V. ゴキブリ神経節しゃ断作用

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イソメ毒の殺虫作用機構を解明するために，ワモンゴキブリ神経に対する作用を調べた．摘出した腹部神経索における第6腹部神経節節前神経線維（尾毛神経索）刺激に対応する節後線維（腹部神経索）の活動電位は 2×10^{-6}M 以上の濃度のイソメ毒の処理により抑制された．しかし，神経索伝導は尾毛神経においても，また腹部神経においてもイソメ毒によって影響されなかった．したがって，上記の活動電位抑制作用は神経節伝導をしゃ断することによると判断される．

イソメ毒による節しゃ断作用は節前線維に与える閾刺激の強さの増大となって現われた．この作用をさらに検討するため，第6腹部神経節より興奮性後シナプス電位（e. p. s. p.），節の静止電位および索の活動電位を節に接触させた1本の細胞外電極によって誘導した．その結果，イソメ毒は明らかに e. p. s. p. を抑制し，それにともなって索の活動電位が消失するが，静止電位は変化しないことが認められた．イソメ毒と異なって，ニコチンおよびエゼリンは静止電位を増大せしめ，その結果正常な興奮伝達が阻止されることが認められた．イソメ毒により抑制された e. p. s. p. は刺激強度の増大とともに発生し，それにともなって活動電位が発生するから，イソメ毒は刺激伝達物質（Ach）と競合してシナプス後膜をしゃ断すると思われる．

脚筋神経系に対してイソメ毒は全く影響しないので，イソメ毒の昆虫麻痺，致死作用は中枢神経系における節シナプスしゃ断作用に基づくと推定される．

Introduction

Nereistoxin is a toxic substance which was firstly isolated by Nitta[1] from a dead body of a marine annelid, *Lumbriconereis heteropoda*. The chemical structure of this substance was determined as 4-*N*, *N*-dimethylamino-1, 2-dithiolane by Okaichi and Hashimoto[2,3]. Its synthesis was accomplished by Hagiwara *et al*[4,5]. Some of the toxicological and pharmacological properties of the toxin were reported by Nitta[1,6], and by Okaichi and Hashimoto[7]. The latter two authors tested its insecticidal activity preliminarily and reported the toxin paralysed insects.

Recently the author investigated on the insecticidal properties of nereistoxin, and found that it was highly toxic against lepidopterous larvae including rice stem borers, *Chilo suppressalis*, cabbage worms, *Pieris rapae*, and diamond back moths, *Pluttella maculipennis*[8].

Works have been continued also on the toxicology of the derivatives of nereistoxin by the author and his co-workers. The results revealed that 1, 3-di(thiocyanato)-2-(*N*, *N*-dimethylamino) propane[9] and 1, 3-bis (carbamoylthio)-2-(*N*, *N*-dimethyl amino) propane[10] were practically effective to control rice stem borers, citrus leaf miners, *Phyllocnistis citriculus*, and some other pest insects.

From the recent works in the author's laboratory, an interesting result was obtained on the bilogical conversion of one of the derivatives, the dithiocyanato compound. Namely, it was shown the derivative was converted to nereistoxin through the reaction with sulfhydryl radicals in animal and plant tissues[11]. This conversion seems to us to be an "activation", because the derivative itself does not antagonize the action of acetylcholine (Ach) which contract the isolated rectus abdominis muscle of frog, unless the derivatve was appllied after the incubation with cysteine (Sakai, unpublished data ; ref. Sakai[12]). Therefore, to study

the mechanism of insecticidal action of nereistoxin is important not only for the demonstration of the toxicological properties of this compound itself, but also for the acquirement of a generalized theory of the insecticidal mechanism of the derivatives.

In a previous report of this series[13], it was suggested that the mechanism of the insecticidal action of nereistoxin was a particular one which was regarded to be much dissimilar to those of common insecticides such as chlorinated hydrocarbons and organophosphates. This possibility was obtained from the observation on the symptoms of nereistoxin-poisoned insects; the finding was that the symptoms were not in a similar aspect to those of the common insecticides.

In a further investigation, it was revealed that the anti-cholinesterase activity of nereistoxin was not active to the extent being lethal to the insects[14].

Only one approach to reveal the mechanism of the insecticidal action of nereistoxin is the finding that the toxin blocks competitively the Ach-receptor in the rectus abdomines muscle of frog rendering the muscle inexcitable to externally applied Ach[12].

Consequently, this antagonistic action in the receptor site suggests that nereistoxin may suppress the activity of cholinergic synapses in insects of which existence in the insect central nervous system has been supported by many investigations (ref. Yamasaki and Narahashi[15], and Colhoun[16]). On the other hand, if nereistoxin blocks cholinergic synapses selectively, the insect peripheral neuromuscular junction would not be a site of action of the toxin, because evidences have been presented to show the muscle is not innervated by cholinergic nerve endings[16~23].

The present study has been conducted with the special references to the effect of nereistoxin on the nervous conduction of the American cockroach, *Periplaneta americana*, and showed that nereistoxin blocks the ganglionic transmission by its specific mechanism.

Materials and Methods

Insect Male American cockroaches were used throughout the experiments. They were reared in a room of the temperature at 28°C.

Toxicity of nereistoxin To test the toxicity of the toxin to the cockroach, the solution of nereistoxin prepared with a Ringer solution was injected into the abdominal part of the insect. The volume of the injected solution was 1.2 μl/insect. Ten insects were applied by a single dose, and they were kept in a glass jar supplied with water.

Nerve preparations In the first series of the experiments, a prepartion of the central nervous system which included the cercus, the cercal nerve, the abdominal nerve cord and the third thoracic ganglion was isolated from the cockroach. The preparation was mounted on electrodes which were made of silver wire (0.2 mm in the diameter). Postsynaptic responses were recorded from the connectives between the fifth and the sixth abdominal ganglion with a pair of the electrode. Presynaptic responses were recorded from the cercal nerve just before entering the sixth abdominal ganglion with a pair of the electrode.

Electrical stimuli were delivered from a pair of the electrode touched on the peripheral end of the cercal nerve.

For the observation of axonal conduction, the connectives between the fifth and the sixth abdominal ganglion were stimulated, and the action potentials were recorded from the connectives between the fourth and the fifth abdominal ganglions. In this experiment, the cercal nerve was not included in the preparation.

As a neuromuscular preparation, the head, the wings, the other legs and the intestine were removed from the cockroach, and the insect was pinned on a dissecting tray with its ventral side uppermost. The thoracic and the abdominal nerve-cords were exposed by removing the overlying cuticle. The right hind-leg was fixed on the tray with pins. The crural nerve running from the third thoracic ganglion (nerve no. 5) was hooked by a pair of the electrode for the stimulation, and a pair of the electrode was inserted into the femur muscle through small holes on the cuticle for the recording of the action potentials of the muscle. Most part of the cuticle on the ventral side of the coxa and a part of that of the femur were removed off for the application of nereistoxin.

For the recording of the potentials in the sixth abdominal ganglion, a different electrode was in contact with the dorsal side of the ganglion and an indifferent electrode was in contact with the third thoracic ganglion which had been crushed by forceps to make the recorded potentials monophasic. This method has been proved to be an excellent technique for the recording of the synaptic potentials ; the resting potentials and the excitatory postsynaptic potentials[15,24,25].

Stimulation and recording For the stimulation, square pulses having the duration of 0.3 msec were applied to the nerve cords. A CR-coupled amplifier (UB-203B, San'ei Instrument Co.) of which time constant was adjusted at 0.05 sec was used for the recordings.

Ringer solution and drugs The Ringer solution used contained 159.6mM Na^+, 3.1mM K^+, 1.8mM Ca^{++}, 160.1mM Cl^-, 0.2mM $H_2PO_4^-$, and 1.8mM HPO_4^{--}, and showed pH of 7.2. Nereistoxin used was the synthesized hydrogen oxalate. In some of the experiments, eserine salicylate and nicotine sulfate were tested for the comparison to nereistoxin. The drugs were dissolved in the Ringer solution, and the solutions were applied onto a certain part of the nerve or the muscle preparation with the aid of an injection needle attatched to an syringe.

Temperature All experiments were held in the room of the temperature at 25°±1°C.

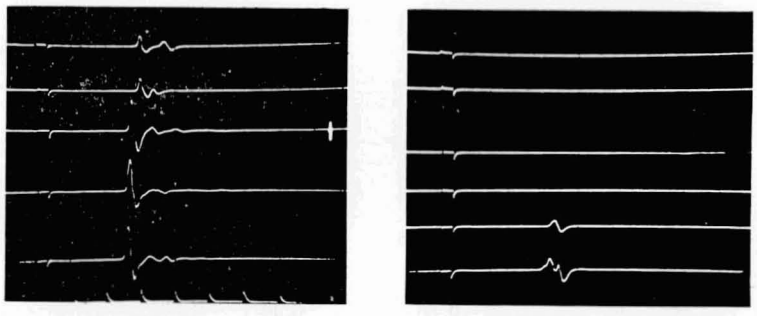
Results

Toxicity of nereistoxin to the cockroach The result of injection test is shown in Table 1. All dosages tested were effective to knock down the

Table 1. Toxicity of nereistoxin hydrogen oxalate to the adult male American cockroach, *Periplaneta americana*, in the injection test. 25°C.

Dose (μg/g)	Number* of knocked down insects at various times after the injection (min.)							
	5	30	60	90	120	180	18hr	72hr
Control	0-0	0-0	0-0	0-0	0-0	0-0	0-0	0-0
50	10-0	10-0	10-0	10-0	10-0	10-0	6-4	5-3
12.5	10-0	10-0	10 0	10-0	10-0	6-4	2-3	2-0
3.13	10-0	10-0	9-1	9-1	6-4	4-5	0-0	0-0
1.56	6-0	8-0	4-4	2-5	2-2	0-0	0-0	0-0

* Numerals on the left side of bars are the number of completely immobilized insects. On the right side, the number of sluggish insects. Ten insects were treated by a single dose.



a b

Fig. 1. Effect of nereistoxin hydrogen oxalate on the postsynaptic response elicited by the stimulation of the cercal nerve in the isolated nerve of American cockroach, *Periplaneta americana*. Nereistoxin : 2×10^{-5} M. 25°C. a: normal (before treatment), b: 4~5min. after the treatment. Stimulus strength increased from top to bottom: 0.43 (threshold in the normal), 0.50, 0.60, 0.80 and 0.90V. Duration of the stimuli: 0.3 msec. Voltage calibration, 2mV. Time marker, 1,000c/sec.

Table 2. Effect of nereistoxin hydrogen oxalate on the threshold of postsynaptic response in the isolated central nervous system of American cockroach, *Periplaneta americana*. 25°C. The cercal nerve was stimulated (duration 0.3 msec). The action potentials were recorded from the abdominal nerve cord.

Concentration of nereistoxin (M)	Time after treatment (min.)	Threshold (Stimulus strength, V) Preparation no. 1	2	3	4
5×10^{-4}	before	0.32-0.25	0.37-0.39		
	2	0.28	0.50		
	5	0.34	0.50		
	10	0.44	0.47		
	15	S.T.*	0.46		
	20	>2.0	S.T.		
	25	>2.0	1.5		
	30	—	2.0		
	35	—	2.0		
	40	2.0	—		
2×10^{-4}	before	0.25-0.26	0.35-0.38	0.49-0.52	
	2	0.36	0.50	0.69	
	5	0.45	0.48	0.80	
	10	0.42	0.45	S.T.	
	15	0.55	0.42	S.T.	
	20	0.50	0.58	0.78	
	25	0.53	0.64	S.T.	
	30	0.55	S.T.	S.T.	
	35	0.57	—	—	
	40	0.76	0.73	>2.0	
1×10^{-4}	before	0.44-0.45	0.35	0.38-0.39	0.38-0.40
	2	0.62	0.42	0.54	0.44
	5	0.63	0.43	0.59	S.T.
	10	0.50	0.49	0.56	S.T.
	15	0.48	0.50	S.T.	2.0
	20	0.55	0.51	S.T.	2.0
	25	0.68	0.55	S.T.	2.0
	35	0.71	S.T.		
	40	0.73	0.59		
5×10^{-5}	before	0.29-0.31	0.29-0.33		
	2	0.40	0.38		
	5	0.42	0.47		
	10	0.41	0.46		
	15	0.42	—		
	20	0.43	0.49		
	25	0.45	0.49		
	30	—	0.51		
2×10^{-5}	before	0.43-0.45	0.33-0.35		
	2	0.51	0.38		
	5	0.80	0.40		
	10	0.80	0.40		
	15	0.75	0.51		
	20	0.76	0.61		
	25	0.77	0.66		
	30	0.79	0.68		
	35	0.60	0.70		
	40	0.69	0.74		
	45	0.81	0.74		
	50	0.81	0.84		

1×10^{-5}	before	0.25-0.27	0.22-0.25	0.64-0.65	0.28-0.31
	2	0.24	0.24	0.76	0.50
	5	0.34	0.28	0.86	1.0
	10	0.51	0.32	0.90	1.1
	20	0.84	0.41	1.0	1.1
	25	0.90	—	1.1	1.2
	30	0.90	—	1.3	—
	35	0.91	—	1.3	—
	40	0.91	—	1.3	—
	45	1.0	—	1.5	—
	50	1.1	—	1.4	—
4×10^{-6}	before	0.75-0.80			
	2	0.75			
	5	0.90			
	10	1.1			
	15	1.2			
	20	1.2			
	25	1.3			
	30	1.5			
	35	1.5			
	40	1.8			
	45	2.0			
	50	2.0			
2×10^{-6}	before	0.53-0.55	0.30-0.31		
	2	0.45	0.31		
	5	0.48	0.32		
	10	0.52	0.32		
	15	0.57	0.35		
	20	0.71	0.38		
	25	0.73	0.37		
	30	0.79	0.41		
	35	0.79	0.45		
	40	0.81	0.46		
	45	0.81	0.45		
	50	0.91	0.57		
2×10^{-7}	before	0.50-0.51	0.41-0.43		
	2	0.52	0.41		
	5	0.53	0.43		
	10	0.51	0.43		
	15	0.51	0.42		
	20	0.50	0.42		
	25	0.55	0.48		
	30	0.58	0.45		
	35	0.56	0.50		
	40	0.59	0.51		
	45	0.59	0.57		
4×10^{-8}	before	0.33-0.34			
	2	0.33			
	10	0.34			
	20	0.31			
	30	0.31			
	40	0.33			
	50	0.34			

* Spike trains appeared.

cockroaches. The dosage of 1.56 μg/g was effective to knock down 80% of the individuals at 60~80 min. after the treatment, but the poisoned insects completely recovered after 18 hr. All insects injected by the dosages of more than 3.13 μg/g of nereistoxin were knocked down, but the lethal effect was not complete even with 50 μg/g. The result showed that the minimum effective concentration of nereistoxin in the insect body is calculated as approximately 1×10^{-5}M (2.5 μg/g). Therefore, in order that a certain site is confirmed as the site of action of nereistoxin, it must be required that the function of the site is disordered by nereistoxin of 1×10^{-5}M or of lower concentrations.

Effect on the nervous conduction in the abdominal nerve preparations The postsynaptic response elicited by the stimulation of the cercal nerve was suppressed by nereistoxin applied on the sixth abdominal ganglion. As shown in Fig. 1, it was noted that the response was not completely depressed. Increase of the stimulus strengh caused the reappearance of the postsynaptic response. Namely, the strength of the threshold stimulation for the postsynaptic potential increased with the application of nereistoxin. This sequence is shown in Table 2. The result showed the variation of the threshold in the preparations applied by different concentrations of nereistoxin.

The increase of the threshold was observed still in the preparation applied by 2×10^{-6} M nereistoxin, but the effect was not clear at 2×10^{-7}M. The application of 4×10^{-8}M did not affect the nervous conduction.

When the concentration of the applied nereistoxin was higher than 1×10^{-4} M, frequently the burst of the spontaneous discharges appeared (Table 2 and Fig. 2). The amplitude and the frequency of the discharges gradually increased forming spike trains. Finally, the conduction was suppressed (Table 2).

In the next experiment, the site where the depression of the conduction occured was investigated. The peripheral end of the cercal nerve was stimulated, and the presynaptic potentials and the corresponding postsynaptic potentials were recorded simultaneously.

As clearly appeared in the records in Fig. 3,

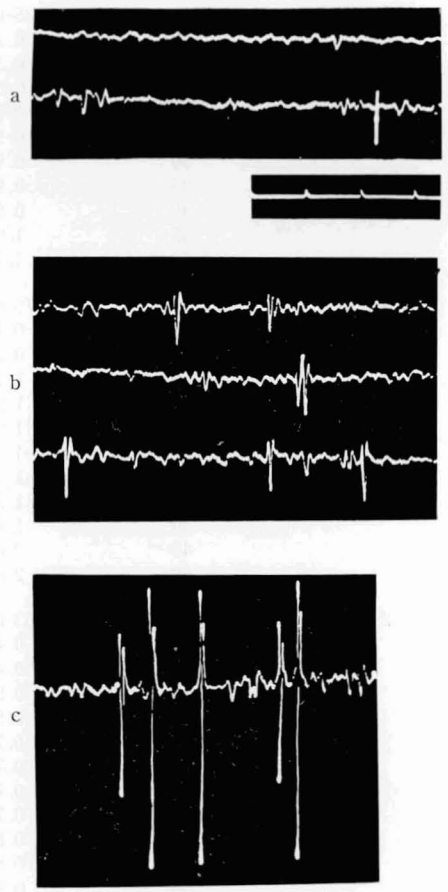


Fig. 2. Effect of nereistoxin hydrogen oxalate of a high concentration (2×10^{-4} M) on the spontaneous discharges of abdominal nerve cord of American cockroach, *Periplaneta americana*. 25°C.
a : normal (before treatment), b : 2 min. after the treatment, c : 4 min. Voltage calibration, 40 μV. Time marker; 1,000c/sec.

the presynaptic response was not affected by the application of nereistoxin even at the time when the postsynaptic response disappeared. The postsynaptic response was considerably depressed and only apperared by a stronger stimulus.

In the experiment with the abdominal nerve cord, no effects of nereistoxin were observed on the axonal conduction (Fig. 4).

The results described above apparently show that the depression of the nervous conduction in the cockroach central nerveous system treated by nereistoxin occured at the sixth abdominal

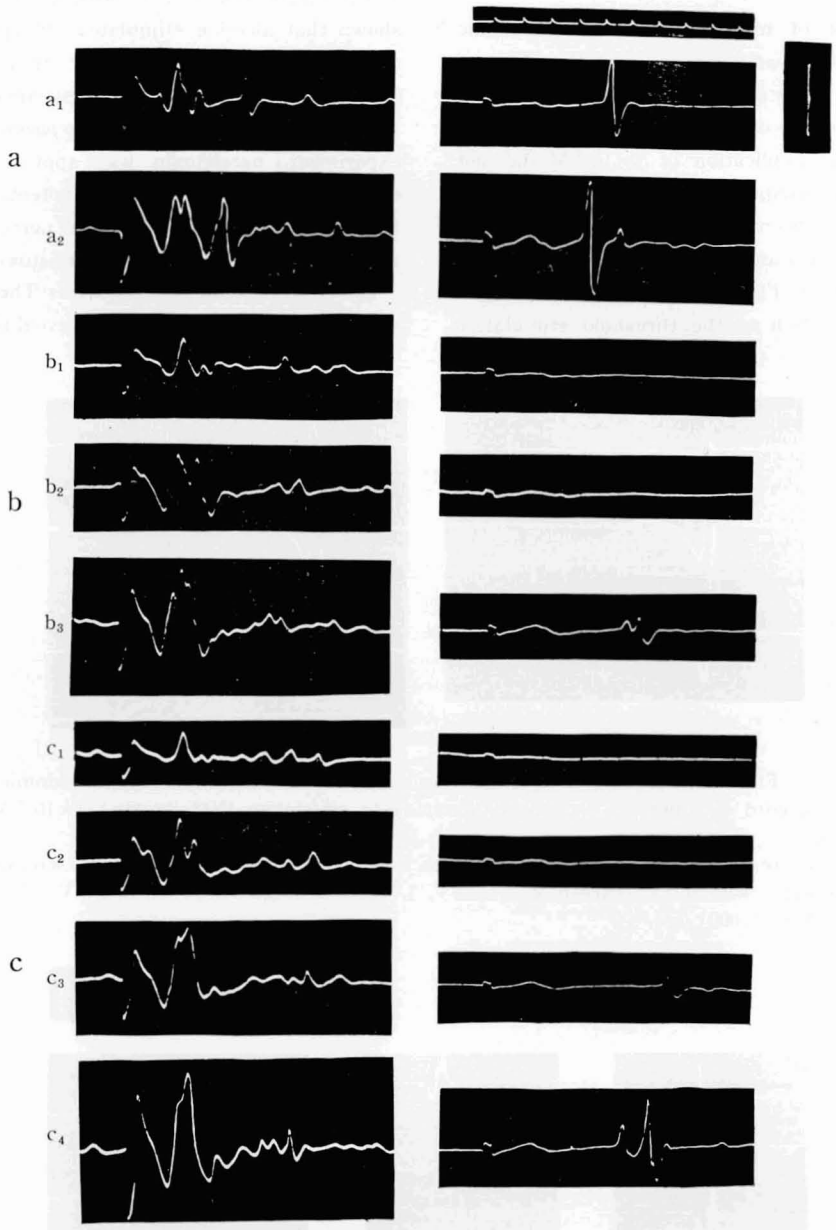


Fig. 3. Effect of nereistoxin hydrogen oxalate on the presynaptic (cercal nerve) potential (right) and the corresponding postsynaptic (abdominal nerve cord) potential (left) in the isolated nerve of American cockroach, *Periplaneta americana*. 25°C. Nereistoxin: 2×10^{-5}M. The cercal nerve was stimulated (duration, 0.3 msec). The responses were recorded simulataneously.
a: normal; a_1: stimulus strength, 0.28 V (threshold of the postsynaptic response), a_2: 0.55 V. b: 2~4 min. after the treatment; b_1: 0.28 V, b_2: 0.55 V, b_3: 1.1 V. c: 20~23 min; c_1: 0.28 V, c_2: 0.55 V, c_3: 1.1 V, c_4: 2.1 V. Voltage calibration, 1 mV. Time marker, 1,000 c/sec.

ganglion.

The effect of nicotine, a potent ganglionic blocking agent, was compared with the effect of nereistoxin in the synaptic pathway. This drug showed somewhat different action on the nervous system. The application of 1×10^{-6} M did not affect the postsynaptic potentials. The application of a higher concentration suddenly elicited the burst of the spontaneous discharges, then blocked the transmission (Fig. 5). Table 3 shows the result of the observation on the threshold stimulation in the preparation treated with nicotine. It was shown that nicotine stimulated the spontaneous discharge initially, and after that suddenly depressed the response to the presynaptic stimulus.

Effect on the neuromuscular preparation In this experiment, nereistoxin was applied onto the exposed leg muscles. The action potentials elicited by the stimulation of the crural nerve were not affected by the application of nereistoxin (Fig. 6).

Effect on the synaptic potentials The following experiments were conducted to reveal the mecha-

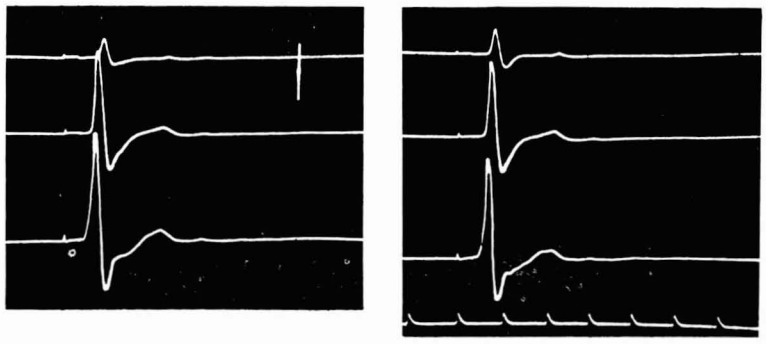


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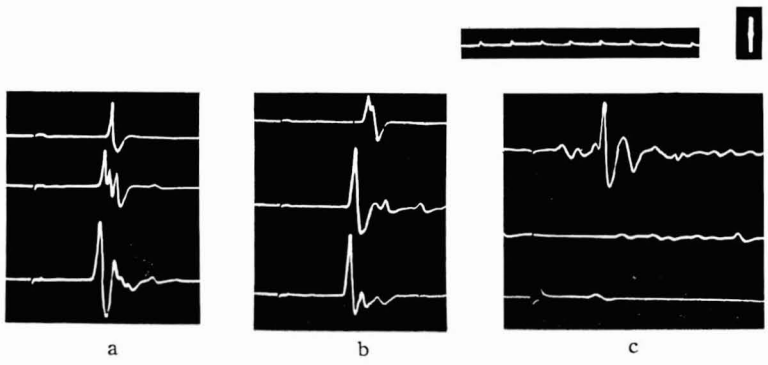
Fig. 4. Effect of nereistoxin hydrogen oxalate on the axonal conduction of abdominal nerve cord of American cockroach, *Periplaneta americana*. Nereistoxin : 4×10^{-5} M. 25°C.
a: before treatment. b: 20 min. after the treatment. From top to bottom, stimulus strength was 0.3 V (threshold). 0.38 V. 1.52 V. Voltage calibration, 2 mV. Time marker, 1,000 c/sec.



a b c

Fig. 5. Effect of nicotine sulfate on the postsynaptic response elicited by the stimulation of the cercal nerve in the isolated nerve of American cockroach, *Periplaneta americana*. 25°C.
a: normal (before treatment) from top to bottom, stimulus strength: 0.50 (threshold), 0.60, 0.70 V. b: 15 min after the treatment of 1×10^{-5} M; from top to bottom, stimulus strength : 0.50, 0.60, 0.70 V. c : after 19 min. from the application of 1×10^{-5} M, 8×10^{-5} M was applied. Top : 1 min., 0.50 V Middle : 2.5 min., 0.50 V. Bottom : 3 min., 1.5 V. Voltage calibration, 1mV, time marker, 1,000 c/sec.

Table 3. Effect of nicotine sulfate on the threshold of postsynaptic response in the isolated central nervous system of American cockroach, *Periplaneta americana*. 25°C. Methods are same to Table 2.

Concentration of nicotine (M)	Time after treatment (min.)	Threshold (Stimulus strength, V) Preparation no. 1	2	3
8×10^{-5}	before	0.34–0.36	0.55	0.28–0.30
	2	S. T. *	0.53	S. T., 0.52
	5	>1.0	S. T.	>1.0
	10	>2.0	>2.0	>1.0
	15	—	—	>2.0
1×10^{-5}	before	0.50	0.32–0.35	0.23
	2	0.50	0.33	0.23
	5	0.50	0.35	0.24
	10	0.48	0.32	0.25
	20	0.47	0.32	0.22
	30	0.49	0.34	0.23
	40	0.45	>1.0	>0.5
1×10^{-6}	before	0.29	0.46–0.48	
	2	0.30	0.47	
	10	0.27	0.47	
	20	0.28	0.46	
	40	0.26	0.47	

* Spike trains appeared.

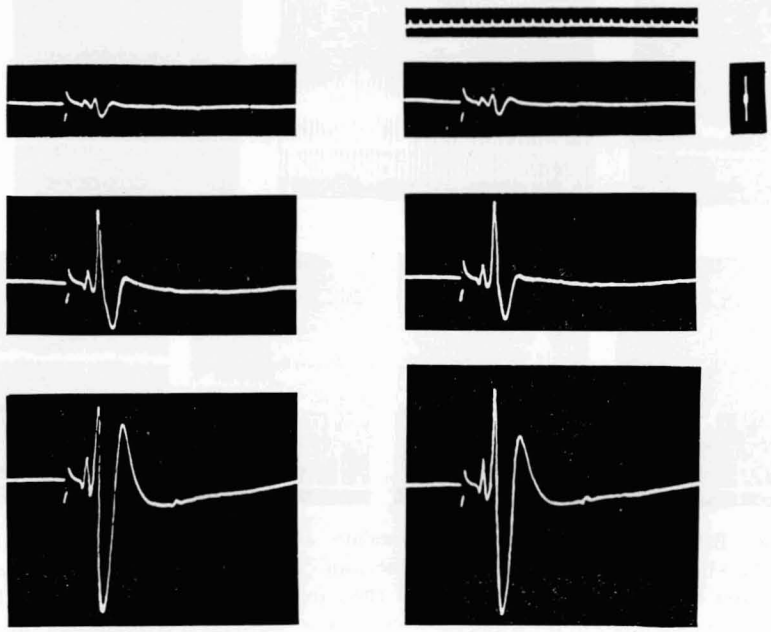


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Fig. 6. Effect of nereistoxin hydrogen oxalate on the action potentials of the femur muscle of American cockroach, *Periplaneta americana*. Nerve no. 5 was stimulated (duration 0.3 msec). Nereistoxin: 2×10^{-4} M. 25°C.
a : before treatment; b : 20 min. after the treatment. From top to bottom, stimulus strength increased; 0.18, (threshold), 0.20, 0.25 V. Voltage calibration, 1 mV. Time marker, 1,000 c/sec.

nism of the ganglionic blocking action of nereistoxin. As comparison the actions of nicotine and eserine were investigated at the same time. The cercal nerve was stimulated at the frequency of 0.5 c/sec. The potentials recorded with the aid of an electrode being contact with the sixth abdominal ganglion were composed of the resting potential, the excitatory postsynaptic potential (e. p. s. p.) and the action potentials of the axonal conduction [15,24,25]. The resting potential was observed with the aid of a base line which was also employed for the recording of time markers (Fig. 7). The e. p. s. p.'s were recorded as slow potentials, and the action potentials were fast one which superimposed on the e. p. s. p.'s.

The result shown in Fig. 7. revealed that in the preparation treated by nereistoxin, the e. p. s. p. diminished, and in parallel with this change the action potentials disappeared. The application of nereistoxin did not change the resting potential. A stronger stimulus was able to produce the e. p. s. p. of which potential was enough to discharge the axonal action potentials (Fig. 7a).

However, nicotine initially accelerated the

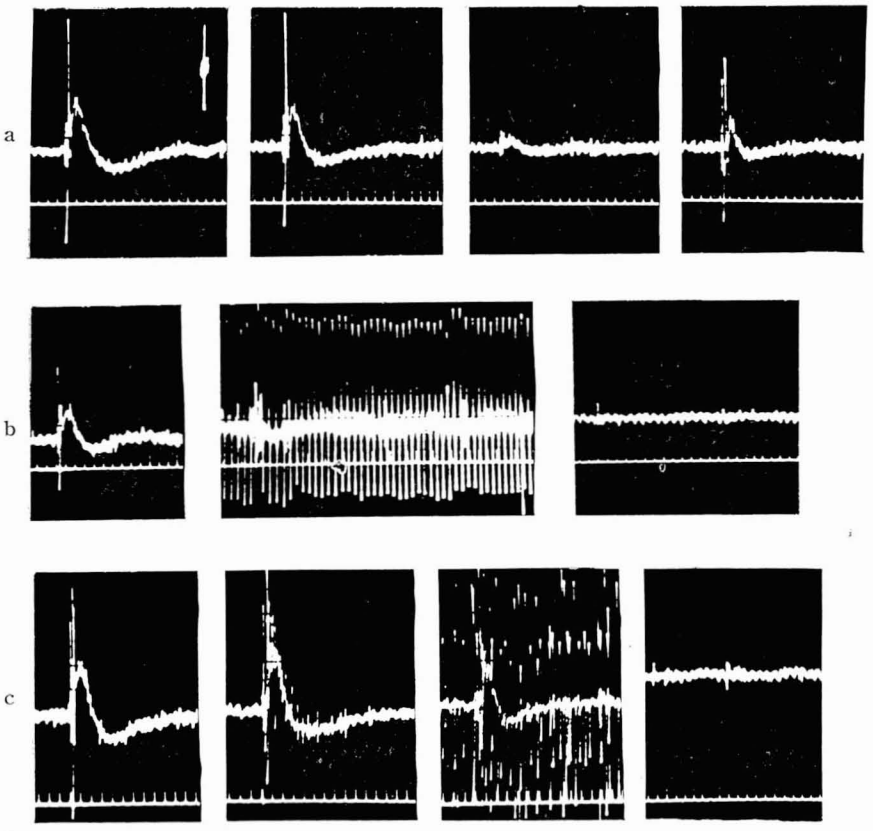

Fig. 7. Effect of nereistoxin hydrogen oxalate, nicotine sulfate and eserine salicylate on the sixth abdominal ganglion of American cockroach, *Periplaneta americana*. A different electrode was in contact with the ganglion. The recorded potentials were the action potentials in the axon, the excitatory postsynaptic potentials and the resting potential. 25°C.
a : nereistoxin 2×10^{-5} M. From left, normal (before treatment), 2 min. and 10 min. Record on the right side was obtained by a stronger stimulation at 13 min. b : nicotine 2×10^{-5} M. Normal, 11 min., 15 min. and 17 min. c: eserine 5×10^{-5} M. Normal, 24 min., 29 min. and 41 min. Lines with time markers are also adopted as base lines for the observation of the resting potential. Voltage calibration, 400 μV. Time marker, 100 c/sec.

occurance of spike discharges which gradually formed trains. After further advance of time, the resting potential was increased to an abnormal extent where the synapse no longer returned to the original reporalized phase. Then, the synaptic transmission remained blocked (Fig. 7b).

Eserine, a cholinesterase inhibitor, increased the frequency of the after-dischrges superimposed upon the e. p. s. p. With the advance of time, even without the stimulation, the discharges in the postsynaptic axons appeared to form the trains. Finally the resting potential increased to a greater extent where the response to the presynaptic stimulation ceased (Fig. 7c).

Discussion

The results of the present electrophysiological investigations revealed the ganglionic blocking action of nereistoxin in the central nervous system of the American cockroach. It is also confirmed in the experiments that the toxin does not affect the axonal conduction (Fig. 4 and Fig. 7), and the functions in the neuromuscular system of the leg. (Fig. 6).

It has been known that some of insecticidal compounds affect the synaptic transmission in insects with their particular ways respectively. Namely, DDT facilitates the synaptic transmission [26~30]. Gamma-BHC elicites the increase of the after-discharges in the postsynaptic response[31~36]. Anti-cholinesterases, such as organophosphates, block the synaptic transmission by their esterase-inhibitory action which renders the accumulation of the transmitter substance[37~39]. The action of nicotine in insects has been believed to be synaptic block by its occupation of Ach-receptors as well as in vertebrates[37,38,40]. Indeed, the present experiments (Table 3, Fig. 5 and Fig. 7) showed the block by this insecticide was proceeded by the increase of the nervous discharges; in other words, the block by nicotine is thought to be the result of the abnormally augmented deporalization of the postsynaptic membrane.

Comparing nereistoxin with these insecticidal compounds in the action to the synaptic transmission, it is evident that the mode and the mechanism of the dlocking action of nereistoxin is much different from the others.

As shown in Table 1, Figs. 1 and 7, the effect of nereistoxin appeared as the increase of the threshold of the postsynaptic response. The result obtained in the experiment recording the ganglionic potentials revealed that nereistoxin acts to reduce the e. p. s. p. without any effect on the membrane resting potential (Fig. 7a). The diminished e.p.s.p. should be unable to produce impulses which excite the postsynaptic nerve cords. However, the experiment apparently showed that a stronger stimulus was effective to set up the e. p. s. p. of which intensity was enough for the deporalization of the axonal membrane to discharge the action potentials (Fig. 7a).

All these features in the synaptic events would be safely explained by such a way that nereistoxin occupies the postsynaptic membrane without deporalizing it, because nereistoxin did not increase the resting potential (Fig. 7a). It can be estimated that the occupation by nereistoxin can overcome the activity of transmitter substance which normally deporalize the postsynaptic membrane to manifest the e. p. s. p., when the presynaptic stimulus is of a low intensity. However, a stronger stimulation should liberate much amount of the transmitter with which nereistoxin is no longer able to compete in the occupation.

In the isolated rectus abdominis muscle of frog, it has been pointed out by the author that nereistoxin blocks the Ach-receptor by the competition with Ach[12]. The higher the concentration of applied Ach is, the suppressive effect of nereistoxin on the contraction of the muscle is the lower.

At the present time, although the nature of the synaptic transmitter substance in the insect central nervous system is not yet determined firmly, many investigations have presented the results which confirmatively approve the presence of the cholinergic transmission in the ganglion (ref. Yamasaki and Narahashi[15] andColhoun[16]). In this respect, it is noticeable that nereistoxin which blocks the cholinergic system of the frog rectus abdominis muscle also excerts a blocking action on the insect ganglion. This is to say, the ganglionic blocking action of nereistoxin supplies an eveidence that the transmitter substance in the insect ganglion is Ach.

Accordingly, it must be a matter of course that nereistoxin does not block the neuromuscular transmission in the crural muscle of the cockroach, since the muscle is regarded to be innervated by non-cholinergic nerve-endings[16~20]. Furthermore, recent works by Kerkut and his co-workers have shown the possibility that L-glutamic acid is the transmitter[21~23].

As mentioned before, the potentials led off from the last abdominal ganglion clearly demonstrated the difference of the mechanism of the blocking action between nereistoxin, nicotine and eserine. The final action of the latter two drugs is the ganglionic blocking, though of cours the mechanism is different each other. Nicotine deporalizes the postsynaptic membrane acting directly to the membrane, and as the results the membrane becomes to a deporalized phase where the action of the transmitter substance cannot produce the e. p. s. p. Eserine is a cholinesterase inhibitor, and induces the accumulation of the transmitter substance which magnifies the membrane potential of the synapse to a greater extent. These two drugs act substantially to block the synaptic transmission non-competitively, but on the contrary nereistoxin blocks it competitively with the transmitter without deporalizing the synaptic membrane.

Although some of the synaptic blocking agents are commonly known to be active against vertebrates, these are inactive against insects. D-tubocurarine applied on the cockroach ganglion did not affect the ganglionic transmission[41]. Hopf[42] injected glasshoppers with d-tubocurarine, atropine, etc., and O'Brien and Fisher[43] treated several species of insects with the blocking agents. However, the drugs were poorly insecticidal. Accordingly, it would be able to say that nereistoxin is the firstly found competitive ganglionic blocking agent which is lethal to insects.

Nereistoxin of a high concentration elicited the burst of discharges in the axonal conduction (Fig. 2). However, further investigations must be required to elucidate the mechanism of this action.

For leading off the conclusion that the ganglionic blocking action is the mechanism of the insecticidal action of nereistoxin, it is necessary to prove the parallelism between the concentration to block the synaptic transmission in the isolated nerve preparation and the dosage to knock down the insects.

In the injection experiments (Table 1) the minimum dosage of nereistoxin to knock down or paralyse the insect was 3.13μg/g. Because it is reasonable to assume that the injected nereistoxin distributed homogeneously all over the insect body, the body concentration to paralyse the insect is calculated as approximately 1×10^{-5} M. As shown in Table 2, the treatment of the nerve preparation with 1×10^{-5}M brings steady block of the transmission, and moreover even 2×10^{-6} M nereistoxin still suppressed the transmission.

Therefore, basing upon the parallelism discussed above, it can be safely concluded that nereistoxin paralyses the insect by its ganglionic blocking action on the central nervous system.

Summary

The effect of nereistoxin hydrogen oxalate on the nervous conduction in the adult male American cockroach, *Periplaneta americana*, was investigated to reveal the mechanism of the insecticidal action.

The postsynaptic response across the sixth abdominal ganglion was considerably suppressed by nereistoxin at the concentration of more than 2×10^{-6} M. On the other hand, the axonal conduction in the cercal and the abdominal nerve cords was not affected by the toxin. The blocking action of nereistoxin appeared in the increase of the intensity of the threshold stimulation applied on the presynaptic (cercal) nerve. Of the synaptic potentials led off from the sixth abdominal ganglion, the excitatory postsynaptic potential (e. p. s. p.) was reduced to a extent which does not discharge the axonal action potentials. The resting potential of the synapse was not affected by nereistoxin.

On the contrary, nicotine and eserine augmented the resting potential rendering the synaptic membrane not to be deporalized by the transmitter. The fact that a strong stimulation was effective to discharge the e. p. s. p. in the nereistoxin-treated ganglion revealed the block by nereistoxin which was competitive with the transmitter.

The neuromuscular transmission, or the muscular contracture in the crural muscle was not affected by nereistoxin.

From these results, it was safely concluded that

nereistoxin paralysed the insect with its ganglionic blocking action on the central nervous system.

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