INVOLVEMENT OF CHOLINERGIC MECHANISM IN THE ACTION OF ALLETHRIN IN INSECT CNS

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Abstract.....Allethrin had a stimulating action on spontaneous discharges in the cockroach sixth abdominal ganglion superfused with an insect saline solution. This action at a low concentration (5×10^{-8} M) of allethrin was abolished by either of d-tubocurarine, hexamethonium or atropine at 5×10^{-4} M. It was also abolished by the treatment of ganglia with hemicholinium-3 or by low-calcium-high magnesium insect saline solution. However, treatment of these blocked ganglia with allethrin at more than 5×10^{-7} M overcame the block, producing increased spontaneous activity. Allethrin had no effect on insect cholinesterase activity.

These results may suggest that the stimulating action at a low concentration of allethrin may be mediated by the release of ACh from cholinergic terminals in ganglia.

Key words; pyrethroid, acetylcholine, ganglion, cockroach.

INTRODUCTION

Allethrin is a potent neurotoxic insecticide, which chemically belongs to pyrethroids. The action of the pyrethroid has been extensively studied on giant axons of the cockroach by Narahashi et al. (1965), who observed an increase in and a prolongation of the depolarizing (negative) afterpotential and a train of repetitive activity. At higher concentrations, the action potential was reduced in amplitude and eventually blocked. However, recent studies (Burt and Goodchild, 1971; Gamougis, 1973; Narahashi et al., 1977; Wouters and van den Bercken, 1978) have suggested that the pyrethroid acts on synaptic junctions in central nervous system (CNS) rather than axons. Biochemical studies (Desai et al., 1975; Schneider, 1975) have revealed that DDT and some pyrethroids are capable of inhibiting Na^+ , K^+ and Mg^{2+} -ATPase from insect nervous tissue. The inhibition of the enzyme in presynaptic membranes would enhance the release of transmitter (Vizi, 1978). ACh has been postulated as an excitatory transmitter in

insect CNS (Pichon and Callec, 1970 : Gerschenfeld, 1973 : Shankland et al., 1971). But the ACh released or accumulated by various insecticides has been suggested to act an endogenous toxic substance (Tobias et al., 1946 : Shankland, 1978).

In this report, the possible involvement of cholinergic mechanism in the action of allethrin in insect CNS was studied.

MATERIALS and METHODS

The excised abdominal nerve cord of the male adult cockroach *Periplaneta americana* L. was used, since Burt and Goodchild (1971) reported that changes in spontaneous discharges in the sixth abdominal ganglion closely paralleled with the time course of allethrin poisoning in the cockroach.

Recording of spontaneous discharges in ganglia.

The nerve chamber used is diagrammatically shown in Fig. 1. It is made of acrylic

Fig. 1. Schematic diagram illustrating the principle of the recording techniques; The sixth abdominal ganglion was continuously superfused with an insect saline and the potential difference (spontaneous discharges) between the ganglion (A6) and the anterior nerve cord were recorded. For the monitor of synaptic transmission the cercal nerves were stimulated.

resin and consist of three compartments. The cercal nerves were mounted on a pair of a platinum wire stimulation electrode equipped in the left compartment. The sixth abdominal ganglion was placed in the central compartment (1.5 mm in diameter) equipped with an inlet and an outlet for perfusing medium and in contact with the one of a pair of an Ag-AgCl wire recording electrode. The other electrode was in contact with the anterior nerve cord in the right compartment. A small groove made on the top of each partition wall for the nerve to pass over was sealed with vaseline. The both side compartments were moistened with an insect saline solution and covered by a glass plate to prevent the nerve from drying up. The spontaneous discharges were displayed on a

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cathode ray oscilloscope (Nihonkohden VC-8) via a preamplifier (Nihonkohden AVB-8) and continuously recorded by Nihonkohden oscilloscopic camera. The spontaneous discharges were maintained fairly constantly for 4 hours or longer.

The penetration of various anticholinergic drugs into ganglia was examined by testing their effect on transmission across the cercal nerve-giant fiber synapses. When the cercal nerves were stimulated with 0.01 msec and supramaximal voltage, the compound action potentials of the cercal nerves (Pre-fiber, about 1 mV in amplitude) and of the giant fiber (post-fiber, about 10 mV in amplitude) were observed.

Application of drugs.

Drugs were dissolved in an insect saline solution (Pichon and Callec, 1970) for the superfusion of ganglia. Allethrin was dissolved in ethanol at 10^{-2} M as stock solution and diluted with the insect saline solution before use.

Insect saline solution used was composed of 210 mM NaCl : 3.1 mM KCl : 1.8 mM CaCl_2 : 1.8 mM NaH_2PO_4 : 0.2 mM Na_2HPO_4 : 20 mM glucose. The composition of a low calcium-high magnesium saline solution used was the same as the insect saline solution except that it contained 0.18 mM CaCl_2 and 10 mM MgCl_2 .

Assay of acetylcholinesterase activity.

Ten-twenty cockroach abdominal nerve cords were homogenized in 400 volumes of 0.1 M phosphate buffer (pH, 7.2) and used as an enzyme preparation. The assay of the enzyme activity was performed by the colorimetric method of Ellman et al. (1961). The result was expressed as acetylthiocholine hydrolysed / min / nerve cord.

RESULTS

Fig. 2 shows the effect of 5×10^{-8} M allethrin on spontaneous discharges in the cockroach sixth abdominal ganglion irrigated continuously with an insect saline solution. The spontaneous discharges in control were in the order of 30–60 μV in amplitude and of 3–60 in frequency per second. Allethrin substantially increased the spontaneous discharges more than double in the frequency and up to more than 100 μV in amplitude, sometimes the bursts of spikes appeared, whereas at less than 10^{-8} M it had a slight or no effect. According to the criteria of Shankland (1978), the spontaneous activity in control was mostly in non-cholinergic small fibers, but allethrin appeared to stimulate both non-cholinergic and cholinergic fibers.

Shankland et al. (1971) reported that transmission was blocked by either drug of d-tubocurarine, bexamethonium or atropine at the cercal nerve-giant nerve fiber synapses of the cockroach. In this study, the penetration of these blockers was examined by testing their blocking action on transmission. Either drug at 5×10^{-4} M completely blocked the synaptic transmission within 60 min. In the blocked ganglia, the stimulating action of allethrin at 5×10^{-8} M on spontaneous discharges was abolished (Fig. 3). However, the cholinergic blockers used are known to have a non-cholinergic suppressive action on the excitable membranes (Yeh and Narahashi, 1974; Katz and Miledi, 1978; Colquhoun et al., 1979). Then the action of allethrin was examined on the ganglia depleted in stored ACh by repeated stimulation of the cercal nerves in the

Fig. 2. Spontaneous discharges of the cockroach sixth abdominal ganglion before and after application of allethrin.

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Fig. 3. Effect of various cholinergic blockers on the stimulating action of allethrin. Preliminary experiments have shown that either cholinergic blocker at $5 \times 10^{-4} M$ blocks completely the transmission at the cercal nerve-giant nerve fiber synapses within 60 min. Each point represents a count during a period of 1 sec.

presence of 10^{-3} M hemicholinium-3 as described by Shankland et al. (1971). In the treated ganglia, the transmission was completely abolished, but the spontaneous discharges in non-cholinergic small fibers remained in frequency almost at a previous level. Allethrin at 5×10^{-8} M was without effect (Fig. 4).

At more than 5×10^{-7} M, the action of allethrin was not abolished even with the combination of all the three cholinceptor blockers (Fig. 5). Whether this action was

Fig. 4 Effect of 5×10^{-8} M allethrin on spontaneous discharges in the cockroach sixth abdominal ganglion treated with 10^{-3} M hemicholinium. Synaptic transmission was completely abolished, but spontaneous basic activity was remained at control level. Each point represents a count during a period of 1 sec.

Fig. 5 Effect of combined three cholinceptor blockers on the stimulating action of allethrin. Each point represents a count during a period of 1 sec.

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non-cholinergic nerve fibers or not was examined. Since it has been suggested that calcium ions are necessary for the release of transmitter from presynaptic terminals and magnesium ions may compete with calcium ions to ionic channels to suppress the release of transmitter (Katz and Miledi, 1970), the effect of allethrin was examined on the ganglia superfused with a low calcium-high magnesium insect saline (Fig. 6). The frequency in spontaneous discharges in control appeared to be reduced. The action of allethrin at higher concentrations was not abolished, whereas it at 5×10^{-8} M was abolished.

Since acetylcholinesterase inhibitors have been known to have a stimulating action on the spontaneous discharges in ganglia, the effect of allethrin was examined on cholinesterase activity from insect. Allethrin was found to have no inhibitory action on cholinesterase activity from the cockroach nervous tissue (Fig. 7).

Fig. 6. Effect of a low calcium-high magnesium insect saline solution on the stimulating action of allethrin. Ten mM MgCl_2 was added and CaCl_2 was reduced to 0.18mM in an insect saline solution. Superfusion with this insect saline solution blocked completely the transmission at the cercal nerve-giant nerve fiber synapses.

Each point represents a count during a period of 1 sec.

Fig. 7 Effect of allethrin on cholinesterase activity.

The cockroach nervous tissue homogenate was used as enzyme source. The activity was assayed by the colorimetric method of Ellmann et al. (1961). The results were expressed as nmol acetylthiocholine hydrolysed/min /nerve cord. Each column and vertical bar represent the mean and SEM respectively (n=4).

DISCUSSION

Burt and Goodchild (1971) have suggested that the fetal lesions caused by pyrethrin I may be within the ganglion rather than associated with axonic conduction in the cockroach. The results presented in this study showed that the stimulating action at a low concentration of allethrin on spontaneous activity in the cockroach sixth abdominal ganglion was abolished in the presence of cholinergic blockers (Fig. 3) and by the treatment with hemicholinium-3 (Fig. 4). In addition, allethrin had no inhibiting effect on cholinesterase activity from the insect nervous tissue (Fig. 7) and no agonistic action on the *Torpedo* nicotinic ACh receptors (Abbassy et al., 1982). These findings may suggest that the stimulating effect of allethrin at a low concentration is mediated by the release of ACh from cholinergic terminals within the ganglion. The related cholinergic receptor might be a mixed type similar to that in the cercal nerve-giant nerve fiber synapses (Shankland et al., 1971). However, treatment of these blocked ganglia with allethrin at more than $5 \times 10^{-7} M$ overcame the block, producing increased spontaneous activity (Fig. 5). Furthermore, in ganglia superfused with a low calcium-high magne-

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sium insect saline solution, 5×10^{-8} M allethrin had no stimulating effect, but at more than 5×10^{-7} M it did (Fig.6). This latter action is presumably of non-specific on both cholinergic and non-cholinergic fibers. The voltage clamp studies of Narahashi et al. (1976) have shown equivocally that pyrethroids act upon axonal membranes. The present results may suggest that cholinergic terminals may be more sensitive for allethrin than the other axonal membranes and the effect of allethrin at a low concentration might be potentiated by ACh released from cholinergic terminals. Since such an indirect action at a low concentration of allethrin would interfere the basic rhythms involved in the vital movements of respiration and locomotions (Kammer, 1976), it might be an important mode of action in the allethrin poisoning in insects.

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