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Mode of Action of Pyrethroids *

T. NARAHASHI [1]

Since pyrethroids are potent neuropoisons, their mechanism of action on the nervous system can best be studied by means of electrophysiological techniques. The nerve excitation occurs as a result of changes in nerve membrane permeabilities to sodium and potassium ions, and therefore any effect of pyrethroids can be interpreted in terms of such permeabilities. Detailed analyses of the action of allethrin on the giant axons of the cockroach, the crayfish, and the squid performed by means of intracellular microelectrode and voltage clamp methods have revealed the ionic basis of the action of allethrin on the nerve.

When an insect is intoxicated with pyrethroids, it quickly develops hyperexcitation and tremors, which are followed by paralysis. These symptoms of poisoning imply that pyrethroids act primarily on the neuromuscular system. It has in fact been shown that, when applied directly on to the excised tissue, pyrethroids cause hyperexcitation and block of the nerve (Lowenstein, 1942; Welsh & Gordon, 1947; Yamasaki & Ishii, 1952).

As will be described later in this paper, the nerve excitation is not directly dependent upon the metabolic energy. Therefore, pyrethroids can be assumed to act directly on the nerve membrane where excitation takes place. As a matter of fact, no enzymatic inhibition by pyrethroids is known to account adequately for the neurotoxic action. Since the change in membrane potential is the principal parameter that can be observed during nerve excitation, the mechanism of action of pyrethroids can best be studied by means of electrophysiological techniques.

This paper describes and reviews the results of experiments conducted with the aid of intracellular microelectrode and voltage clamp techniques. It will be shown that the action of allethrin on the nerve fibre can be interpreted in terms of the permeability of nerve membranes to sodium and potassium ions.

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STUDIES WITH INTRACELLULAR MICROELECTRODES

It was not until 1962 that modern electrophysiological techniques were fully utilized for the study of the action of pyrethroids on the nerve (Narahashi, 1962a, 1962b). Three major actions of allethrin on the giant axon of the cockroach were revealed. At a low concentration (1 μM), allethrin augmented and prolonged the negative after-potential that followed the spike phase. When the temperature was relatively high (about 25°C or above), repetitive after-discharges were superimposed on the increased negative after-potential. The resting potential underwent little or no change under these conditions.

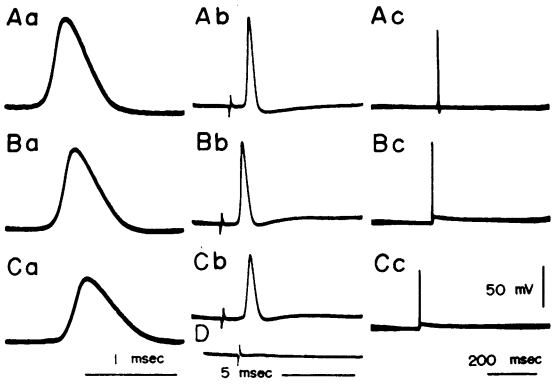
At a slightly higher concentration (3 μM), the negative after-potential was augmented and prolonged, the action potential was gradually suppressed in magnitude, and the nerve conduction was eventually blocked. An example of such an experiment is illustrated in Fig. 1. The resting potential was slightly and progressively decreased, but the depolarization was not large enough to account for the conduction block. These three actions of allethrin—i.e., the increase in negative after-potential, repetitive after-discharge, and conduction block—can adequately account for the hyperexcitation and paralysis of the poisoned insect.

MECHANISM OF NERVE EXCITATION

In order to explore the mechanism of action of pyrethroids on the nerve membrane, one has to know how the nerve excitation occurs. The nerve cell and fibre generally contain a high concentration of potassium and a relatively low concentration

Fig. 1

Action potentials recorded from a giant axon of the cockroach by means of an intracellular microelectrode *



* Aa to Ac: Control records before the application of allethrin; Ba to Bb, Ca to Cc, and D: recordings obtained 2.5, 4, and 10.5 minutes, respectively, after the application of allethrin at a concentration of 3 μM. Temperature 19.5°C. (Reproduced, by permission of the Wistar Press, from Narahashi, 1962a.)

of sodium. In the external fluid, the K/Na ratio is usually reversed. Thus, concentration gradients for sodium and potassium are established across the nerve membrane. At rest the nerve membrane is permeable to potassium, but only sparingly so to sodium and chloride. Therefore, the potential difference across the nerve membrane approaches the equilibrium potential for potassium (E_K), which is defined by the Nernst equation,

$$E_K = \frac{RT}{F} \ln \frac{[K]_o}{[K]_i} \quad (1)$$

where R, T, and F are the gas constant, the absolute temperature, and the Faraday constant, respectively, and $[K]_o$ and $[K]_i$ are the concentrations of potassium outside and inside, respectively. The values of the E_K and the resting potential are usually in the range of -50 mV to -100 mV, the inside being negative with respect to the outside.

When the resting potential decreases owing to the application of an outward current across the nerve membrane, the permeability of the latter to sodium increases rapidly, so that the membrane now becomes almost exclusively permeable to sodium. Thus, the membrane potential is allowed to approach the equilibrium potential for sodium (E_{Na}), forming the rising phase of the action potential. E_{Na} is defined by the Nernst equation,

$$E_{Na} = \frac{RT}{F} \ln \frac{[Na]_o}{[Na]_i} \quad (2)$$

where $[Na]_o$ and $[Na]_i$ represent the outside and inside sodium concentrations, respectively. Since E_{Na} is near $+50$ mV, the membrane potential is temporarily reversed in polarity during the action potential. Sodium ions enter the axon during this phase, according to the electrochemical gradient. Then the increased sodium permeability begins to decrease and the potassium permeability begins to increase beyond its resting value, so that the membrane again becomes almost exclusively permeable to potassium, bringing the membrane potential back to the original resting level. This is the falling phase of the action potential. Potassium ions leave the axon during this phase, according to the electrochemical gradient. These changes in membrane ionic conductances during the action potential are illustrated in Fig. 5.

The membrane permeability to sodium and potassium can be measured as the membrane conductance for each ion. Therefore, the sequence of events during the action potential can be summarized as follows. The increase in membrane sodium conductance (g_{Na}) is directly responsible for the rising phase of the action potential, and the decrease in g_{Na} and the increase in membrane potassium conductance (g_K) are directly responsible for the falling phase (Hodgkin, 1958). Any change in the action potential, such as a suppression of the action potential and a prolongation of its falling phase, can be explained in terms of these three conductance parameters. The mechanism whereby the increased g_{Na} is lowered towards the resting value is called " sodium inactivation ".

ROLE OF METABOLISM

It should be emphasized that the nerve excitation caused by changes in membrane ionic conductances is not directly supported by the metabolic energy. However, there is a mechanism by which the internal sodium is pumped out and the internal potassium is retained so as to maintain the ionic concentration gradients across the membrane. This activity is stimulated by an increase in the internal concentration of sodium as a result of excitation, thereby restoring the ionic concentration gradients. This mechanism is called the " sodium pump ", and its energy is supplied from high-energy phosphates such as those formed from ATP. Metabolic inhibitors block the sodium pump, but the excitability remains unimpaired as long as the ionic concentration gradients are maintained (Hodgkin & Keynes, 1955).

Depolarization and block occur only after the concentration gradients are reduced below the critical level.

VOLTAGE CLAMP

Voltage clamp is the most straightforward method for measuring the membrane ionic conductances. The concept was originally developed by Marmont (1949) and by Cole (1949), and the method was extensively and successfully used by Hodgkin, Huxley, and Katz (Hodgkin, Huxley & Katz, 1952; Hodgkin & Huxley, 1952a,b,c,d). Later, the technique was greatly improved by Moore & Cole (1963). The basic idea of voltage clamp is to measure the membrane current associated with a controlled step change in membrane potential with the aid of an electronic feedback circuit. Since the currents carried by ions through the resistance component of the membrane are much slower than the current flowing across the membrane capacity, the ionic currents can easily be measured (see Fig. 4). Moreover, the current carried by sodium ions is transient in nature, whereas the current carried by potassium ions starts more slowly and attains a steady state (see Fig. 2 and 4). Therefore the sodium and potassium currents can be measured separately. Since we can now measure the sodium and potassium currents and the membrane potential, the membrane conductances for sodium and potassium are easily calculated.

EFFECTS OF ALLETHRIN ON CONDUCTANCE INCREASE MECHANISMS

Giant axons of the squid, *Loligo pealei*, having a diameter of 400–500 μm, were used as material. Some experiments were performed with internally perfused squid giant axons. This was achieved by squeezing out the axoplasm by means of a small roller, and inflating it with artificial internal perfusates whose major component was potassium glutamate. Therefore, allethrin could be applied either to the outside or to the inside of the axon (Narahashi & Anderson, 1967).

Allethrin, applied either outside or inside in a concentration of 10–30 μM, produced effects very similar to those produced in the cockroach giant axon. The negative after-potential was increased, repetitive after-discharges occurred, and the action potential was eventually suppressed.

Fig. 2 illustrates families of membrane currents associated with various magnitudes of step depolarization from the holding membrane potential of -80 mV under voltage clamp conditions. Upon 30-mV depolarization (from -80 mV to -50 mV), a small transient inward current (downward deflection) occurred and was followed by a small steady-state outward current (upward deflection). The former is sodium current and the latter potassium current. The capacitative current was too brief to be reproduced in the figure. With increasing

Fig. 2

Membrane currents associated with step depolarizations from the holding membrane potential of –80 mV in a voltage-clamped squid giant axon before and during the external application of 10-μM allethrin, temperature=8°C*

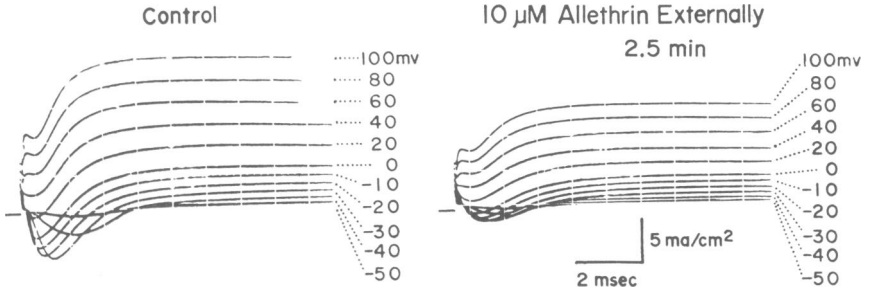


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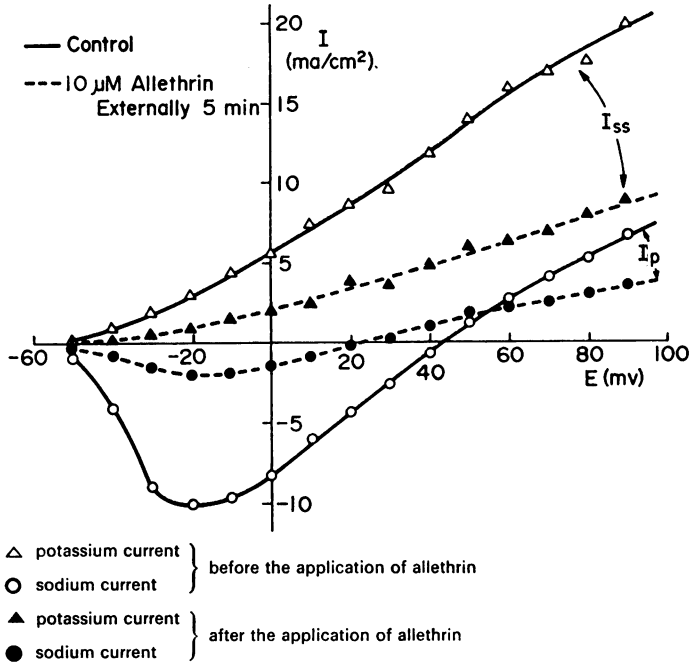
Fig. 3

Current–voltage relationships for the peak transient sodium current (I_p) and for the steady-state potassium current (I_{ss}) in a voltage-clamped squid giant axon before and during the external application of 10-μM allethrin; temperature = 8°C*



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depolarization, the peak transient sodium current and the steady-state potassium current increased in magnitude (depolarizations to −40 mV and −30 mV). With further increasing depolarizations, the peak sodium current decreased (depolarizations to −20 mV, −10 mV, and 0 mV), and finally changed its polarity (+40 mV, +60 mV, +80 mV, and +100 mV). The steady-state potassium current kept increasing with an increase in depolarization. After the application of allethrin externally at a concentration of 10 μM, both the peak sodium current and the steady-state potassium current were suppressed in magnitude (Fig. 2).

The peak amplitude of the sodium current and the steady-state amplitude of the potassium current are plotted as a function of the membrane potential in Fig. 3. Before the application of allethrin, the peak sodium current becomes zero at the membrane potential of +43 mV. This is the equilibrium potential for sodium. The potassium current increases with depolarization. After the application of allethrin, both currents are suppressed appreciably. The inhibition of the sodium current is directly responsible for the conduction block.

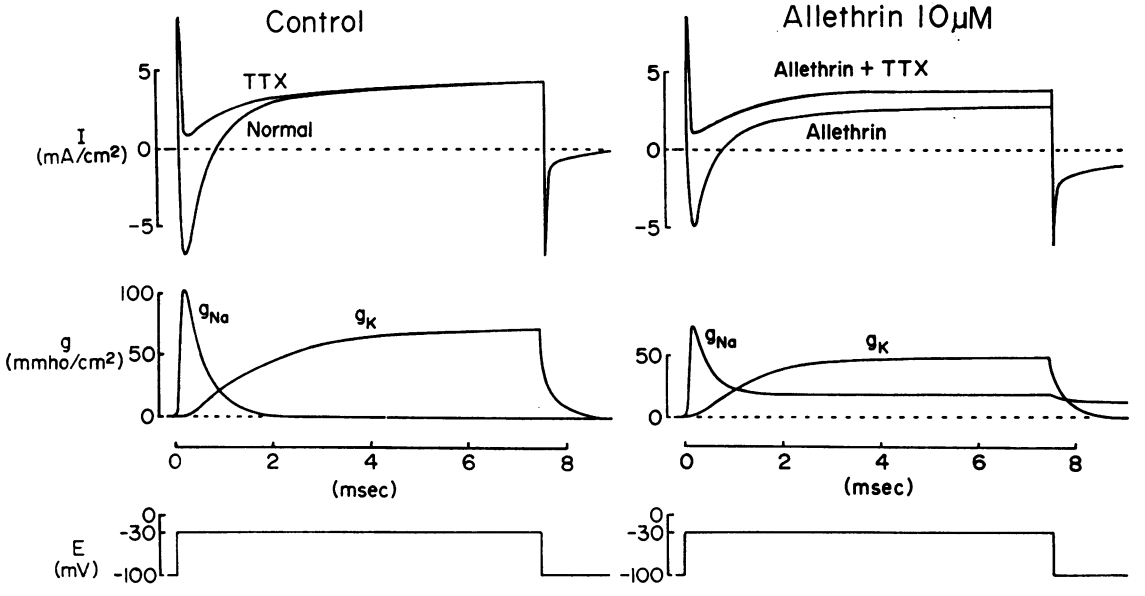
When allethrin was applied internally to the squid axon, the increase in negative after-potential was more pronounced than with external application. Under voltage clamp conditions, the falling phase of the peak sodium current was slowed. Such a prolongation of the sodium inactivation was studied in more detail with the giant axon of the crayfish, which was somewhat more sensitive to allethrin than the squid axon.

EFFECTS OF ALLETHRIN ON THE SODIUM INACTIVATION MECHANISM

Since giant axons from the crayfish, *Procambarus clarkii*, were more sensitive to allethrin than the squid giant axon in producing large negative after-potentials, the effect of allethrin on sodium inacti-

Fig. 4

Membrane currents and membrane conductances associated with a step depolarization from the holding membrane potential of —100 mV to —30 mV in a crayfish giant axon before and during the external application of 10-μM allethrin. Top row: membrane currents (I) before and after the addition of tetrodotoxin (TTX); middle row: membrane conductances for sodium (g_{Na}) and potassium (g_K) calculated from the membrane currents in the top row; bottom row: step depolarization of the membrane. Temperature = 12°C



vation was expected to be greater in the crayfish giant axon than in the squid giant axon.

The membrane current associated with a step depolarization of 70 mV from the holding membrane potential of −100 mV is illustrated in Fig. 4. In the control experiment, before the application of allethrin, an initial large capacitative current flowing outwards (upward deflection) across the membrane was followed by a transient inward sodium current, which in turn was followed by a steady-state outward potassium current (current tracing designated " Normal "). Upon cessation of the depolarization, a brief inward capacitative current flowed and was followed by a small inward tail current, which declined slowly. Since the equilibrium potential for potassium in the crayfish giant axon has been estimated to be −87 mV (Murayama, Abbott & Narahashi, unpublished data), the tail current is carried by potassium ions.

In order to separate the membrane ionic current into sodium and potassium components more clearly, tetrodotoxin (TTX) was applied. TTX, the poison of the puffer fish, and has been shown selectively to inhibit the sodium current without affecting the potassium current (Narahashi, Moore & Scott, 1964). As shown in the " Control " curves of Fig. 4, TTX completely abolished the peak transient current without affecting the steady-state current. Therefore, the difference between the " Normal " membrane current and the " TTX " membrane current in this figure should represent the sodium component of the membrane current, and the membrane current remaining in " TTX " should represent the potassium current.

The membrane conductances for sodium (g_{Na}) and potassium (g_K) can be calculated from the equations:

$$g_{Na} = \frac{I_{Na}}{E - E_{Na}} \tag{3}$$

$$g_K = \frac{I_K}{E - E_K} \tag{4}$$

where I_{Na} and I_K are the sodium and potassium currents, respectively, and E is the membrane potential. The sodium and potassium conductances thus calculated from the membrane currents in the upper set of curves in Fig. 4 ares hown in the lower set. The sodium conductance increases rapidly upon step depolarization, but starts decreasing after attaining a peak. The former phase represents the mechanism of the sodium conductance increase, and the latter phase the mechanism of the sodium inactivation. The potassium conductance increases with a certain delay, and keeps increasing slowly until it attains a steady state. This represents the mechanism of the potassium conductance increase. There is no mechanism of potassium inactivation. Upon cessation of the depolarization, the potassium conductance declines exponentially.

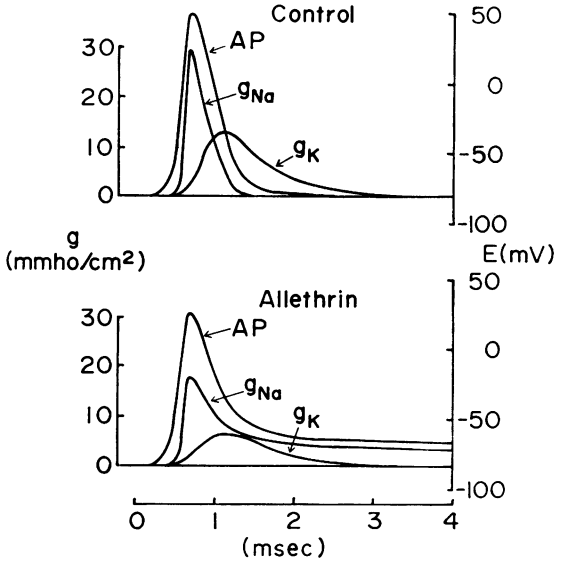
The membrane currents after the external application of allethrin at a concentration of 10 μM are also illustrated in Fig. 4. Two changes in membrane current are easily noted: both the peak transient current and the steady-state current are somewhat suppressed (" Allethrin " curve). When TTX was applied to the allethrin-poisoned axon, the peak current disappeared completely, and the steady-state current increased. Thus, the difference between " Allethrin " and " Allethrin+TTX " curves represents the sodium current in the allethrin-poisoned axon; the sodium current is somewhat suppressed in peak magnitude but is substantially prolonged in the falling phase. The steady-state potassium current is slightly suppressed.

The sodium and potassium conductances in the allethrin-poisoned axon are illustrated in the lower diagrams of Fig. 4. Three changes are apparent: (*a*) the peak amplitude of the sodium conductance is somewhat suppressed, (*b*) the sodium inactivation is greatly prolonged, and (*c*) the potassium conductance is slightly suppressed. Effect (*a*) above is directly responsible for the decrease in the amplitude of the action potential, and effects (*b*) and (*c*) are responsible for the increase and prolongation of the negative after-potential. Diagrams of the action potential and the associated sodium and potassium conductance changes are shown in Fig. 5 for the control and allethrin-treated axons.

Another notable effect of allethrin on the membrane conductances is a shift of the steady-state sodium inactivation curve along the potential axis in the direction of hyperpolarization. The mechanism of the sodium conductance increase is a function of the membrane potential, and decreases

Fig. 5

Schematic drawings of the time courses of the action potential (AP), the membrane sodium conductance (g_{Na}), and the membrane potassium conductance (g_K) before and during the application of allethrin



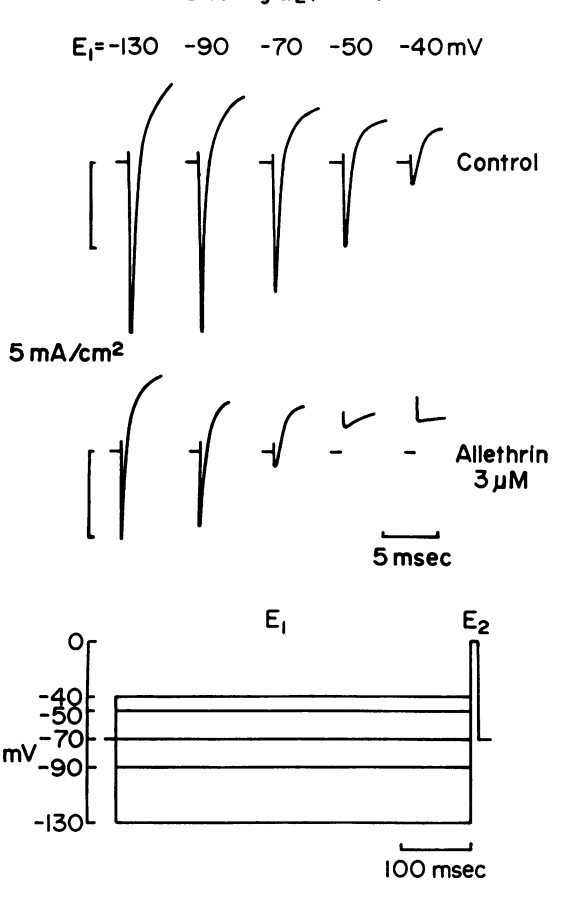
with depolarization. Upon step change of the membrane potential, the sodium conductance mechanism attains a new level with a time constant of 10–20 msec (Hodgkin & Huxley, 1952c). Therefore, when the conditioning depolarizing or hyperpolarizing pulses of varying magnitudes and a 500-msec duration are followed by a brief test depolarizing pulse, the peak sodium current associated with the test pulse gives a measure of the sodium conductance mechanism at the various membrane potential levels.

Fig. 6 illustrates families of the membrane currents associated with a test depolarization following various conditioning pulses, whose membrane potentials are indicated at the top of the figure. It will be seen that the sodium current associated with the test pulse decreases in magnitude with a decrease in the membrane potential of the conditioning pulse. The same tendency is observed in the allethrin-poisoned axon.

The peak sodium current associated with a test pulse, expressed as a ratio to its maximum value, is plotted as a function of the membrane potential of the conditioning pulse in Fig. 7. Such a curve

Fig. 6

Families of membrane currents associated with a test depolarizing pulse following conditioning pulses of various magnitudes and of 500-msec duration in a crayfish giant axon before and during the external application of 3-μM allethrin

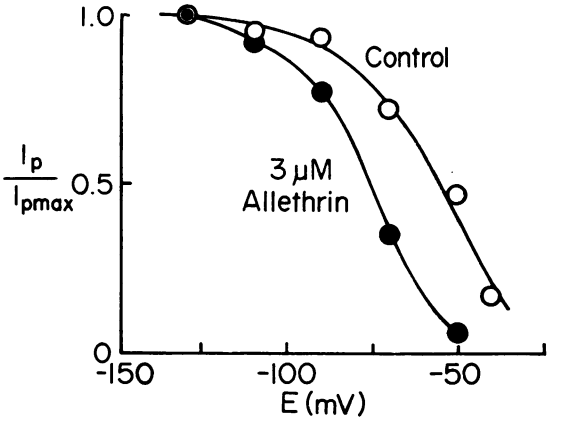


is called the " steady-state sodium inactivation curve ". Allethrin shifts the curve in the direction of a more negative potential inside the membrane, or hyperpolarization.

The shift of the steady-state sodium inactivation curve by allethrin also contributes to the suppression of the action potential. At the same resting potential, the availability of the sodium conductance mechanism decreases after the application of allethrin, thereby suppressing the action potential.

Fig. 7

Steady-state sodium inactivation curve in a crayfish giant axon before and during the external application of 3-μM allethrin



STRUCTURE–ACTIVITY RELATIONSHIP

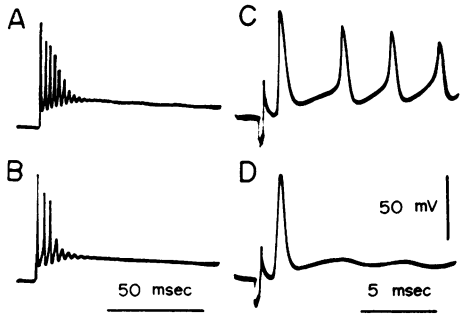
The ketone analogue of allethrin, 2-allyl-4-[[[2,2-dimethyl-3-(2-methylpropenyl)cyclopropyl]carbonyl]methyl]-3-methyl-2-cyclopenten-1-one, has been found to be a fairly potent insecticide and to show fairly pronounced activity on the nerve (Berteau, Casida & Narahashi, 1968). The effects on the action potential of the crayfish giant axon were very similar to those of allethrin. Voltage clamp experiments with the crayfish giant axon revealed that the ionic mechanism of the ketone analogue was also essentially the same as that of allethrin. These studies suggest that the interaction of the pyrethroids with the receptors in the nerve membrane is not absolutely dependent on an ester group of the pyrethroid molecule. It appears that the configuration of the molecule — i.e., its appropriate size and shape for interaction with the membrane receptor—is one of the critical factors for pyrethroid-like action on the nerve.

EFFECT OF TEMPERATURE

It has been known for many years that the insecticidal activity of pyrethroids increases as the temperature is lowered (Blum & Kearns, 1956; Chamberlain, 1950; Guthrie, 1950; Harries, DeCoursey & Hofmaster, 1945; Hartzell & Wilcoxon, 1932). Increased metabolic activity of insects at high temperatures probably contributes to the more rapid

Fig. 8

Intracellularly recorded action potentials in a cockroach giant axon poisoned with 1-μM allethrin. Each set of records shows the response to a single electrical stimulus. Temperature: A, 33°C; B, 28°C; C, 26.5°C; D, 26°C)*



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degradation of pyrethroids, thereby lowering their insecticidal activity. However, it is also possible that the sensitivity of the nervous system varies with temperature, as is the case with DDT (Yamasaki & Ishii, 1954).

The ability of allethrin to initiate repetitive after-discharges was found to increase as the temperature was raised (Narahashi, 1962a). Fig. 8 depicts the action potentials produced by single stimuli in the cockroach giant axon poisoned with allethrin. At 33°C, a single stimulus elicited a burst of action potentials (record A). At 28°C, the number of action potentials produced by the same stimulus decreased (record B). At 26.5°C, a repetitive activity could still be seen (record C), but lowering the temperature to 26°C completely abolished the repetitive activity (record D). Only a small oscillation of membrane potential could be observed. Such a positive temperature coefficient of the action of allethrin in producing repetitive after-discharges reflects an intense excitatory effect on insects at high temperatures, but does not directly affect the mortality of the poisoned insects.

The nerve-blocking action of allethrin is no doubt directly responsible for the paralysis of the poisoned insects. This action has indeed been demonstrated to be highly temperature-dependent in the cockroach nerve cord, a decrease in temperature intensifying the blocking action (Narahashi, 1971). Moreover, this effect of temperature was completely reversible, so that a cycle of block and recovery could be repeated many times by changing the temperature. Similar results were obtained with squid giant axons.

No detailed ionic mechanism for this negative temperature coefficient of allethrin action is known. Some preliminary experiments were performed with the giant axon of the cockroach (Narahashi, 1971). The maximum rate of rise of the action potential was observed as a measure of inward sodium current (Narahashi, 1961), and the steady-state sodium inactivation curves were compared at low and high temperatures in the allethrin-poisoned axon. It was found that lowering the temperature shifted the curve in the direction of a more negative potential inside the membrane. In other words, a smaller amount of the sodium conductance mechanism becomes available at low temperatures. This effect of temperature is at least partly responsible for the increased nerve-blocking activity of allethrin at low temperature. Other factors that may be involved in the negative temperature coefficient of allethrin action remain to be studied.

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* Former name of T. Narahashi.

DISCUSSION

HOBBIGER: Is the concentration of allethrin used of the same order as that which produces toxic action in insects? What are the effects of allethrin, in the concentrations used, on mammalian nerve fibres? Are other sites—e.g., ganglia—more susceptible to allethrin than are the nerve fibres? How do the effects of allethrin on nerve fibres compare with those of veratrum alkaloids? And is it possible that allethrin produces its effects by changing the binding and distribution of Ca^{++} in the nerve membrane or its conductance there?

NARAHASHI: The concentration of allethrin we used was somewhat higher than that found in the intoxicated insect. In England, Dr Burt found that the ganglia of the cockroach were much more sensitive to the action of pyrethroids than was nerve fibre. The ganglia could be the site of action. We have used the giant axon and relatively high concentrations of allethrin for experimental convenience. We have no data on mammalian nerve fibres. The effects of veratrum alkaloids on nerve fibres are similar to those of allethrin. There is evidence that the "sodium gate" of the nerve membrane is kept open by veratrum, and this action can account for the prolonged action potential and repetitive discharges. One of the possible mechanisms whereby allethrin might exert its effects would be a disturbance of the binding of Ca^{++} with the membrane phospholipids. A similar mechanism has been suggested by Blaustein & Goldman for the action of local anaesthetics.

BARNES: Rats poisoned by Dr Elliott's synthetic pyrethroids by simple injection display signs of poisoning very similar to those produced by DDT—an uncoordinated tremor. This suggests that these compounds may exert similar effects at neutral membranes. Has Dr Narahashi observed any blocking action with high doses of DDT?

NARAHASHI: We have not observed any blocking action of p,p′-DDT, even at concentrations of 10^{-4}M