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TEMPERATURE- AND STRUCTURE-DEPENDENT INTERACTION OF PYRETHROIDS WITH THE SODIUM CHANNELS IN FROG NODE OF RANVIER

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(1) The interaction of a series of pyrethroid insecticides with the Na^+ channels in myelinated nerve fibres of the clawed frog, *Xenopus laevis*, was investigated using the voltage clamp technique. (2) Out of 11 pyrethroids 9 insecticidally active compounds induce a slowly decaying Na^+ tail current on termination of a step depolarization, whereas the Na^+ current during depolarization was hardly affected. These tail currents are most readily explained by a selective reduction of the rate of closing of the activation gate in a fraction of the Na^+ channels that have opened during depolarization. (3) The rate of decay of the Na^+ tail current varies considerably with pyrethroid structure. After α-cyano pyrethroids the decay is at least one order of magnitude slower than after non-cyano pyrethroids. The decay always follows a single-exponential time course and is reversibly slowed when the temperature is lowered from 25 to 0°C. Arrhenius plots in this temperature range are linear. (4) These results indicate that the relaxation of the activation gate in pyrethroid-affected Na^+ channels is governed by an apparent first order, unimolecular process and that the rate of relaxation is limited by a single energy barrier. Application of transition state theory shows that after α-cyano pyrethroids this energy barrier is 9.6 kJ/mol higher than after non-cyano pyrethroids. (5) Differences in rate of decay of the Na^+ tail current account for the reported differences in repetitive nerve activity induced by various pyrethroids. In addition, the effect of temperature on the rate of decay explains the increase in repetitive activity with cooling.

Introduction

Pyrethroids are typical neurotoxic compounds structurally related to the natural pyrethrins, which have been synthesized during the last decade for their excellent insecticidal properties [1,2]. In various nerve preparations pyrethroids cause a depolarizing afterpotential following the action potential and they often induce repetitive nerve activity [3–7]. Voltage clamp experiments have shown that this excitatory effect is due to a marked prolongation of the transient increase in Na^+ permeability associated with depolarization of the nerve membrane [8–11]. In myelinated nerve fibres of the clawed frog, *Xenopus laevis*, the pyrethroids allethrin and permethrin induce a large, slowly decaying Na^+ tail current after repolarization, whereas the Na^+ current during depolarization is only slightly affected. It was suggested that the rate of closing of the activation gate is reduced by an effect of pyrethroids on open Na^+ channels [11].

In cultured mouse neuroblastoma cells pyrethroids by themselves have no detectable effect on $^{22}Na^+$ influx. However, when applied in combination with toxins that are known to keep

Na^+ channels open, like veratridine, batrachotoxin and grayanotoxin, they stimulate $^{22}Na^+$ entry [12]. This agrees with the finding that in myelinated nerve fibres pyrethroids preferentially interfere with open Na^+ channels [11]. From the synergism between pyrethroids and a variety of other drugs on $^{22}Na^+$ influx it has been concluded that pyrethroids bind to a site which is different from the binding sites of all other toxins known to affect the gating system of the nerve membrane Na^+ channel [12].

Several studies have revealed pronounced differences in poisoning symptoms and neurophysiological effects of α-cyano-3-phenoxybenzyl pyrethroids and pyrethroids without an α-cyano substituent [7,13–15]. We have found, however, that these two groups of pyrethroid induce qualitatively similar trains of repetitive nerve impulses in the lateral-line sense organ of *Xenopus laevis* [7]. A most interesting common feature is that the repetitive activity induced by α-cyano as well as non-cyano pyrethroids in this sense organ increases dramatically with cooling. This negative correlation between temperature and repetitive activity, which may be related to the increased toxicity of pyrethroids to insects at low temperature [16,17] has not yet been accounted for properly.

The main objectives of the present work study were to study the influence of temperature on the interaction of pyrethroids with the Na^+ channel in myelinated nerve fibres and to investigate the effect of the α-cyano substituent. A preliminary account of some of the results has been published [11].

Materials and Methods

Sodium current measurements. Large diameter myelinated nerve fibres were dissected from the sciatic nerve of the clawed frog, *Xenopus laevis* [18]. Fibres were mounted across three partitions in a Perspex nerve chamber modified after Nonner [19]. The partitions were covered with silicone grease and typical seal resistances ranged from 5–10 MΩ. The ends of the fibre were cut at a distance of 0.8 mm and 0.6 mm from the node in an axoplasma substitute. The nerve chamber was mounted on a Peltier element for temperature control. To minimize thermal gradients the chamber was surrounded by 7 mm thick aluminum and covered with a Perspex lid in which electrodes, a miniature thermistor and a suction cannula were mounted. Ag-AgCl electrodes made contact with the saline pools through 120 mM CsCl agar bridges. The pool with the node was continuously perfused at a rate of 0.3–0.5 ml/min. The temperature of the solution was initially kept at 15°C and was varied during the experiments between 0°C and 25 ± 0.1°C.

The voltage clamp circuit was designed after the original of Dodge and Frankenhaeuser [20]. The solution in the B-pool was actively held at ground potential by an Ag-AgCl and a Pt black electrode connected to the input and the output of a virtual ground circuit, respectively. The difference in junction potential between the Ag-AgCl electrodes in the B- and C-pool was corrected with a DC-balance network in the virtual ground circuit and drift was minimized by filling both pools with the same solution. Current clamp amplifier and control amplifier were equipped with variable gain and frequency networks. The final DC-gain of these amplifiers was $5 \cdot 10^5$ and the closed loop gain of the current clamp amplifier ranged from 3 to 10 in different experiments. A phase lead-lag combination network was adjusted at the beginning of each experiment to obtain a critically damped response of the control amplifier to a 60 mV hyperpolarizing pulse. The fast capacitive transient in the current signal of a well-tuned fibre usually had a width of 5–10 μs at half maximum amplitude. Linear capacitive and leakage currents were subtracted by an analog circuit that generated a differentiated signal proportional to the pulse amplitude with three variable time constants of variable amplitude and a DC-level. The membrane was held at the potential at which $h_\infty = 0.7$ (−75 mV). Potassium currents were eliminated by external tetraethylammonium chloride and by substituting Cs^+ for K^+. Na^+ currents were low-pass filtered at 10 or 5 kHz and calibrated assuming a 10 MΩ internal resistance. Holding current was continuously monitored and the repetition rate of membrane depolarizations was chosen low enough to avoid cumulative effects (2–0.1 Hz). Voltage steps were generated by a programmable, microprocessor-controlled square wave generator (amplitude resolution 1 mV; error < 0.03 mV; mini-

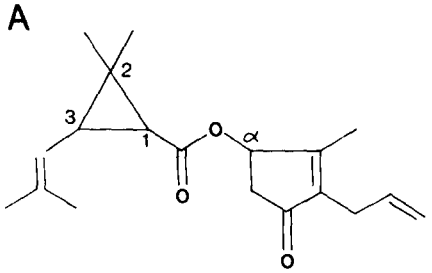
mum pulse duration 10 μs). Unless otherwise noted all depolarizing pulses were preceded by a pre-pulse of 100 ms to −125 mV to remove resting Na^+ channel inactivation. Data collection was under control of a minicomputer (HP1000) which was programmed to synchronize the collection of current samples from a transient recorder (10 bits; aperture time 15 ns) and pulse information from the square wave generator. The membrane current was sampled at a fixed interval of 1 μs to 1 ms, depending on the time course of the signal measured. For every step depolarization pulse parameters together with 2048 current samples were stored on disc, to be analyzed later by means of several interactive programs. The time constants of the Na^+ tail current decay were calculated using a least-squares method. The exponentially decaying phase of the Na^+ tail current was extrapolated back to the moment of repolarization in order to obtain a measure of the slow tail conductance at zero time.

Solution and chemicals. The composition of the external solutions was: Normal Ringer: 116 mM NaCl/2.4 mM KCl/2 mM $CaCl_2$/3 mM Hepes buffer; pH was adjusted to 7.3 with approximately 1.1 mM NaOH; osmolality 230 mosmol/l. Cs-Ringer had the same composition except for KCl that was replaced by CsCl. The axoplasma sub-

TABLE I

CHEMICAL STRUCTURE OF PYRETHROIDS USED

A



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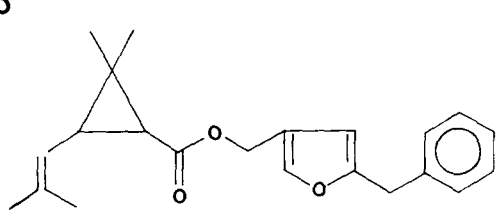

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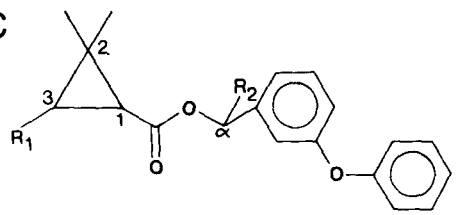

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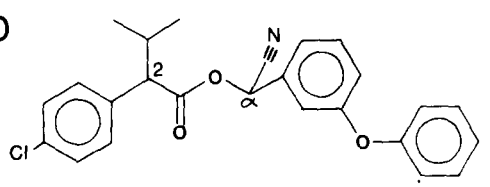

Compound	Structure	R_1	R_2	Stereo-chemistry	Purity (%)
Non-cyano pyrethroids					
Allethrin	A	–	–	1*RS* 3*RS* α*RS*	> 90
Cismethrin	B	–	–	1*R* 3*S*	99
Permethrin	C	$-C{=}C(Cl)_2$	H	1*RS* 3*RS*	93
cis-Permethrin	C	$-C{=}C(Cl)_2$	H	1*R* 3*S*	95
trans-Permethrin	C	$-C{=}C(Cl)_2$	H	1*R* 3*R*	> 99.5
α-Cyano pyrethroids					
Cypermethrin	C	$-C{=}C(Cl)_2$	$-C{\equiv}N$	1*R* 3*R* α*S*	98
Deltamethrin	C	$-C{=}C(Br)_2$	$-C{\equiv}N$	1*R* 3*S* α*S*	99
S/S Fenvalerate	D	–	–	2*S* α*S*	94.4
RS/S Fenvalerate	D	–	–	2*RS* α*S*	97.6
R/R Fenvalerate	D	–	–	2*R* α*R*	99.6
RS/R Fenvalerate	D	–	–	2*RS* α*R*	98.9

stitute consisted of 120 mM CsCl, or 106 mM CsCl and 14 mM NaCl; osmolality 220 mosmol/l. Tetraethylammonium chloride was mixed by volume from a 120 mM stock solution with Cs-Ringer in a concentration of 6 or 10 mM.

The pyrethroids are listed in Table I. Stock solutions of these compounds in ethanol, except for deltamethrin that was dissolved in acetone, were kept in the refrigerator at 4°C. In each experiment a small amount of stock solution was dispersed through a hypodermic needle in Cs-Ringer and this was vigorously shaken to produce a fine suspension of pyrethroid. Pyrethroids were applied in a concentration of 1–20 μM. As these compounds are practically insoluble in water these figures refer to the amount of pyrethroid initially dispersed. Because of the high lipid solubility, effects of pyrethroids are quasi-irreversible and recovery experiments were not performed. The amount of solvent in Ringer was always less than 0.5% (v/v) which had no detectable effect in control experiments. Between experiments the nerve chamber and the perfusion equipment were thoroughly cleaned to avoid contamination.

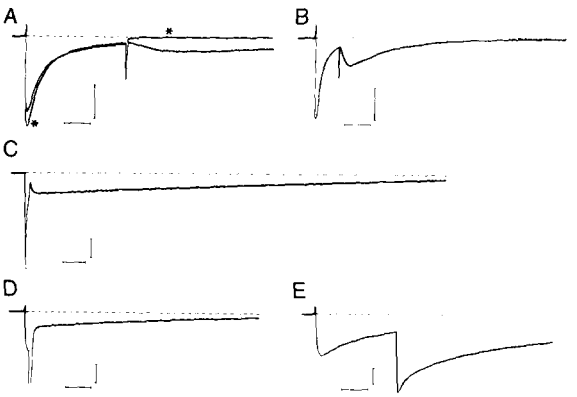

Fig. 1. Effects of pyrethroids on Na^+ currents evoked by a step depolarization to a membrane potential of −5 mV. Temperature 15°C. (A) Na^+ current before (*) and after treatment with 10 μM permethrin. On termination of the depolarizing pulse the Na current is rapidly turned off in the normal node, whereas after permethrin a prolonged Na^+ tail current remains. (B) Na^+ current in the same node after permethrin recorded at a slower time base. (C) Na^+ current and Na^+ tail current in a node treated with 1 μM cypermethrin. (D) and (E) Na^+ current and Na^+ tail current evoked by depolarizations of different duration in a node treated with 10 μM cismethrin. Horizontal calibrations: (A, D, E) 2 ms; (B) 10 ms and (C) 50 ms. Vertical calibrations: (A, B) 10 nA; (C) 1 nA and (D, E) 6 nA.

Results

Interaction of pyrethroids with the Na^+ channel

In the normal node the Na^+ current is always rapidly turned off on termination of a step depolarization. After treatment with pyrethroids a marked, slowly decaying Na^+ tail current remains after the end of a step depolarization, whereas Na^+ current during depolarization is hardly affected as illustrated in Fig. 1A, B for permethrin. The other non-cyano pyrethroids allethrin and cismethrin induce similar Na^+ tail currents. These Na^+ tail currents stabilize in a few minutes after the perfusion with pyrethroid suspension is started. The α-cyano pyrethroids cypermethrin, deltamethrin, *RS/S* and *S/S* fenvalerate also induce marked Na^+ tail currents, without seriously affecting Na^+ currents during depolarization, but the decay of these tail currents is very slow and they last much longer than after the non-cyano pyrethroids (Fig. 1C). The two insecticidally inactive isomers of fenvalerate (*R/R* and *RS/R*) fail to induce a detectable Na^+ tail current.

The amplitude of the Na^+ tail current induced by the various pyrethroids depends on amplitude and duration of depolarization. After short depolarizations only small tail currents remain and with increasing duration of depolarization tail current amplitude gradually increases (Fig. 1D, E). The duration of depolarization necessary to evoke maximum tail current amplitude varies with pyrethroid structure and when the depolarization is further prolonged, tail current amplitude slowly decreases. When the number of Na^+ channels that are activated during depolarization is varied by changing the amplitude of the test pulse while keeping its duration constant, Na^+ tail current amplitude varies proportionally (Fig. 2A). Na^+ tail current amplitude is also proportional to the number of non-inactivated Na^+ channels when the amplitude of the pre-pulse is varied while keeping the test pulse constant (Fig. 2B). These results show that the amplitude of the Na^+ tail current induced by deltamethrin is proportional to the degree of activation as well as to the degree of inactivation during depolarization; i.e. to the number of Na^+ channels that are open during de-

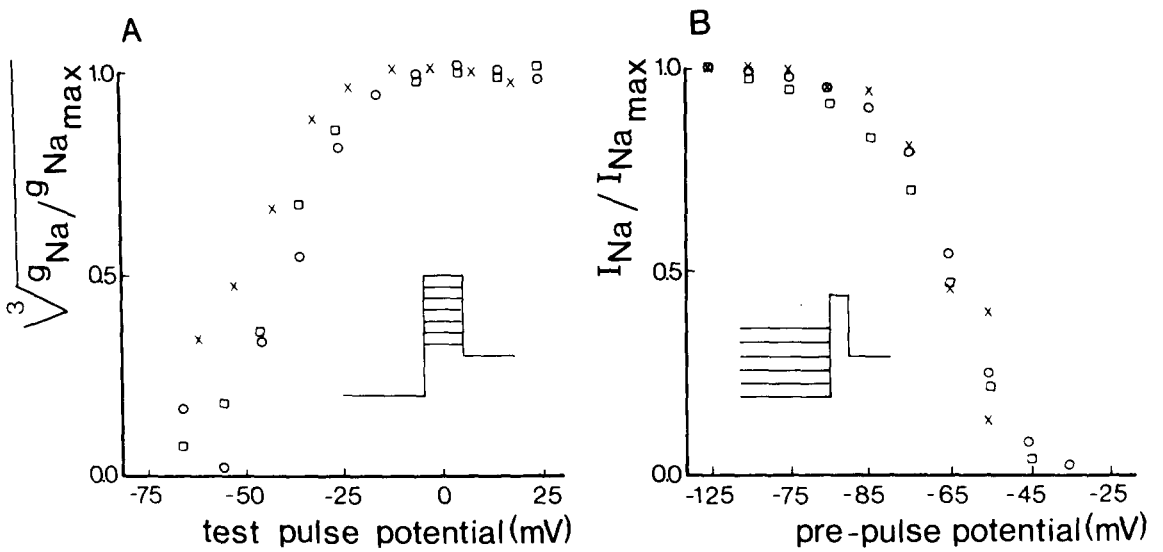

Fig. 2. Dependence of Na^+ tail current amplitude on Na^+ channel activation (A) and on Na^+ channel inactivation (B). Normalized values are calculated from Na^+ currents before (○) and after (□) treatment with 2 μM deltamethrin and from Na^+ tail currents (×). Temperature 15°C. In (A) Na^+ conductance extrapolated back to the beginning of 8 ms depolarizing test pulses of various amplitudes were normalized to the maximum conductance measured. The same procedure was used to normalize Na^+ tail conductance at the moment of repolarization. In (B) the amplitude of the 100 ms pre-pulse was varied and Na^+ currents were elicited by a constant test pulse to −5 mV for 3 ms. In this case Na^+ current amplitude was normalized. Insets indicate pulse patterns.

polarization. As the Na^+ tail current amplitude is proportional to the number of Na^+ channels that displays modified closing kinetics after treatment with deltamethrin, it can be concluded that a direct proportionality exists between the number of Na^+ channels affected by deltamethrin and the number of Na^+ channels that were open during the preceding depolarization. Similar results have been obtained with allethrin and permethrin [11] and with the other pyrethroids tested. Thus it appears that non-cyano as well as α-cyano pyrethroids preferentially interfere with Na^+ channels of which both the activation gate and the inactivation gate are in the open configuration.

Furthermore, following membrane depolarizations that cause a high degree of Na^+ channel inactivation the tail current shows an initial rise before it starts to decay (e.g. Fig. 1B). These hooked tail currents are most readily explained by postulating that on termination of a step depolarization the inactivation gate reopens, while the closing of the activation gate is delayed. These results corroborate our earlier hypothesis that all active pyrethroids selectively reduce the rate of closing of the activation gate of a fraction of the Na^+ channels and that the slow decay of the Na^+ tail current reflects the relaxation of the activation gate in pyrethroid-affected Na^+ channels [11].

TABLE II

TIME CONSTANTS OF EXPONENTIAL DECAY OF PYRETHROID-INDUCED Na^+ TAIL CURRENTS (MEAN ± S.D.) FOR THE VARIOUS PYRETHROIDS AT 15°C

Number of nodes in parentheses. n.d., no detectable Na^+ tail current.

Compound	Concn. (μM)	τ_{tail}
Non-cyano pyrethroids		
Allethrin	20	9.8 ± 1.2 (6)
Cismethrin	10	20.7 ± 5.1 (3)
Permethrin	10	17.4 ± 0.3 (3)
cis-Permethrin	5; 20	30.3; 26.7 (2)
trans-Permethrin	10; 20	5.4; 7.3 (2)
α-Cyano pyrethroids		
Cypermethrin	1	610; 1431 (2)
Deltamethrin	2	1453 ± 370 (3)
S/S Fenvalerate	5; 10	652; 628 (2)
RS/S Fenvalerate	20	463; 627 (2)
R/R Fenvalerate	20	n.d. (2)
RS/R Fenvalerate	20	n.d. (2)

Pyrethroid structure and Na^+ tail current decay

The decaying phase of the tail current always follows a single exponential time course with a time constant that is independent of tail current amplitude. This indicates that the relaxation of the activation gate in pyrethroid affected channels is determined by an apparent first order, unimolecular process. Table II shows the time constants of exponential decay for the various pyrethroids. After α-cyano pyrethroids the Na^+ tail current decays much more slowly that after non-cyano pyrethroids and the difference in time constant is at least one order of magnitude. Much smaller differences in tail current decay exist between compounds that belong either to the non-cyano or the α-cyano pyrethroids. The rate of decay of the tail currents is not correlated with pyrethroid concentration within the range tested.

The inability of the (*R*)-α-cyano isomers of fenvalerate to induce a detectable Na^+ tail current in contrast to the (*S*)-α-cyano isomers indicates that the interaction of pyrethroids with the Na^+ channel gating system is a highly stereospecific process. This is also supported by the finding that Na^+ tail currents induced by either the *cis*- or *trans*-isomer of permethrin, or by a mixture of these isomers have different time constants. These results show that the rate of relaxation of the activation gate in pyrethroid-affected channels is characteristic of each individual compound and that the relaxation process is particularly slow after α-cyano pyrethroids.

Influence of temperature on Na^+ tail current decay

The decay of pyrethroid-induced Na^+ tail currents is markedly slowed when the temperature is lowered as illustrated in Fig. 3 for permethrin. A similar decrease in the rate of decay of the tail current with cooling occurs after the other pyrethroids. Between 0 and 25°C this effect of temperature is readily reversible. In this temperature range the decay of the tail current is always fitted by a single exponential function, although Na^+ current amplitude and, consequently, tail current amplitude varies with temperature.

The rate of relaxation of the activation gate of pyrethroid-affected channels is positively correlated with temperature. The rate constant, i.e. the reciprocal value of the time constant of decay of the Na^+ tail current, is directly related to absolute temperature by the Arrhenius equation:

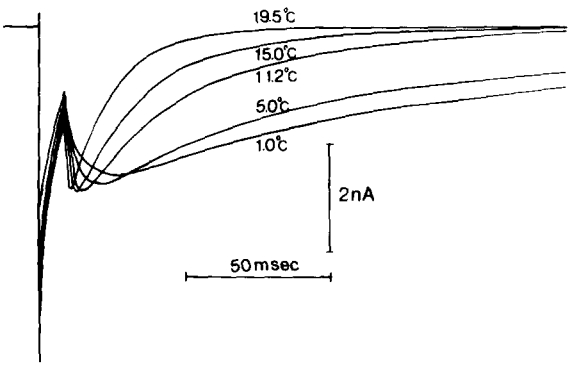

Fig. 3. Influence of temperature on Na^+ tail current decay. Na^+ currents in a node treated with 10 μM permethrin evoked by a step depolarization to −5 mV at the temperatures indicated.

$$r = A \exp(-E_a/RT) \quad (1)$$

Arrhenius plots of the rate of decay of pyrethroid-induced tail currents are shown in Fig. 4. The experimental results of individual nerve fibres were fitted by straight lines and variance analysis [21] was performed to test the linearity of fit if sufficient values for the rate constant were available. For 13 lines thus tested the hypothesis of linearity was not rejected at the $P = 0.10$ level (two-sided test; *F*-table), but for one line which yielded a value of $P = 0.04$. The straight lines in Fig. 4 represent the least-squares fit to the lumped experimental results of each pyrethroid and variance analysis showed no deviation from linearity tested at the $P = 0.10$ level.

The linearity of the Arrhenius plots indicates that a single energy barrier limits the rate of relaxation of the activation gate in pyrethroid-affected Na^+ channels. The slopes of the lines in Fig. 4 correspond to Q_{10} values that range from 2.9 for allethrin to 6.4 for deltamethrin. These values are large compared to the Q_{10} of 1.7 for β_m, the rate constant of relaxation of the activation gate, in the normal node of *Xenopus laevis* [22,23]. At low temperatures no breaks are observed in the Arrhenius plots, which is also the case for Arrhenius plots of the maximum Na^+ conductance and of the time constant of Na^+ inactivation

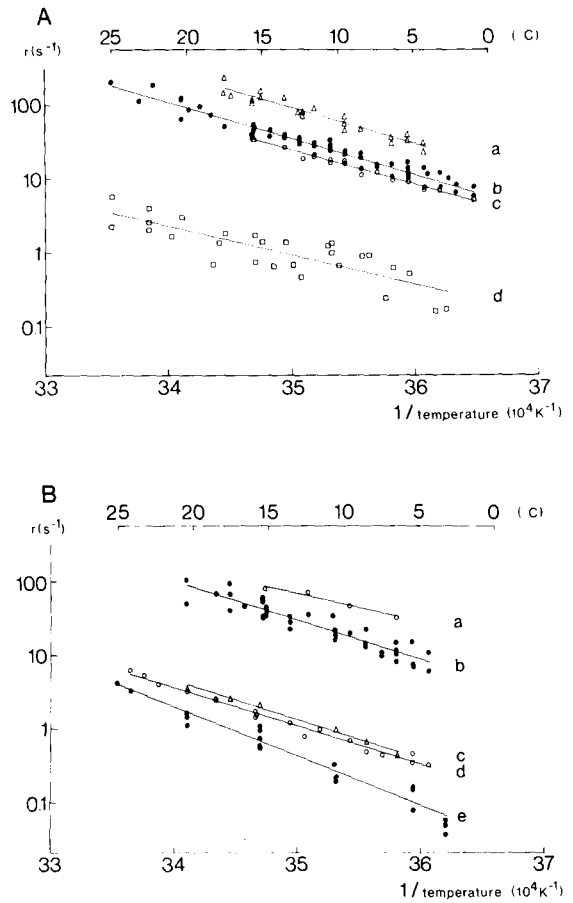

Fig. 4. Arrhenius plots of the rate of decay of pyrethroid-induced Na^+ tail currents. (A) *trans*-Permethrin(a), permethrin(b), *cis*-permethrin(c) and cypermethrin(d). (B) Allethrin(a), cismethrin(b), *RS/S* fenvalerate(c), *S/S* fenvalerate(d) and deltamethrin(e). Same concentrations as in Table II.

(τ_h) in nerve fibres of *Rana pipiens* [24] and *Rana esculenta* [25]. Breaks have been reported between 5° and 8°C in Arrhenius plots of τ_m, τ_h and time to peak Na^+ current of nerve and muscle fibres of four species of *Rana*, when the temperature range was extended as low as −6°C [26]. The absence of a break in the Arrhenius plot of the present results could be due to the limited low temperature range investigated, to the different type of nerve fibre used, or to an effect of pyrethroids on the transition temperature. However, any hysteresis in the temperature curves that would obscure a break in the Arrhenius plot was not observed.

Discussion

The decay of pyrethroid-induced Na^+ tail current is described by an apparent first-order, unimolecular process to which transition state theory can be applied. According to this theory [27,28], all chemical reactions pass through an intermediate state or activated complex, which is always in equilibrium with the initial state. For the crossing of a single energy barrier, which is the case for the relaxation of the activation gate in pyrethroid-affected Na^+ channels, the rate constant r is given by the following equation:

$$r = \kappa kT/h \exp(\Delta S_a/R) \exp(-\Delta H_a/RT) \quad (2)$$

Here κ is a transmission coefficient that is unity for simple energy barriers, which means that all molecules reaching the top of the barrier move in the forward direction along the reaction coordinate. The other symbols in Eqn. 2 have their usual meaning; the subscript 'a' refers to the activated complex. For the relatively narrow temperature range we have investigated, ln r is linearly related to $1/T$. A comparison of Eqn. 2 with the Arrhenius equation (Eqn. 1) shows that ΔH_a and E_a are approximately equal when E_a is large compared to RT. Therefore, ΔH_a and ΔS_a can be calculated from the Arrhenius plots (Fig. 4). The free energy of activation ΔG_a may then be calculated from:

$$\Delta G_a = \Delta H_a - T\Delta S_a \quad (3)$$

The calculated energy changes associated with the formation of the activated complex are listed in Table III. All ΔS_a values are positive and relatively large, which indicates that the relaxation process is favoured by an increase in activation entropy. The values of ΔH_a and ΔS_a do not systematically vary with pyrethroid structure. There is, however, a statistically significant difference between the ΔG_a values obtained for non-cyano and α-cyano pyrethroids ($P = 0.01$; Wilcoxon test). This difference averages 9.6 kJ/mol, which is in the same order of magnitude as a single H-bond [29]. The large difference in the rate of decay of Na^+ tail currents induced by non-cyano and α-cyano pyrethroids is apparently due to a relatively small difference in the height of the energy barrier that has to be crossed for the relaxation of the activa-

TABLE III

VALUES FOR ENTHALPY (ΔH_a), ENTROPY (ΔS_a) AND FREE ENERGY OF ACTIVATION (ΔG_a) AND Q_{10} VALUES OF THE PROCESS OF RELAXATION OF THE ACTIVATION GATE IN PYRETHROID-AFFECTED Na^+ CHANNELS

Compound	ΔH_a (kJ/mol)	ΔS_a (J/K/mol)	ΔG_a (kJ/mol)	Q_{10}
Non-cyano pyrethroids				
Allethrin	76.8	60.9	59.3	2.9
Cismethrin	105.1	152.8	61.1	4.4
Permethrin	98.2	129.5	60.9	4.0
cis-Permethrin	94.3	112.8	61.8	3.8
trans-Permethrin	97.7	136.2	58.4	3.9
α-Cyano pyrethroids				
Cypermethrin	78.4	28.6	70.2	3.0
Deltamethrin	132.4	211.7	71.5	6.4
S/S Fenvalerate	103.2	118.3	69.1	4.3
RS/S Fenvalerate	105.7	127.8	68.9	4.4

tion gate in pyrethroid-affected channels.

We have previously shown that the action of the insecticide DDT on Na^+ channel gating in the frog node of Ranvier is very similar to that of the non-cyano pyrethroids [11]. DDT also selectively reduces the rate of closing of the activation gate in a fraction of the Na^+ channels that open during depolarization of the membrane. The temperature dependence of the rate of decay of DDT-induced Na^+ tail currents indicates that the free energy of activation (ΔG_a) of DDT-affected channels is about equal to that after the non-cyano pyrethroids. The activation entropy has a small negative value which could mean that entropy changes play only a minor role in the relaxation of the activation gate in DDT-affected channels (Vijverberg, H.P.M., unpublished observations).

The inactive pyrethroids are of particular interest, since these compounds may bind to the Na^+ channel without interfering with the gating mechanism. Some pyrethroids which do not affect $^{22}Na^+$ influx in mouse neuroblastoma cells prevent the effect of active pyrethroids and it has been suggested that the interaction of pyrethroids with the Na^+ channel proceeds in two successive steps, i.e., the initial binding to the receptor and the subsequent modification of the gating system [12]. From the present results it is clear that the α-(*R*) isomers of fenvalerate fail to interfere with the gating system, but they may bind to the same site as the active α-(*S*) isomers. Although pyrethroids modify Na^+ channel gating in a highly stereospecific way, the Na^+ channel binding site can accommodate a variety of pyrethroids and possibly also DDT-like molecules. This may be related to the flexibility of these ligands [30], but receptor modulation may also be involved [32]. A mutual, conformational adjustment of ligand and receptor which may occur in a number of discrete, successive steps, would increase the probability of binding of different molecules to the same site of the Na^+ channel [32].

Pyrethroids also induce persistent Na^+ tail currents in the giant axon of the squid and of the crayfish [9,10]. These tail currents could also be due to a reduced rate of closing of the activation gate of pyrethroid-affected Na^+ channels, but the time constants of tail current decay are much larger. In contrast to frog node of Ranvier, pyrethroids slow down Na^+ channel inactivation in invertebrate axons. It seems reasonable to assume that the reduced rate of closing of the activation gate is primarily responsible for the excitatory action of pyrethroids and probably also for their insecticidal action. A number of Na^+ channels that open during a nerve impulse will remain open for a longer time after treatment with pyrethroid and the action potential will be followed by a depolarizing afterpotential. The prolonged Na current following the nerve impulse may also give rise to repetitive activity. The α-cyano pyrethroids will induce a longlasting depolarizing afterpotential, which summates during rapid nerve stimulation causing gradual depolarization of the nerve mem-

brane. In nerve fibres this results in a frequency-dependent suppression of the action potential amplitude [7,13]. In the lateral-line sense organ of *Xenopus laevis* all active pyrethroids induce trains of afferent nerve impulses [7]. For the various pyrethroids there appears to be a good correlation between the rate of decay of the Na^+ tail current in the nodal membrane and the duration of nerve impulse trains in the lateral-line sense organ. In particular, the large differences in the rate of tail current decay between non-cyano and α-cyano pyrethroids is reflected by a difference of one order of magnitude in nerve impulse train duration. In addition, the duration of nerve impulse trains in the lateral-line sense organ induced by both types of pyrethroid increases dramatically with cooling, which is accounted for by the further prolongation of pyrethroid-induced Na^+ tail currents at low temperature reported here.

Several studies have shown that the *trans*-isomers of pyrethroids are less toxic than their corresponding *cis*-isomers [5,14,33]. This is in keeping with the faster rate of decay of the Na^+ tail current induced by *trans*-permethrin as compared to *cis*-permethrin. The α-(*R*)-isomers of fenvalerate, which fail to induce an Na^+ tail current in the node of Ranvier, have a low toxicity to insects and in the locust they cause neurophysiological effects only at high concentrations compared to the insecticidally active isomers [5].

In conclusion, pyrethroids selectively reduce the rate of relaxation of the activation gate of the Na^+ channel in a highly stereospecific way. The rate of relaxation depends on the structure of the pyrethroid molecule and on temperature. Numerous structural analogs of pyrethroids are available and further investigation into their structure-activity relationship will contribute to a better understanding of the physico-chemical characteristics of the Na^+ channel protein.

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