

Short communication

FREQUENCY-DEPENDENT EFFECTS OF THE PYRETHROID INSECTICIDE DECAMETHRIN IN FROG MYELINATED NERVE FIBRES

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The pyrethroid insecticide decamethrin (10^{-6} M) caused a frequency-dependent depression of the action potential in frog myelinated nerve fibres which was associated with a progressive membrane depolarisation brought about by summation of depolarising after-potentials. Voltage clamp experiments with single nodes of Ranvier showed that this afterpotential was most probably due to a long-lasting prolongation of the transient increase in sodium permeability of the membrane. The results indicate that decamethrin, like the other pyrethroids, specifically affects the sodium channels of the nerve membrane.

Decamethrin	Depolarising after-potential	Frequency-dependent depression
Frog node of Ranvier	Prolongation of sodium permeability	Pyrethroids

1. Introduction

In recent years great progress has been made in the development of synthetic pyrethroid insecticides (Elliott, 1977). One of the most promising new compounds is decamethrin (NRDC 161). This compound is extremely toxic to insects; it has an LD_{50} in the order of 0.03 mg/kg and its mammalian (oral) to insect (topical) toxicity ratio is greater than 2500. Since the symptoms of decamethrin poisoning in rats differed significantly from those of other pyrethroids, it has been suggested that this pyrethroid has a novel site of action (Barnes and Verschoyle, 1974; Elliott et al., 1974).

It is generally assumed that allethrin, one of the early synthetic pyrethroids, acts primarily on the sodium channels of the nerve membrane of invertebrates as well as of vertebrates causing a prolongation of the transient increase in sodium permeability during excitation which may result in pronounced repeti-

tive activity (Narahashi, 1976; Wouters and Van den Bercken, 1978). The present results clearly indicate that like allethrin, decamethrin specifically affects the sodium channels of frog myelinated nerve membrane.

2. Materials and methods

Compound action potentials of intact sciatic nerves of the clawed frog, *Xenopus laevis*, were recorded in a moist chamber by means of silver wire electrodes.

To measure membrane potentials of single nodes of Ranvier, large myelinated fibres were dissected from the sciatic nerve, mounted in a Perspex chamber across three partitions and sealed with silicone grease. The internodes were cut in isotonic KCl (120 mM). Ag—AgCl electrodes were used for potential measurement and current injection. Membrane potentials were recorded under current clamp conditions as originally described by Franken-

haeuser (1957). Additional observations were made on voltage-clamped nodes of Ranvier (Dodge and Frankenhaeuser, 1958; Hille, 1971).

Decamethrin (OMS-1998; gift from WHO, Geneva) was dissolved in acetone as a 10^{-2} M stock solution. Small amounts of this solution were squirted through a hypodermic needle into Ringer solution. The maximum concentration of acetone was 0.5% (v/v) which was without significant effect in control experiments. The Ringer solution contained (in mM): NaCl 116, KCl 2.4, CaCl_2 2.0, and HEPES-buffer 3.0; the pH was adjusted to 7.3. The experiments on intact nerves were carried out at a room temperature of $20-22^\circ\text{C}$. Single fibres were cooled to $15 \pm 1^\circ\text{C}$.

3. Results

3.1. Effects on intact nerves

After exposure of the sciatic nerve to Ringer solution containing 10^{-6} M decamethrin for 2 h the amplitude of the compound action potential evoked at a rate of less than 1 per 2 sec was only slightly decreased. At higher stimulus frequencies, however, the amplitude of the action potential rapidly declined to a plateau level. If the nerve was subsequently left unstimulated for a few seconds the action potential regained its full amplitude. For a given period of stimulation the degree of depression of the action potential and the time required for recovery increased with increasing stimulus frequency. This frequency-dependent depression, which was already apparent after 30 min of treatment with 10^{-6} M decamethrin, is illustrated in fig. 1A. In some experiments there was a shift of the baseline in the direction of depolarisation during train stimulation. Repetitive activity was not observed in any of the nerves treated with decamethrin.

3.2. Effects on single nodes of Ranvier

After control recordings the normal Ringer solution bathing the node was replaced with

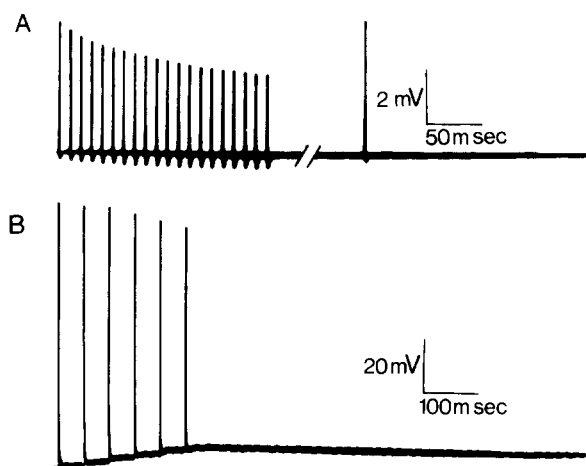


Fig. 1. A. Train of compound action potentials evoked at 100 Hz, followed by a single action potential elicited 1 sec after the end of the train in a sciatic nerve exposed to 10^{-6} M decamethrin for 105 min. B. Train of action potentials evoked at 20 Hz in a single node of Ranvier treated with 10^{-6} M decamethrin for 42 min. Each action potential was followed by a depolarising after-potential that became superimposed upon the previous after-potential. Resting potential was -75 mV (dashed line).

Ringer containing 10^{-6} M decamethrin. At this concentration the pyrethroid did not significantly affect either the resting potential or the shape and amplitude of the nodal action potential evoked at a rate of less than 1 per 2 sec. At high amplification a small depolarising after-potential was seen following the action potential. During stimulation with trains of pulses, however, a progressive depolarisation of the nodal membrane developed, which returned very slowly to the resting potential after the train had ceased (see fig. 1B). Apparently, every action potential in the train was followed by a small depolarising after-potential which became superimposed upon the previous after-potential. The amplitude of the action potential decreased concomitantly with the depolarisation. The amplitude of the depolarisation and the degree of depression of the action potential increased with increasing stimulus frequency. Whether the depression of the action



Fig. 2. Inward sodium current associated with a step depolarisation of 60 mV in a single node of Ranvier after 30 min of treatment with 5×10^{-5} M decamethrin. The dashed line represents the level to which the membrane current quickly returned after a step depolarisation in control experiments. Holding potential -84 mV.

potential was solely due to sodium channel inactivation produced by the depolarisation or whether an inhibitory action of decamethrin on membrane sodium permeability was also involved is a matter for further investigation.

Preliminary voltage-clamp experiments showed that after the end of step depolarisation which evoked a large inward sodium current, the membrane current did not return rapidly to the holding level as in control recordings. Instead, a large inward tail current remained (fig. 2) which persisted for more than 1 sec. The observation that a substantial inward tail current was already apparent after step depolarisations, which evoked no potassium current (see fig. 2), strongly suggests that the tail current flows through the sodium channels of the membrane.

After treatment with 5×10^{-5} M decamethrin the nodal membrane was gradually depolarised by 10 to 20 mV within 1 h. At this concentration the frequency-dependent effects on the action potential and the inward tail currents were qualitatively the same as described above.

4. Discussion

The results demonstrate that the decamethrin-induced frequency-dependent depression of the action potential in frog nerve fibres is associated with a progressive depolarisation of

the nerve membrane due to summation of depolarising after-potentials. The voltage-clamp experiments showed that this depolarising after-potential is most probably brought about by a long-lasting prolongation of the transient increase in sodium permeability of the membrane.

Allethrin, one of the early synthetic pyrethroids, also causes a prolongation of the transient sodium permeability in frog myelinated nerve membrane (Vijverberg et al., 1979). The allethrin-induced sodium tail current, however, decays within 100 msec (at a membrane potential of -80 mV), whereas in the case of decamethrin it takes more than 1 sec before the holding current level is regained. It appears that, despite large differences in tail current kinetics, both pyrethroids affect the sodium channels of frog nerve membrane in essentially the same, specific way.

Additional experiments showed that allethrin, as well as permethrin, did not induce a frequency-dependent depression of the action potential in frog nerves as described here for decamethrin, even after higher concentrations (3×10^{-5} M). On the other hand, cypermethrin — a pyrethroid with a molecular structure identical to that of permethrin, except for an α -cyano group on the 3-phenoxy-benzyl alcohol (Elliott, 1977) — also induced a frequency-dependent depression of the action potential very similar to that observed after decamethrin (H.P.M. Vijverberg, unpublished observations). Decamethrin contains the same α -cyano group. Therefore the suggestion is that the dramatic prolongation of the sodium permeability in frog nerve membrane by decamethrin is related to the presence of this α -cyano group.

Allethrin and permethrin cause pronounced repetitive activity in nerve fibres and in sensory organs of the frog (Van den Bercken et al., 1973; Vijverberg, Van der Zalm and Van den Bercken, to be published). Such repetitive activity was not observed here in decamethrin treated nerve fibres. Very recently, however, we have observed that in the lateral-line sen-

sory organ of the clawed frog decamethrin induced very long trains of impulses which may last for up to 15 sec. This type of repetitive activity is to be expected because of the long-lasting prolongation of the sodium permeability induced by this pyrethroid.

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References

- Barnes, J.M. and R.D. Verschoyle, 1974, Toxicity of new pyrethroid insecticide, *Nature* 248, 711.
- Dodge, F.A. and B. Frankenhaeuser, 1958, Membrane currents in isolated frog nerve fibre under voltage clamp conditions, *J. Physiol.* 143, 76.
- Elliott, M., 1977, Synthetic pyrethroids, in: *Synthetic Pyrethroids*, ACS Symposium Series, Vol. 42, ed. M. Elliott (American Chemical Society, Washington, D.C.) p. 1.
- Elliott, M., A.W. Farnham, N.F. Janes, P.H. Needham and D.A. Pulman, 1974, Synthetic insecticide with a new order of activity, *Nature* 248, 710.
- Frankenhaeuser, B., 1957, A method for recording resting and action potentials in the isolated myelinated nerve fibre of the frog, *J. Physiol.* 135, 550.
- Hille, B., 1971, The permeability of the sodium channel to organic cations in myelinated nerve, *J. Gen. Physiol.* 58, 599.
- Narahashi, T., 1976, Effects of insecticides on nervous conduction and synaptic transmission, in: *Insecticide Biochemistry and Physiology*, ed. C.F. Wilkinson (Plenum Press, New York) p. 327.
- Van den Bercken, J., L.M.A. Akkermans and J.M. Van der Zalm, 1973, DDT-like action of allethrin in the sensory nervous system of *Xenopus laevis*, *European J. Pharmacol.* 21, 95.
- Wouters, W. and J. Van den Bercken, 1978, Action of pyrethroids, *Gen. Pharmac.* 9, 387.