The effects of the pyrethroids deltamethrin and cismethrin on nerve excitability in rats

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SUMMARY Deltamethrin produced a prolonged period of increased excitability following the passage of a nerve impulse, which was dose-related and lasted up to 400 ms. Excitability changes were detected without neurological signs following a single IV injection of 0.5 mg/kg and feeding 200, 100 and 50 ppm in the diet for up to 8 weeks. No changes were detected following 0.3 mg/kg IV or 25 ppm in the diet. No cumulative effects were detected during chronic feeding. Cismethrin produced increased nerve excitability only between 2 and 4 ms after a nerve impulse. Excitability changes after cismethrin were biphasic during the first 20 ms.

Many synthetic pyrethroid insecticides have been developed in recent years and are likely to be increasingly used, since they combine low mammalian toxicity with high insecticidal activity. They have the additional advantage of being rapidly metabolised. It is well known that their insecticidal action is a direct neurotoxic one, inducing hyperexcitability, tremors and eventual paralysis.

Early studies by Narahashi¹ demonstrated that during exposure to allethrin, one of the first pyrethroids to be synthesised, a prolonged negative after-potential followed the passage of a nerve impulse. In voltage clamp studies of giant axons, Narahashi and Anderson² showed that this was due to a prolonged inward sodium current. More details of the effect of allethrin and other pyrethroids on ionic movements have since been worked out using similar methods both on giant axons^{3,4} and on amphibian nodes of Ranvier.⁵ Van den Bercken and Vivjerberg⁵ concluded that deltamethrin held open m (activation) gates of nodes of Ranvier in frog myelinated fibres for a prolonged period. From their work on giant axons, Lund and Narahashi⁴ have concluded that under the influence of tetramethrin a proportion of sodium channels remain normal, but others not only remain open for a prolonged time after a nerve impulse but are

in an abnormal state when inactive as well as when open. Jacques *et al*⁶ have shown that the pyrethroid binding component is distinct from other sodium channel toxin receptors such as batrachotoxin, veratridine and dihydrograyanotoxin II and also from tetrodotoxin.

It was argued in a previous publication⁷ that the effects demonstrated in single fibres would be detectable as activity-related changes in threshold in whole nerve trunks. In normal nerves following the passage of a nerve impulse, excitability is increased for 20 ms after the end of the relative refractory period (super-normal phase) and a period of reduced excitability (subnormality) follows, lasting up to 200 ms. Following a single high dose of deltamethrin given intravenously excitability was found to be increased to a greater extent and lasted for up to 400 ms.⁷

Following the high dose of deltamethrin used in the previous study, severe abnormal writhing and jerking movements developed within 15 minutes. The effects of lower doses of pyrethroids on nerve excitability have now been studied. If excitability changes can be detected in the absence of clinical abnormalities, then application of the technique described here might have a useful role in the investigation of humans exposed to pyrethroids.

It is important to know whether cumulative effects occur during chronic exposure to pyrethroids. To investigate this possibility, deltamethrin was mixed with the animals' diet at various dose levels and for various lengths of time. Serial studies were carried out during and after administration of the diets.

Two distinct clinical syndromes have been described in rats following exposure to different pyrethroids.^{8,9}

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The syndrome, consisting of writhing and jerking movements (choreoathetosis) and salivation, produced by deltamethrin has been called the CS syndrome by Verschoyle and Aldridge.⁹ The other syndrome consists of fine, followed by coarse tremors and has been called the T syndrome.⁹ Although structure-activity relationships are not absolute, the presence of an α -cyano group appears to be associated with the CS syndrome.⁹ In the present experiments, in addition to deltamethrin which produces the CS syndrome, cismethrin has been studied as an example of a substance that produces the T syndrome and has no α -cyano group.

Methods

Animals

Male Sprague-Dawley rats, ranging in age from 3 to 5 months were used. Anaesthesia for intravenous dosing and electrophysiological study was with "Hypnorm" (Janssen) 0.4–0.5 mg/kg given intramuscularly as the main anaesthetic, supplemented where necessary by inhalation of a nitrous oxide/oxygen mixture given through a facial mask.

Administration of pyrethroids

Deltamethrin For intravenous administration, deltamethrin (Roussel) was dissolved in glycerol formal to make solutions containing 2.5 or 5.0 mg/ml. The femoral vein was exposed and the required dose was injected slowly, over 5–10 minutes. For oral administration, deltamethrin was dissolved in 2% arachis oil and mixed with powdered diet in a commercial food mixer. The animals were allowed free access to food throughout the period of dosing.

Cismethrin For intravenous administration cismethrin (Wellcome) was dissolved in glycerol formal to make a solution containing 10 mg/ml. Slow injection was given into the exposed femoral vein.

Electrophysiology

The methods used were similar to those described by Parkin and Le Quesne.⁷ The same stimulating and recording apparatus was used, but the stimulating electrodes consisted of thin strips of silver.¹⁰ These were found to give a better contact with the skin without danger of producing ischaemia than the tinned copper wire electrodes used in the previous study. The subcutaneous temperature of the tail was maintained at 31–35°C by heat provided from coiled polythene tubing situated under the tail and containing water pumped from a thermostatically controlled bath.

Two independently variable stimuli were used. S1 was always supramaximal and, as previously, S2 was adjusted to produce a $\frac{1}{3}$ maximal response. The time interval between stimuli was varied and at each time interval the amplitude of S1 alone was always measured to ensure that the preparation was stable. Minor fluctuations occurred and the stimulus intensity of S2 was adjusted as necessary. Results of an experiment were rejected if S2 did not remain constant. Statistical comparisons of results were made using Student's *t* test.

Results

CONTROL ANIMALS

The changes in amplitude of S2 when preceded by S1 at varying time intervals are illustrated in fig. 1. Forty-two animals were used to establish the control response. The results were similar to those described in the previous study.⁷ Supernormality was present from 4 to 20 ms, the largest response being at 6 ms when the conditioned response was 120% of its unconditioned value. Subnormality lasted from 30 to 200 ms. In order to ensure that the solvent for the pyrethroids had no effect on nerve excitability, five animals were given 0.1 ml glycerol formal IV. One hour later excitability changes were the same as in control animals (table 1).

DOSE RESPONSE EFFECTS OF IV DELTAMETHRIN

Groups of five animals were given single IV injections of deltamethrin in the following doses: 1.5, 1.0, 0.75, 0.5, 0.3 mg/kg.

Clinical features

Following injection of 1.5 mg/kg, excess salivation began within five minutes, followed by severe abnormal movements, consisting of coarse whole body tremors with writhing and jerking movements of the limbs and trunk (the CS syndrome as described by Verschoyle and Aldridge).⁹ Following injection of 1.0 and 0.75 mg/kg, salivation occurred as after the higher dose but the abnormal movements were less intense. Because of the intensity of the movements electrophysiological studies could not be carried out during the first few hours after injection. These animals were therefore studied after five hours. Following administration of 0.5 mg/kg, two out of five animals developed excessive salivation, but none of them had any abnormal movements. These animals were examined at one and five hours after injection. No clinical abnormalities were detected in any of the animals given 0.3 mg/kg, which were examined after one hour.

Electrophysiological findings

Excitability changes observed five hours after injection of 1.5 mg/kg are shown in fig 1 compared with those in control animals. The results are very similar to those found previously by Parkin and Le Quesne.⁷ The peak of supernormality occurred at 6 ms in both deltamethrin and control groups. However the mean value at 6 ms for five deltamethrin animals was 141 (SD 15.7)%, compared with a mean value of 120 (SD 11.5)% for 42 control animals. The difference was highly significant ($p < 0.001$). Supernormality persisted for up to 400 ms.

The results for the different doses of deltamethrin are shown in table 1 and fig 2 from which it can be seen that there was a clear dose-response effect. The

Fig 1 Percentage change in amplitude of response to a submaximal stimulus when preceded by a supramaximal conditioning stimulus at varying time intervals. Open circles—mean results for 42 control animals; closed circles—mean results for 5 animals 5 h after 1.5 mg/kg IV deltamethrin. Stippled area denotes + 2 SD from mean in control animals; vertical lines + 2 SD from mean in deltamethrin-treated animals.

differences between the groups were most evident at stimulus intervals between 30 and 100 ms. In order to increase the number of values for statistical comparison of the effects of different doses, the results at four time intervals (30, 50, 75 and 100 ms) have been combined and the results are shown in table 1. Prolongation of increased excitability was evident at five hours in animals given 1.5, 1.0, or 0.75 mg/kg. Although no neurological abnormalities developed following 0.5 mg/kg, prolonged supernormality was detected one hour after injection but not after five hours. No clinical or electrophysiological abnormalities were detected one hour after 0.3 mg/kg.

Five animals were examined 24 hours after 1.5 mg/kg. A slight persistent effect was detected at this time ($p < 0.05$) (table 1).

CHRONIC EXPOSURE TO DELTAMETHRIN

Groups of animals were given a diet containing deltamethrin in the following concentrations: 200, 100, 50 and 25 ppm for up to eight weeks. No clinical abnormality was detected in any of the animals.

Electrophysiological studies were carried out at intervals during dosing and some animals were examined one or two days after returning to a normal diet. The mean results after one week on different diets are shown in fig 3. There was no increase in supernormality at short stimulus intervals up to 20 ms. The results for four combined stimulus intervals, 30–100 ms, are shown in table 2. Increased excitability was present in animals fed 200, 100 and 50 ppm, but not in those given 25 ppm.

After one week, excitability was increased significantly more in animals fed 200 ppm than those

Table 1 Effects of IV deltamethrin. The values shown are combined results for stimulus intervals 30, 50, 75 and 100 ms. Clinical signs were graded: +++ excess salivation and choreoathetosis; ++ excess salivation and mild abnormal movements; + excess salivation only; — no abnormal signs. p^* — statistical comparison with control; p^{**} — statistical comparison with next higher dose.

Dose mg/kg	Clinical signs	Time (h)	n	S ₂ cond/S ₂ uncond % (SD)	p^*	p^{**}
1.5	+++	5	20	125.8 (8.7)	< 0.001	
1.0	++	5	20	106.7 (5.5)	< 0.001	< 0.001
0.75	++	5	20	104.0 (3.8)	< 0.001	> 0.05
0.5	—	5	20	99.4 (5.5)	> 0.1	< 0.01
0.5	+ or —	1	24	104.4 (4.8)	< 0.001	
0.3	—	1	20	99.7 (4.5)	> 0.1	< 0.01
1.5	—	24	20	100.6 (3.2)	< 0.05	
Glycerol formal	—	1	20	96.8 (6.7)	> 0.1	
Control	—		165	98.4 (4.4)

Fig 2 Mean excitability changes in groups of 5 rats 5 h after IV deltamethrin. ○ — 1.5 mg/kg; ● — 1.0 mg/kg; ▲ — 0.75 mg/kg; △ — 0.5 mg/kg.

Fig 3 Mean excitability changes after 1 week chronic feeding of deltamethrin. ● — 11 animals given 200 ppm; ■ — 4 animals given 100 ppm; □ — 4 animals given 50 ppm; ○ — 5 animals given 25 ppm.

given 100 ppm ($p < 0.05$). Although the effect was less in animals fed 50 than 100 ppm, the results were not significantly different.

In animals fed 200, 100 and 50 ppm abnormalities did not increase with time. Indeed changes were slightly less marked after four weeks than after one week, but significant differences were only found for the 200 ppm group ($p < 0.01$). No electrophysical

abnormality was detected 24–48 hours after return to a normal diet.

CISMETHRIN

Clinical features

Seven animals given 0.3 mg/kg IV cismethrin developed a different type of neurological disorder from that produced by deltamethrin. Within two

Table 2 Effects of exposure to different concentrations of deltamethrin in the diet for one week. The values shown are combined results for stimulus intervals 30, 50, 75 and 100 ms. Statistical comparison with control value: ** = $p < 0.001$; * = $p < 0.05$; NS = not significant.

Time	Dose		Dose		Dose		Dose	
	n	200 ppm S2 cond/ S2 uncond % (SD)	n	100 ppm S2 cond/ S2 uncond % (SD)	n	50 ppm S2 cond/ S2 uncond % (SD)	n	25 ppm S2 cond/ S2 uncond % (SD)
2 days	16	115.8 (13.0)**						
1 week	44	112.5 (7.8)**	16	107.8 (4.2)**	16	105.7 (6.3)**	20	99.2 (3.6) NS
4 weeks	40	107.9 (7.4)**	16	104.6 (6.0)**	16	103.3 (3.2)**		
8 weeks	20	110.2 (4.4)**						
1-2 days after	36	99.4 (3.9) NS						
Control	165	98.4 (4.4)

Fig 4 Mean excitability changes in ● — 5 animals 5 h after 1.5 mg/kg IV deltamethrin; ▲ — 7 animals 5 h after 3.0 mg/kg IV cismethrin; □ — 42 control animals.

Fig 5 Excitability changes 1 h after 3.0 mg/kg cismethrin. — values for individual animals; ● — mean values.

minutes of the injection fine whole body tremor developed and the animals became hypersensitive to sounds and touch (T syndrome of Verschoyle and Aldridge).⁹ No excessive salivation or abnormal movements of the type seen after deltamethrin occurred. Following the dose used in the present study, marked tremors continued for about one hour and then gradually subsided within the next five hours.

Electrophysiological findings

The mean results are shown in fig 4 where the findings five hours after cismethrin administration are compared with those following the highest dose of deltamethrin and control animals. It can be seen that cismethrin also caused an increased excitability, but that this occurred at short stimulus intervals only. At 2 ms, S2 conditioned/S2 unconditioned was 153 (SD 39.2)% in cismethrin treated animals, whereas nerves were still partially refractory in control animals. (S2 conditioned/S2 unconditioned: 84 (SD 28.5)%.) At 4 ms the value for cismethrin animals was 158 (SD

30.0)% compared with 118 (SD 16.1)% for control animals. In both instances the differences were highly statistically significant ($p < 0.001$). Subnormal excitability was present with stimulus intervals of 30 to 200 ms as in control animals.

A striking feature of the excitability changes following cismethrin was the biphasic increased excitability response in the first 20 ms (fig 5). It can be seen that there was considerable individual variation between animals. In some in whom excitability was within the range of that seen in control animals at all time intervals, there was nevertheless a biphasic response. This was never seen in control animals.

Discussion

Following a single intravenous dose of deltamethrin, a clear dose-response effect has been demonstrated on the prolongation of excitability following a nerve impulse. The no-effect level for excitability changes was 0.3 mg/kg single IV dose and exposure to 25 ppm

in the diet. Some electrophysiological effect could still be detected below levels which produced clinically detectable neurological changes, both following a single dose (0.5 mg/kg) and chronic feeding (200, 100 and 50 ppm). The technique may therefore be sufficiently sensitive to be valuable in studying humans with suspected toxicity.

As in the previous study by Parkin and Le Quesne⁷ a slight persistent effect was detected 24 hours after a single injection of 1.5 mg/kg deltamethrin.

In animals receiving deltamethrin in their diet there was a tendency for electrophysiological abnormalities to decrease with time. However no accurate measurement of food intake was made for individual animals and so no record is available of the actual quantity of deltamethrin consumed. Our impression was that the amount of food eaten did fall slightly. In order to study changes on constant exposure, more accurate control of intake would be necessary. However, in the present study there was no suggestion of any cumulative effect nor of any persistent effect when exposure ceased.

Deltamethrin and cismethrin were studied as examples of pyrethroids which produce different clinical syndromes, either the CS or the T syndrome. It has been suggested that the different effects depend on the presence (CS syndrome) or absence (T syndrome) of an α -cyano group in the pyrethroid molecule.⁹ Different physiological effects have also been described between the two groups of pyrethroids. Narahashi¹¹ has commented that in giant axons, compounds without an α -cyano group for example tetramethrin and allethrin, increase the depolarising after-potential and induce repetitive firing to a single stimulus. On the other hand those with an α -cyano group, for example deltamethrin, produce membrane depolarisation without electrical stimulation. He argues that both effects could result from a similar action on sodium channels, but differing in degree. Vivjerberg and Van den Bercken¹² showed that deltamethrin had a much more prolonged effect on the sodium tail current in voltage clamp studies of frog myelinated nodes of Ranvier (up to 1 s) than pyrethroids such as allethrin with no α -cyano group, which prolonged the tail current for less than 100 ms. As predicted from these observations, deltamethrin had a very prolonged effect on nerve excitability as demonstrated by the technique used here, whereas cismethrin increased excitability only during the first few milliseconds.

Following cismethrin, a biphasic change in excitability was present within the first 20 ms. This never occurred in control animals. The reasons for this are obscure. It could be postulated that it was due to an interaction of the factors responsible for increased excitability in normal nerve and those producing the

pyrethroid-induced increased excitability.

There is still some doubt as to the site of origin of abnormal neurological signs. The abnormal movements in the CS syndrome clearly originate in the central nervous system. Both electrophysiological and biochemical abnormalities have been demonstrated in the brain following deltamethrin administration.^{13,14} The origin of tremor in the T syndrome is uncertain. A peripheral nerve origin has been suggested⁹ but this has been questioned by Gray *et al.*¹⁵ who produced tremor by intracerebral injection. Whatever the origin of neurological abnormalities, the present study has shown a clear effect of both types of compound on peripheral nerve origin has been suggested⁹ but this effect on nerve membranes.

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