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STABILIZATION OF SODIUM CHANNEL STATES BY DELTAMETHRIN IN MOUSE NEUROBLASTOMA CELLS

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SUMMARY

1. The effect of the pyrethroid insecticide deltamethrin on sodium channels of mouse neuroblastoma cells was investigated using the patch-clamp technique. The study was aimed at determining how the effects of deltamethrin at the whole cell level would be reflected in the modified properties of single sodium channel currents.

2. Whole cell recordings showed that deltamethrin prolonged sodium currents in neuroblastoma cells by several orders of magnitude.

3. Single channel recordings showed that a variety of channel states were prolonged by deltamethrin. Not only was the open state prolonged by several orders of magnitude but a closed or inactivated state was also prolonged, leading to less frequent channel openings.

4. A subconducting state and a flickering state were observed in the presence of deltamethrin as well as a state in which channels opened with some delay after the termination of a depolarizing pulse.

5. The results are compatible with the hypothesis that deltamethrin stabilizes a variety of channel states by reducing the transition rates between them. This allows states that are normally very brief to be detected more easily.

INTRODUCTION

A number of naturally occurring neurotoxins have been used as tools to characterize various functional aspects of ion channels. Pyrethroids are synthetic derivatives of the natural toxins pyrethrins contained in the flowers of the *Chrysanthemum* species. The pyrethrins as well as the synthetic pyrethroids have been found to drastically prolong sodium current in both vertebrate and invertebrate nerve membranes (Narahashi, 1976, 1982, 1984, 1985; Wouters & van den Bercken, 1978; Ruigt, 1984).

Whereas the effects of pyrethroids on sodium channels have been studied at both the single-channel level (Yamamoto, Quandt & Narahashi, 1983; Yamamoto, Yeh & Narahashi, 1984; Holloway, Salgado, Wu & Narahashi, 1984) and whole cell level (Narahashi & Anderson, 1967; Wang, Narahashi & Scuka, 1972; Lund & Narahashi, 1981*a*, *b*; Vijverberg, van der Zalm & van den Bercken, 1982; Vijverberg, van der Zalm, van Kleef & van den Bercken, 1982; Ruigt, 1984), no study has examined these effects at both levels on the same preparation. We have performed such a study for

the effects of the pyrethroid deltamethrin on sodium channels of mouse neuroblastoma N1E-115 cells.

The goals of this study were to determine (1) how the effects of deltamethrin seen at the whole cell level are reflected in the properties of modified single sodium channels and (2) to determine whether there were any kinetic properties of modified single channel currents which could account for the mechanism of action of deltamethrin, and which could not be obtained simply from examining whole cell currents.

As expected, the prolonged sodium currents observed at the whole cell level were reflected in prolonged open times of single sodium channels. In addition, a subconducting state and a state in which the channel flickers between the open and closed state were seen in the presence of the drug. Similar channel behaviour was also seen in data obtained before drug treatment but as detailed in the Results, this could be explained without invoking different channel states. The single channel data also showed that the closed or inactivated state was stabilized in the presence of the drug, which was not obvious from whole cell current data. The data are consistent with the hypothesis that deltamethrin reduces the rate at which transitions can occur between channel states. Whole cell currents are prolonged due to stabilization of the open state. Stabilization of channel states makes deltamethrin a useful tool in studying channel kinetics which are normally very fast. A preliminary account of these results has been presented (Chinn & Narahashi, 1985).

METHODS

All experiments were performed with mouse neuroblastoma N1E-115 cells. Cells were maintained in tissue culture as described by Quandt & Narahashi (1982).

Whole cell current measurement

Currents were recorded using the patch-clamp technique originally developed by Hamill, Marty, Neher, Sakmann & Sigworth (1981). Electrodes having tip resistances of 0·5–2 MΩ were placed onto the cell surface, and the membrane under the pipette tip was ruptured by applying gentle suction to the pipette. The potential inside the cell was maintained at the ground level. An inverted command voltage (holding potential + command pulse) was applied to the external solution via a bath electrode (3 M-KCl–agar/Ag–AgCl). Currents through the pipette in response to the command voltages were recorded by a current-to-voltage (I/V) converter which was directly connected to the suction pipette via an Ag–AgCl pellet. The I/V converter consisted of a Burr–Brown 3523L operational amplifier with a 10 MΩ feed-back resistor. A part of the output voltage of the current recording was added to the command voltage to compensate for the series resistance. This compensation was performed empirically, by speeding up the capacity transient as much as possible without producing ringing.

Because current–voltage (I–V) curves for the sodium channel shift to more negative potentials during the first few minutes after beginning cell dialysis (Fernandez, Fox & Krasne, 1984), at least 20 min were allowed after rupturing the cell membrane before taking data. For whole cell experiments, the external solution contained (in mM): NaCl, 140; CsCl, 5; $CaCl_2$, 1·8; $MgCl_2$, 0·8; and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES), 5 (titrated with NaOH to pH 7·3). The internal solution contained (in mM): CsF, 145; NaF, 1·4; and HEPES, 5 (titrated to pH 7·0 with CsOH). The whole cell records shown are the raw traces and leakage and capacity currents have not been subtracted. In order to verify that currents recorded from cells exposed to deltamethrin originated from the sodium channels, tetrodotoxin (TTX) was applied at a concentration of 1 μM. For some of the I–V curves, the membrane currents were corrected for leakage and capacity components by subtracting records with TTX from those without TTX. Experiments with

TTX also showed that at potentials more negative than +30 mV the leakage I–V relationship was linear. Therefore, for some cells, the leakage current associated with a 20 mV hyperpolarizing pulse was extrapolated linearly to depolarized potentials up to +30 mV for leakage current correction.

Single-channel current measurement

Single-channel currents were recorded from excised inside-out membrane patches as described by Hamill *et al.* (1981) using a List EPC-5 patch clamp. Electrodes had tip resistances of 5–8 MΩ. For these experiments, the external sodium concentration was increased in order to augment the single-channel current amplitude. The external solution contained (in mM): NaCl, 250; CsCl, 5; $CaCl_2$, 1·8; $MgCl_2$, 0·8; and HEPES, 5 (titrated to pH 7·3 with NaOH). The internal solution contained (in mM): CsF, 145; NaF, 1·4; sucrose, 198; and HEPES, 5 (titrated to pH 7·0 with CsOH). Removal of capacity and leakage currents was performed by subtraction of an average of traces containing no channels from a trace or an average of traces containing channels. In experiments before drug treatment, sodium channels were often activated in all test pulses. In such cases, pulses of smaller amplitude were used to obtain blank traces and the average was scaled up before subtraction.

Test solution

For both whole cell and single channel experiments, deltamethrin (Roussel UCLAF, Marseille, France) was first dissolved in dimethylsulphoxide at a concentration of 10 mM. This stock solution was then diluted with external solution for whole cell experiments and internal solution for single-channel experiments to make the final concentration 10 μM. The test solutions prepared in this way formed a milky suspension. Therefore, whereas the apparent concentration of the suspension was 10 μM, the actual concentration of the drug in solution was unknown. The deltamethrin containing test solution was perfused into the chamber, replacing the solution lacking the drug.

Unless otherwise indicated, both whole cell and single-channel experiments were performed at 11 °C and recordings were filtered with a two-pole low pass filter with a cut-off frequency of 1 KHz. Pulses were generated with the use of a digital-to-analog converter driven by a DEC LSI 11/23. Data were recorded and analysed by means of the computer and subsequently written onto disk. The data are given as the mean ±S.D. where appropriate.

RESULTS

Whole cell experiments

Modification of whole cell sodium current by deltamethrin. Sodium current recorded from a whole cell was drastically modified by deltamethrin. An example of such an experiment is illustrated in Fig. 1*A* in which the sodium currents before and after exposure to 10 μM-deltamethrin are superimposed. Following deltamethrin treatment, the peak current was followed by a steady-state current during the depolarizing pulse. The tail current generated upon repolarization was very small in amplitude and decayed rapidly before drug treatment. However, in the deltamethrin-modified membrane a large tail current was generated and decayed very slowly.

Deltamethrin modification of sodium channel currents occurred gradually, over a period of minutes. Repetitive pulsing at a frequency of 1 Hz accelerated channel modification. Whereas the time course or frequency dependence of channel modification was not studied in detail, the following protocol was used to facilitate modification of sodium channels. Cells were bathed in deltamethrin (10 μM) and depolarized from a holding potential of −100 to −20 mV for 10–50 ms once every 0·5–1 s for approximately 10–15 min. The number of modified channels could be estimated from the increase in the holding current at −100 mV between pulses because the modified channels closed very slowly. After this procedure, the cell was

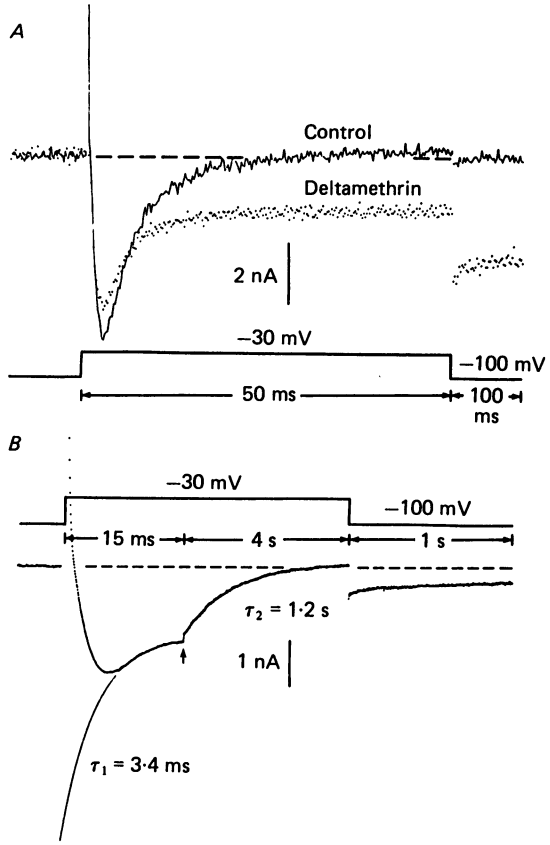

Fig. 1. *A*, whole cell sodium currents associated with a step depolarization from a holding potential of −100 to −30 mV before (continuous line) and after (dashed line) exposure to 10 μM-deltamethrin. Note that the time scale during and after the pulse is different. Records were taken at 12 °C and were not filtered. *B*, the depolarizing pulse was prolonged to 4015 ms so that the full decay phase at −30 mV could be seen. As indicated in the Figure, the time scale changes both during and after the voltage pulse. During the pulse, data for the first 15 ms were taken at a rate of 50 μs per point while the last 4 s (after the arrow) were taken at a rate of 10 ms per point. The current was fitted by the sum of two exponential functions (line through the decay phase). Time constants for the fast (τ_1) and slow (τ_2) phase were 3·4 ms and 1·2 s respectively for this cell. Records were taken at 11 °C and were filtered at 1 kHz. Records in *A* and *B* were not leakage subtracted.

washed with drug-free solution. The effect on channel modification of prolonged exposure to the drug or prolonged wash-out after drug exposure was not examined. However, over a period of at least 15 min after drug wash-out the modified current amplitudes at a given potential remained stable.

The effect of deltamethrin on peak amplitude was variable. Drug application for a period of 15–20 min caused a 21 ± 12 % decrease in five of the eight cells used (e.g. Fig. 1*A*), but in the other three cells it caused a 23 ± 29 % increase. However, in all the cells tested the sodium current was greatly prolonged by deltamethrin.

The slow decay phase of the sodium current during a depolarizing step is seen in

full in Fig. 1*B*. In this experiment the cell membrane was depolarized from a holding potential of −100 to −30 mV for 4015 ms. The record during the first 15 ms is shown on an expanded time scale. After the arrow, the record is shown on a more compressed time scale for the last 4 s. The decay phase was best fitted by the sum

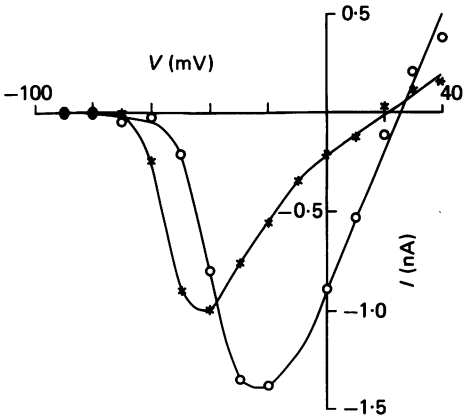

Fig. 2. *I–V* relationships for the sodium current recorded from a whole cell before (○) and after (*) exposure to 10 μM-deltamethrin. The curve obtained before drug treatment is a plot of peak sodium current obtained at each voltage. After exposure to deltamethrin, current amplitude 50 ms after the onset of the voltage pulse is plotted at each potential. Leakage currents were subtracted from both currents.

of two exponential functions, as shown by the line fit to the decay phase. The fast time constant was 3·4 ± 1·7 ms while the slow time constant was 1·20 ± 0·14 s ($n = 6$). Unmodified cells also displayed two time constants of decay, the fast one being 3·3 ± 1·0 ms and the slow one being 18·4 ± 14·4 ms ($n = 5$).

I–V relationships. *I–V* relationships before and after deltamethrin application are shown in Fig. 2. These *I–V* curves were obtained after subtraction of leakage currents. Before drug treatment, the membrane was held at −100 mV and step depolarized for 50 ms to various levels once every 3 s. The peak current amplitude is plotted against pulse potential in the Figure. To obtain the deltamethrin-modified *I–V* curve, channels were first modified as described previously. The membrane was then depolarized from a holding potential of −100 mV to various levels for 50 ms once every 30 s. The long interpulse interval allowed all of the modified channels to close. The modifed channel current was measured at the end of the depolarizing pulse (50 ms after the onset), a time at which normal channels were largely or completely inactivated. It should be noted that whereas the *I–V* curve before drug treatment plots the amplitude of the peak current, because the modified current was determined at a fixed time after the pulse onset, the peak of modified channel activity was not necessarily measured. This does not affect the conclusions drawn below.

As with other pyrethroids (Lund & Narahashi, 1981*a*; Salgado & Narahashi, 1982; Ruigt, 1984) the modified channels could be activated at a potential 10–20 mV more negative than normal channels. The reversal potential for sodium current did not seem to be affected (within ±5 mV) by deltamethrin. In four other cells, TTX was

found to block the inward currents of both normal and deltamethrin-treated cells, indicating that we are here dealing with the sodium channels.

Unlike pyrethroid data obtained by other investigators (Lund & Narahashi, 1981*a*, *b*; Ruigt, 1984), the *I–V* curve of modified channels rectified slightly between −10 and +20 mV (Fig. 2). One possible mechanism of this rectification is a change

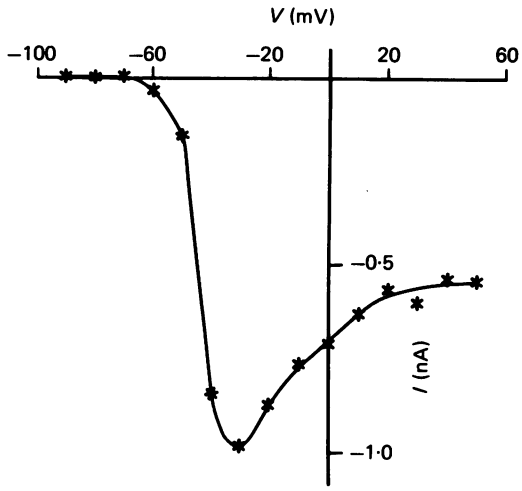

Fig. 3. Tail current amplitude plotted as a function of the membrane potential during a depolarizing step in a cell exposed to 10 μM-deltamethrin. The step depolarized for 50 ms to various membrane potential levels. Note that the number of channels open at each potential, as judged by the tail current amplitude, is greatly voltage dependent but at membrane potentials more positive than +10 mV this potential dependence is less pronounced.

in the number of channels activated at these potentials. The peak amplitude of the tail current was measured at −100 mV after a 50 ms depolarizing step to various potential levels. Because the tail current amplitude was always measured at the same potential, a change in this amplitude reflects a change in the number of channels opened 50 ms after the onset of the depolarizing pulse. The tail current amplitude is plotted against the membrane potential in Fig. 3. Indeed the tail amplitude, and hence the number of open channels, diminishes at the potential ranges from −30 to +10 mV, and reaches a limiting value at more positive potentials. Similar results were obtained in four other cells. The data shown in Fig. 3 were obtained from a cell different from that in Fig. 2 in which the voltage dependence at more positive potentials was not as pronounced.

Although the number of modified channels open does vary with pulse potential, it is possible to find points in the *I–V* curve (usually at potentials more positive than +10 mV) where the number of channels open, as judged by the tail current, is approximately constant and from which a reversal potential can be extrapolated. For all five cells tested, this extrapolated reversal potential for modified channel currents was approximately the same within ±5 mV as that for unmodified channel currents. This was confirmed by single channel conductance data to be discussed later.

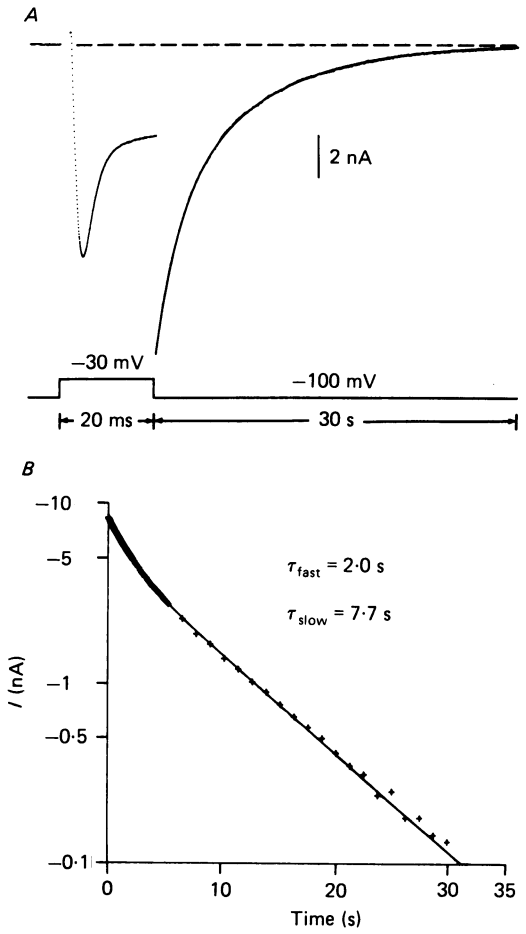

Fig. 4. Modified sodium channel tail current. *A*, time course of the tail current from a cell exposed to 10 μM-deltamethrin. The cell was depolarized from a holding potential of −100 to −30 mV for 20 ms and repolarized to −100 mV for 30 s. The tail current decay can be fitted by the sum of two exponential functions as shown by the line through the data. The time scales during the voltage pulse and during the tail current are different. *B*, semilogarithmic plot of points taken from the tail current shown in *A*. 130 points were taken from the initial 5·3 s of the tail current and 20 points from the remaining 24·7 s. The points were best fitted by the sum of two exponential functions (line through the points). τ_{fast} and τ_{slow} are time constants of the falling phase of the tail current.

Effect on tail currents. As has been found in squid and crayfish giant axons (Lund & Narahashi, 1983; Brown & Narahashi, 1985), deltamethrin greatly prolonged the sodium tail current upon repolarization in neuroblastoma cells. However, in contrast to squid and crayfish axons, the time constant of decay was of the order of seconds rather than minutes. Fig. 4*A* shows the decay of sodium current at −100 mV after a 20 ms voltage step to −30 mV. The time scale during the pulse and during the tail current is different. The tail current decay was fitted by a double exponential function as detailed below which overlies the current in Fig. 4*A*.

Points taken from the tail current in Fig. 4*A* are plotted in Fig. 4*B* on a semilogarithmic scale. The overlap of fast and slow components of the tail current was most prominent during the first several seconds of the current. Therefore, to better visualize the contribution of each to the over-all curve, 130 points were taken from the initial 5·3 s of the tail current whereas only 20 points were taken from the remaining 24·7 s. The line through the points shows the double exponential fit with decay time constants of 2·0 and 7·7 s. The amplitude of the fast phase was 3·6 nA and that of the slow phase was 4·7 nA. In the five cells tested, the tail current decayed with time constants of $2{\cdot}4 \pm 0{\cdot}5$ s and $6{\cdot}7 \pm 1{\cdot}4$ s. For these five cells, the fast phase comprised 44 ± 27 % of the tail current amplitude whereas the slow phase comprised 56 ± 27 % of the tail current.

Single channel experiments

Effect on kinetics. Single sodium channel currents recorded from an excised inside-out membrane patch before deltamethrin application are illustrated in Fig. 5*A*. Channels were activated by depolarizing the membrane from a holding potential of −100 to −30 mV for 140 ms every 3 s. Data were taken at a rate of 100 μs per point. In this and all other single channel traces, leakage and capacitive currents have been subtracted out (see Methods). In this membrane patch, channels were activated in all traces before drug treatment.

To modify the sodium channels by deltamethrin, the following procedure was used: a membrane patch was repetitively depolarized from a holding potential of −100 to −30 mV for 3140 ms. The interpulse interval was 3 s. The prolonged depolarizing pulses were used because of the slow time constants for channel inactivation as estimated from the whole cell data. This protocol ensured that channels activated during a pulse would be ready for activation by the onset of the next pulse.

Single sodium channel currents recorded from the same membrane patch as that in Fig. 5*A* but after 25 min of exposure to 10 μM-deltamethrin are shown in Fig. 5*B*. The time scale varies during the pulse. During the first 140 ms of the pulse, data were taken at a rate of 100 μs per point. During the remaining 3 s of the voltage pulse, data were taken at a rate of 10 ms per point. After exposure to deltamethrin, three changes are apparent. First, there are channels (traces 2 and 5) having short open times which may derive from unmodified channels. Secondly, there are channels whose open times are greatly prolonged (traces 3 and 4). Thirdly, in contrast to data before drug treatment, not all traces show channel activity (traces 1, 6, 7 and 8). With prolonged drug exposure ($\geqslant 1$ h) the number of channels having normal open times decreased drastically and the number of blank traces increased greatly. In five membrane patches, the percentage of blank traces increased from 1 ± 1 % before drug application to 57 ± 32 % in traces examined between 20 and 40 min after continuous drug exposure. Changing the pulse duration from 3140 to 140 ms did not alter the ability of deltamethrin to block or prolong channel activity.

The lack of channel openings observed after application of deltamethrin as shown in Fig. 5*B* was not due to repeated pulse applications or a prolonged experimental period. To avoid problems with sodium channels entering quiescent or hibernating periods, following the example of Horn & Vandenberg (1984) and Horn, Vandenberg

& Lange (1984), long interpulse intervals (at least 3 s) were used. Also, membrane patches which contained a large number of channels and exhibited high channel activity with multiple channel overlaps before drug treatment, were chosen for the experiments (Fig. 5*A*). In these experiments no changes in channel activity resulting in multiple blank sweeps were noticed before drug exposure. Furthermore, membrane

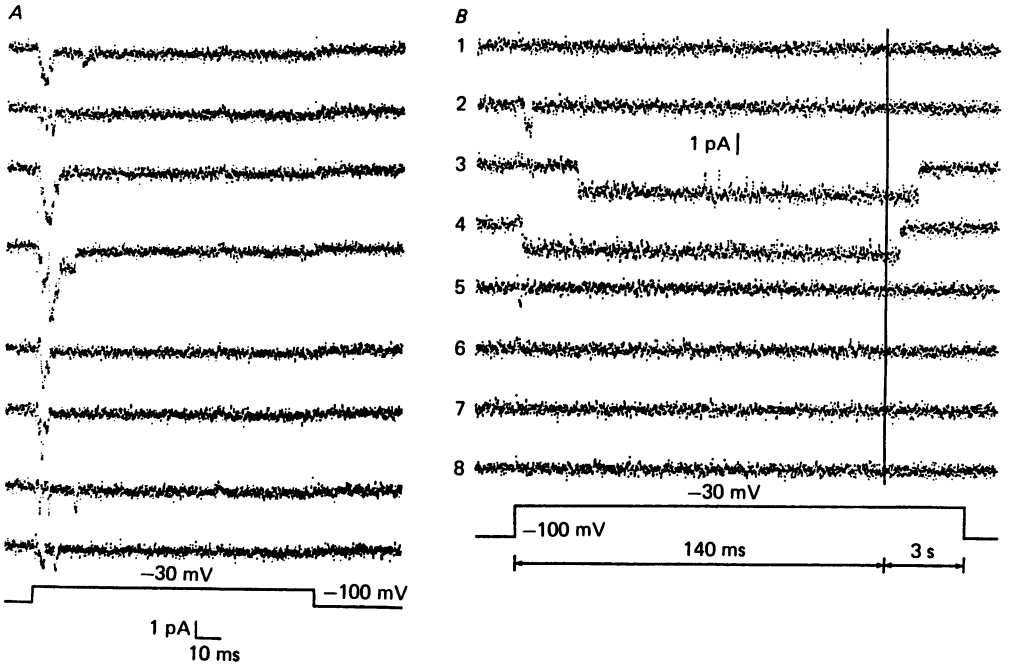

Fig. 5. Effects of deltamethrin on single sodium channel currents. *A*, currents from a cell before drug treatment in response to 140 ms depolarizing steps from a holding potential of −100 to −30 mV with a 3 s interpulse interval. Records were taken at a rate of 100 μs per point. *B*, currents after exposure to 10 μM-deltamethrin. The membrane patch was depolarized for 3140 ms from a holding potential of −100 to −30 mV. The interpulse interval was 3 s. The time scale changes during the voltage step as indicated in the Figure. During the first 140 ms, data records were taken at a rate of 100 μs per point and after the vertical line records were taken at a rate of 10 ms per point.

patches not exposed to the drug and which exhibited high channel activity remained active for periods similar to the duration of the experiments with deltamethrin.

Deltamethrin markedly slowed the time course of channel activity. This was demonstrated by comparing an averaged trace from normal single channels with an average of traces from modified channels after exposure to deltamethrin at high time resolution (100 μs per point) (Fig. 6*A*). Data from only the first 140 ms after the onset of the pulse are shown in the Figure. The traces containing modified channels were taken at a time when almost all of the open channels appeared to be modified as judged by their long open times, which ranged from a minimum of approximately 20 ms to several seconds. In the Figure, the peak amplitudes of the normal and drug-modified currents have been normalized to each other. It can be seen that the

peak of activity of drug-modified channels occurs later than normal channels. In five membrane patches the peak of the current before drug treatment occurred 8·2 ± 3·7 ms after the beginning of the depolarizing pulse, and after drug application it occurred 30·4 ± 12·0 ms after the onset of the pulse. Fig. 6*B* shows an average of

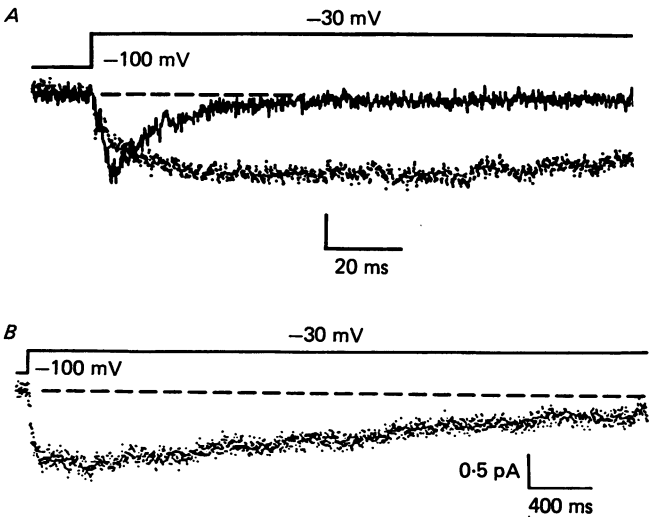

Fig. 6. *A*, an average of single channel current traces before (continuous line) and after (dotted line) exposure to 10 μM-deltamethrin. The peaks of the traces are normalized to each other. Note that deltamethrin increases the time to peak of the averaged current trace. Data were taken at a rate of 100 μs per point. For the curve before drug treatment, ninety-three traces were used in making the average and twelve blank traces were used for leakage and capacitive current subtraction as detailed in the Methods. For the deltamethrin modified curve, thirty-four traces were used to produce the average and fifty-nine blank traces were used for leakage and capacitive current subtraction. The vertical calibration bar represents 0·6 pA for the normal trace and 0·53 pA for the deltamethrin-modified trace. *B*, an average of current traces from a membrane patch in response to a 3·9 s depolarizing pulse from a holding potential of −100 to −30 mV. Data were taken at a rate of 2 ms per point. Forty-three traces were used in making the average and eleven blank traces were used for leakage and capacitive current subtraction.

modified channels from another patch in which the slow decay of channel activity is seen. In this experiment, the patch was depolarized from −100 to −30 mV for 3·9 s and the interpulse interval was 10 s. Channel activity was almost completely over after 3·9 s. Similar results were obtained in four other membrane patches.

The time course of inactivation of modified channels was studied with an open time histogram using data taken from six membrane patches in the presence of the drug. Membrane patches were depolarized from −100 to −30 mV for 3·9 s and the interpulse interval was 10 s. The number of modified channels having a given open time is plotted in Fig. 7. Because deltamethrin reduced channel activity, the total number of channels involved in this histogram is small (n = 335). Nevertheless, the data can be fitted by a single exponential function with a time constant of 1·1 s, similar to the slow time constant of decay of whole cell current at −30 mV (1·2 ± 0·1 s) (Fig. 1*B*).

Channel openings after a voltage pulse. The sodium channels modified by delta-

methrin opened not only during a depolarizing step but also after repolarization. This might explain the presence of a dual time constant of decay of whole cell tail current (Fig. 4*B*). One time constant would be due to closing of the activation gate and the other due to channels which opened after returning to the holding potential.

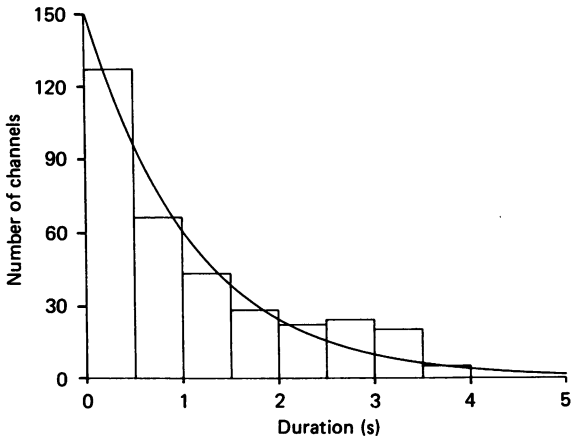

Fig. 7. Open time histogram of single sodium channel currents recorded from six membrane patches exposed to 10 μM-deltamethrin. The number of channels having a given open time is plotted. The histogram can be fitted by a single exponential decay time constant of 1·1 s.

A membrane patch containing modified channels was depolarized from a holding potential of −100 to −30 mV for 3 s and then repolarized for 6 s. Current records taken from one such experiment are shown in Fig. 8*A*. Recordings were made during the 3 s pulse and for the first 3 s after repolarization. It can be seen in all traces except trace 4 that, although no channels were open at the end of the pulse, channels opened after repolarization. It can also be seen in trace 8 that, while no channels were open during the depolarization, channels opened after repolarization. Late openings were observed in all four membrane patches examined. Despite late openings after terminating a depolarizing step, no channels opened spontaneously without depolarizing steps (Fig. 8*B*).

Single channel characteristics. Single sodium channel conductance was not significantly affected by deltamethrin. For each membrane patch, the slope conductance was calculated from the average single-channel current amplitude at −30 and 0 mV at 11 °C, both before and after deltamethrin exposure. The average of the slope conductances obtained was 14·9 ± 1·6 pS before and 16·4 ± 1·4 pS after (n = 4 membrane patches) exposure to deltamethrin.

The histogram of current amplitude of single deltamethrin-treated channels can be fitted with a single Gaussian distribution normalized to the data such that the total area enclosed by the histogram and the area under the Gaussian curve are the same. Fig. 9*A* shows an amplitude histogram at −30 mV using data from six membrane patches. The mean amplitude of the single channel currents was 1·37 ± 0·10 pA (n = 309).

In eight membrane patches, 5 % of the channels (n = 425) showed a subconducting

state during opening (Fig. 9*B* at the arrow). Because this state was rarely observed and brief in relation to the usual duration of a modified channel, it did not significantly affect the mean amplitude of either an individual channel or of the histogram. At −30 mV the mean amplitude and open time of single channel currents

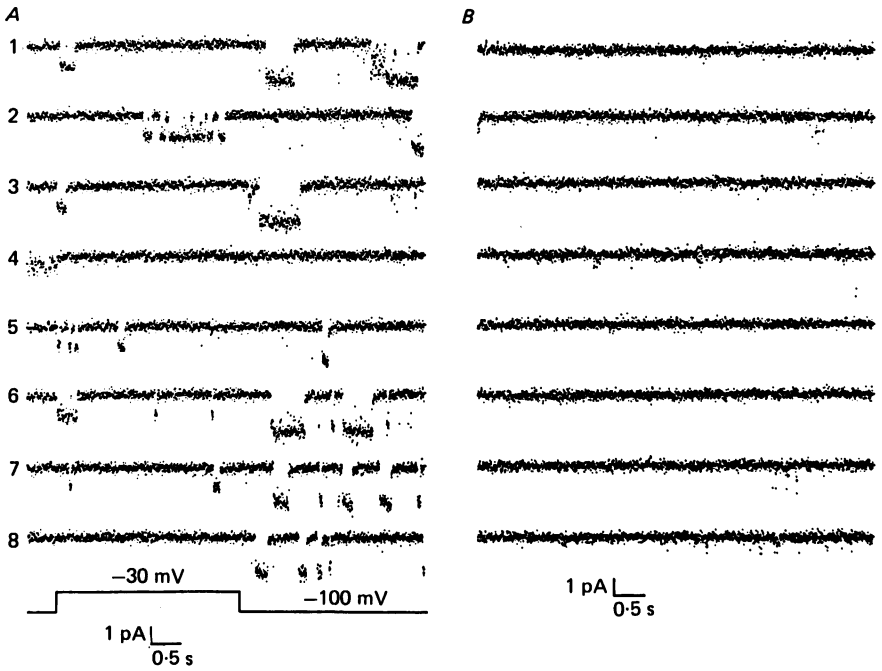

Fig. 8. Sodium channel openings after a voltage pulse in the presence of 10 μM-deltamethrin. *A*, a series of consecutive current traces taken from a membrane patch depolarized from a holding potential of −100 to −30 mV for 3 s and then repolarized for 6 s (only a 3 s period after each voltage step is shown). *B*, a series of consecutive current traces taken after the stimulus pulse was turned off. Note the lack of spontaneous channel activity at −100 mV. Data in *A* and *B* were taken at a rate of 10 ms per point.

in the subconducting state were estimated to be 0·91 ± 0·17 pA and 74 ± 54 ms (n = 20), respectively. Whereas channel currents of similar amplitude could be seen before drug treatment, it was not possible to determine if they represented a true subconductance state or were simply part of the normal distribution of single channel amplitudes. Normal channels were not seen to enter into a subconducting state from the fully open state as they did after modification. However, detecting this may have been hampered by their short lifetimes.

In eight membrane patches, flickerings of delthamethrin-modified sodium channels between the open and closed states were observed in 5% of the traces with channel opening (n = 425, 11 °C). Fig. 9*C* shows a channel flickering at 11 °C in which the first 140 ms of the 3140 ms voltage step are shown at an expanded time scale. Fig. 9*D* shows a channel flickering at 21 °C. Because the time scale is more compressed than the first 140 ms of Fig. 9*C*, the individual channel openings and closings are not resolved. The amplitudes of single-channel currents increased with in-

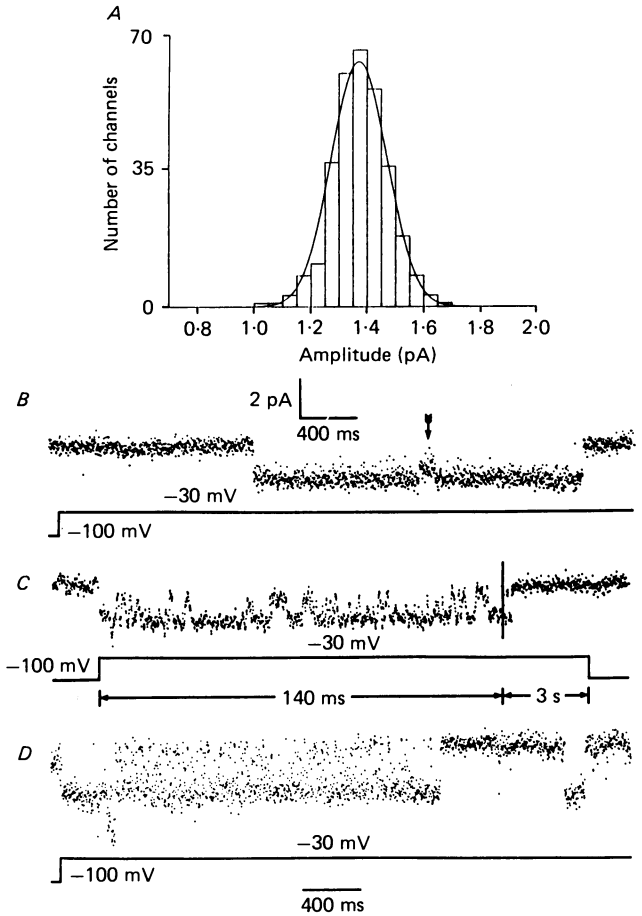

Fig. 9. Characteristics of single sodium channels modified by 10 μM-deltamethrin. In all experiments, channels were activated by depolarizing the membrane patch from −100 to −30 mV. In *A–C*, the data were taken at 11 °C. *A*, amplitude histogram of modified channels which is fitted by a single Gaussian distribution. *B*, subconducting state (at arrow) of a channel. The record was taken at a rate of 2 ms per point. *C*, flickerings of a channel in a different membrane patch from *B*. The time scale changes during the pulse as indicated in the Figure. During the first 140 ms of the pulse, data were taken at a rate of 100 μs per point and after the vertical line data were taken at a rate of 10 ms per point. *D*, flickerings of a channel in a different membrane patch at 21 °C. This record contains a non-flickering channel which opens 3·5 s after the pulse onset and shows that flickering does not affect current amplitude. Data were taken at a rate of 2 ms per point so that individual channel openings and closings of the flickering channel are not resolved.

creasing temperature. Fig. 9*D* shows that the single channel current amplitudes of both flickering and non-flickering channels were the same. Because the flickering state occurred in only 5% of the current traces a detailed analysis of this state has not yet been performed.

Current traces from unmodified membrane patches sometimes exhibited behaviour which appeared to be flickering. However, this behaviour was short lived (< 10 ms)

and could be due to sequential openings of several different channels. The flickering of modified channels cannot be due to multiple openings of different channels because a large number of channels would be required to exist in the membrane patch.

DISCUSSION

At the whole cell level, deltamethrin prolonged the open state of the sodium channel during a depolarizing pulse and after repolarization. The $I-V$ curve was shifted to more negative potentials by deltamethrin. Similar results have been reported previously with deltamethrin and other pyrethroids (Lund & Narahashi, 1981*a*; Salgado & Narahashi, 1982; Ruigt, 1984). In addition, the $I-V$ curve in deltamethrin rectified at potentials between -10 and $+20$ mV due to a decrease in the number of open channels in this potential region.

The prolonged sodium current observed at the whole cell level is reflected in extremely prolonged open times of single sodium channels during a depolarizing step and after repolarization. Similar results were obtained with another pyrethroid, fenvalerate (Holloway *et al.* 1984). In the presence of deltamethrin, delayed channel openings after a depolarizing voltage pulse, a subconducting state and a flickering state were observed. Deltamethrin was also found to reduce channel activity.

The effect of deltamethrin on the amplitude of the peak whole cell current was variable. This may be due to two opposing factors. First, deltamethrin increases channel open time. This increases the probability of channels being open simultaneously and would increase the current amplitude. An increase in sodium current has been previously hypothesized to occur after channel lifetime is prolonged by removal of inactivation (Armstrong, Bezanilla & Rojas, 1973; French & Horn, 1983). An increase in sodium current was sometimes observed after treatment of axons with pronase which removes inactivation (Salgado, Yeh & Narahashi, 1985). The second factor is that deltamethrin reduces channel activity. This would reduce whole cell current. The net result on the peak amplitude of whole cell current would thus depend not only on the number of channels which had been modified but also on these two opposing factors. The variation among membrane patches in the reduction of channel activity may also contribute to the variable effect of deltamethrin on peak amplitude.

Whereas we cannot present a detailed mechanism for the action of deltamethrin on the channel, a simplistic scheme consistent with the effects of deltamethrin on single-channel kinetics is that the drug stabilizes the various channel states by severely decreasing the rates of transition between them. All of the channel states observed in deltamethrin are assumed to be present in unmodified channels, but the drug greatly prolongs the period of time during which the channel remains in each state. This would explain both the prolonged open and closed states of the channel.

The occurrence of both the subconducting and flickering events in the presence of deltamethrin was rare, and this may explain why we did not observe normal channels entering a subconducting state and only rarely observed what appeared to be a flickering state in normal channels in which the lifetime of opening was much reduced. However, two conducting states for sodium channels have been reported in neuroblastoma cells (Nagy, Kiss & Hof, 1983; W. McCarthy & J. Z. Yeh, personal

communication), and a state of channel flickering was reported by Patlak & Ortiz (1985) in sodium channels of adult rat heart.

A lower rate of transition between the closed and open state would explain the increase in the time to the peak of activity in the averaged trace of modified single-channel current. However, because inactivation as well as activation occurs during a depolarizing step, the early peak of normal channels may be due to a faster inactivation of these channels. A better determinant of activation kinetics would be to compare the latency to first opening of channels in the normal and modified states. However, deltamethrin effectively reduces the number of active channels. For a multichannel membrane patch, the average latency to the first channel opening is increased as the number of channels in the patch decreases. To obtain a correct first opening time in this situation, one must measure the time to first opening of each channel in one patch. Such an analysis is complicated due to the possibility of channel reopenings. This problem does not exist for a patch containing only one channel. However, this experiment is difficult because (1) the patches containing only a single channel are rarely obtained and (2) the number of openings of that channel after deltamethrin modification would be reduced by the drug. Although a quantitative comparison of the latency for the first opening of a channel in the normal and the drug-modified state cannot be easily made, it can be stated that after exposure to deltamethrin, channels can activate at times when no normal channels would be activated, i.e. more than 1 s after the beginning of the depolarizing step (Fig. 8*A* trace 2, Fig. 9*B*). This observation is consistent with a lower transition rate between the closed and open states.

The scheme is also consistent with the observation of channel openings after a depolarizing pulse. A channel that has closed after activation during the depolarizing pulse would be in a state in which the inactivation (h) gate was closed and the activation (m) gate was open (state A). If deltamethrin stabilized state A and after repolarization the h gate opened before the m gate closed, this would result in the channel opening after the pulse. Stabilization of state A during the interpulse interval could result in a situation in which channels opened after but not during the pulse (Fig. 8*A* trace 8).

Channels remaining open at the end of the pulse would contribute to a single exponential decay time constant in the whole cell tail current. Channels which open after repolarization could contribute to the second exponential decay seen in the whole cell tail current in the presence of deltamethrin. Although tail currents observed in the presence of some pyrethroids have only a single exponential decay time constant (Vijverberg & van den Bercken, 1982), this would depend on (1) how long after the onset of the depolarizing pulse state A was stabilized and (2) the number and timing of late openings. In fact, late opening channels were postulated by Vijverberg & van den Bercken (1982) to be responsible for a hump in whole cell tail currents seen in vertebrate nodes of Ranvier exposed to some pyrethroids.

The multiple actions of deltamethrin on several channel states may be exerted by alteration of the interactions of the channel with its environment. Being a very lipophilic substance, deltamethrin may have access to the channel gating machinery via the lipid phase of the cell membrane. In keeping with this hypothesis, pyrethroids have recently been found to have no effect on the open sodium channel properties such as cation permeation and cation block (Yamamoto, Yeh & Narahashi, 1986).

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