Kinetics of Modulation of Tetrodotoxin-Sensitive and Tetrodotoxin-Resistant Sodium Channels by Tetramethrin and Deltamethrin

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ABSTRACT

Pyrethroid insecticides may be classified into two groups: type I pyrethroids lack a cyano group in the α-position, whereas type II pyrethroids have a cyano group. Both types prolong the sodium channel current thereby causing hyperexcitability, yet details of modulation of current kinetics remain largely to be seen. The mechanism of pyrethroid modulation of sodium currents was studied by the whole-cell patch-clamp technique with rat dorsal root ganglion neurons. Both deltamethrin (type II) and tetramethrin (type I) acted on both tetrodotoxin-sensitive and tetrodotoxin-resistant channels in a qualitatively similar manner and some quantitative differences were derived from different kinetics. During repetitive stimulation in the presence of deltamethrin, leak current increased due to accumulation of prolonged tail currents, explaining the apparent use-dependent modification. For tetramethrin-modified channels, such accumulation was much less because of faster kinetics. Slowing of the kinetics of sodium channel activation by deltamethrin was revealed even after the fast inactivation had been removed by papain. The kinetics of deltamethrin-modified sodium channels was fitted better by the equation that contained two activation components than that with one component. Deltamethrin caused a large shift of the conductance-voltage curve in the direction of hyperpolarization. Cell-attached patch-clamp experiments revealed that deltamethrin had much smaller mobility in the cell membrane than tetramethrin. It was concluded that the apparent use dependence of deltamethrin modification of sodium channels was due primarily to the accumulation of prolonged tail currents during repetitive stimulation and that the sodium channel activation mechanism is the major target of pyrethroids.

Pyrethroids are synthetic derivatives of pyrethrins, toxins contained in the flowers of some Chrysanthemum species, and are widely used as insecticides due to their high insecticidal potency, low mammalian toxicity, and biodegradability. They may be classified into two groups: type I pyrethroids (such as tetramethrin and allethrin) do not have a cyano group in the α-position, and type II pyrethroids (such as deltamethrin and fenvalerate) contain an α-cyano group. Previous studies of type I and type II pyrethroids have disclosed several important features of their mechanism of action on the sodium channels (Vijverberg and van den Bercken, 1980; Narahashi, 1992, 1996). It is generally observed that type II pyrethroids are more potent and slower in the onset, offset, and slowing of action than type I pyrethroids (Salgado et al., 1989; Tabarean and Narahashi, 1998). The prolongation of single sodium channel currents is more pronounced in type II than type I pyrethroids (Yamamoto et al., 1983, 1984; Chinn and Narahashi, 1986; Holloway et al., 1989), and type II pyrethroids slow the deactivation (the tail currents upon repolarization) of sodium channels to a greater extent than type I pyrethroids.

Rat dorsal root ganglion (DRG) neurons express both tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) sodium channels (Kostyuk et al., 1981; Roy and Narahashi, 1992; Elliott and Elliott, 1993; Ogata and Tatebayashi, 1993). The modulation of these two channel types by type I and type II pyrethroids has been described (Ginsburg and Narahashi, 1993; Tatebayashi and Narahashi, 1994; Tabarean and Narahashi, 1998).

Removal of fast inactivation (by proteases applied to the cytoplasmic side of the membrane) causes a negative shift in the voltage dependence of activation of sodium channels in preparations of neuronal origins (Gonoi and Hille, 1987; Cota and Armstrong, 1989). Although it is clear that the modulation of sodium channels by pyrethroids cannot be explained by modification of fast inactivation alone, there is a possibility that some of the effects observed (negative shift and slowing in rise time) and the effects on activation were actually caused via modification of fast inactivation.

We tested this hypothesis by applying tetramethrin or deltamethrin to sodium channels having fast inactivation

ABBREVIATIONS: DRG, dorsal root ganglion; TTX-S, tetrodotoxin-sensitive; TTX-R, tetrodotoxin-resistant.
removed with papain. The activation kinetics was slowed by deltamethrin even after fast inactivation was removed by papain. The sodium channel activation mechanism was deemed to be the major target of pyrethroids. The apparent use dependence of deltamethrin modification of sodium channels was found to be due primarily to the accumulation of prolonged tail currents during repetitive stimulation. These data are deemed important to gain further insight into the molecular mechanisms of action of pyrethroids on the sodium channels, and their utilization as chemical tools in the study of sodium channels.

Materials and Methods

Cell Preparation. Neurons were isolated from dorsal root ganglia as described previously (Tatebayashi and Narahashi, 1994). Rats (2–6 days postnatal) were anesthetized with methoxyflurane–channels, and their utilization as chemical tools in the study of prolonged tail currents during repetitive stimulation. These deemed to be the major target of pyrethroids. The apparent deltamethrin even after fast inactivation was removed by removed with papain. The activation kinetics was slowed by glucose, and 5 mM HEPES-acid. The pH was adjusted to 7.4 with 23°C). Narahashi, 1992). All experiments were performed at room temper-

Electrophysiological Recording. Sodium currents were recorded using the whole-cell patch-clamp technique (Hamill et al., 1981). Patch pipettes (0.4–1.2 MΩ) were made of borosilicate glass capillary tubes (1.5 mm inner diameter) by using a two-step vertical puller (model PP83; Narishige, Tokyo, Japan). The currents were recorded using a List EPC 7 patch-clamp amplifier (List Medical, Darmstadt, Germany). Currents filtered at 3 kHz with an eight-pole Bessel filter were digitized using an A/D converter (Digidata 1200; Axon Instruments, Foster City, CA) and stored on the hard disk of a computer. Voltage-pulse protocols were generated using a D/A converter (Digidata 1200; Axon Instruments). The data acquisition software was pClamp6 (Axon Instruments). The series resistance was compensated up to 50% of the pipette access resistance. Current signals were corrected for linear capacitive currents with the compensation circuits of the amplifier and the residual capacitive and leakage currents were corrected by linear subtraction.

For voltage-clamp experiments, the pipette solution contained 70 mM CsF, 65 mM CsCl, 10 mM NaCl, and 5 mM HEPES-acid. The pH was adjusted to 7.0 with CsOH. The external solution contained 25 mM NaCl, 20 mM tetraethylammonium-Cl, 75 mM tetramethylammonium-Cl, 5 mM CsCl, 1.8 mM CaCl2, 1 mM MgCl2, 25 mM MgCl2, and 5 mM HEPES-acid. The pH was adjusted to 7.4 with tetraethylammonium-OH. Lanthanum chloride (3 μM) was used to block calcium channel currents. Tetrodotoxin (200 nM) was used to separate TTX-R sodium current from TTX-S sodium current. For the study of the TTX-S currents, cells that expressed only TTX-S currents were used. In DRG neurons TTX-S currents can be distinguished easily from TTX-R currents by their faster kinetics (Roy and Narahashi, 1992). All experiments were performed at room temperature (21–23°C).

Perfusion System. Glass tubing was used instead of plastic tubing to prevent the pyrethroids, which are highly lipophilic compounds, from sticking to the inner surface of the perfusion system. The perfusion system was described in detail elsewhere (Tatebayashi and Narahashi, 1994). After each experiment the perfusion system and the bath were washed for 30 min with ethanol to remove the residual pyrethroids.

Chemicals. Stock solutions of deltamethrin (Roussel UCLAF, Marseille, France) and (+)-trans-isomer of tetramethrin (Sumitomo Chemical Co., Takarazuka, Japan) were made in dimethyl sulfoxide at a concentration of 10 mM. Dimethyl sulfoxide (0.1%, v/v) alone did not affect the sodium currents. All the other chemicals were pur-chased from Sigma. All washout experiments were performed using pyrethroid-free solution that contained the same concentration of dimethyl sulfoxide as the test solution.

Data Analysis. For the kinetic description of current traces, the following equation was used:

\[
I_{Na}(t) = (V - V_{rev})G_{Na}^e(1 - e^{-t/\tau_{m}})e^{-t/\tau_{h}}
\]

where \(I_{Na}\) is the peak Na\(^+\) current elicited by the voltage pulse; \(V\) is the test potential; \(V_{rev}\) is the reversal potential; \(\tau_m\) and \(\tau_h\) are the time constants of activation and inactivation, respectively; and \(G_{Na}^e\) is the maximum Na\(^+\) conductance. This equation is similar to the one from the Hodgkin-Huxley model (for which \(n = 3\)). Also a modified version, which includes a second activation component (\(G_{Na}^{e1}, \tau_{n1}, n1\), was also used:

\[
I_{Na}(t) = (V - V_{rev})[G_{Na}^e(1 - e^{-t/\tau_{m}})n + G_{Na}^{e1}(1 - e^{-t/\tau_{n1}})n1]e^{-t/\tau_{h}}
\]

For estimation of the apparent gating charge, the following equation was used (Sigworth, 1995):

\[
Q_{app} = kT\frac{dlnp_e}{dV}
\]

where \(Q_{app}\) is the apparent gating charge, \(p_e\) is the open probability of the channel, \(T\) is the absolute temperature, and \(k\) is the Boltzmann constant. This equation gives a lower bound of the total gating charge, approaching it in the limit of very negative potential (Sigworth, 1995).

Curve-fitting was done and graphs were produced withSigmaplot4.0 (Jandel Scientific, San Rafael, CA).

Results

Amplitude of Tail Current Induced by Pyrethroids Increases during Repetitive Stimulation. To assess the possible use dependence of the deltamethrin effect, a series of 50 consecutive depolarizing voltage steps (of 4- and 15-ms duration for TTX-S and TTX-R channels, respectively) from -110 to 0 mV were applied. In these experiments no interepi-sode leak subtraction was used to prevent the possible effect of the voltage steps required by this operation on the current elicited during the following episode. After deltamethrin treatment there was an increase (in the negative direction) in the current level upon repolarization (Fig. 1A), corresponding to the slow tail current induced by deltamethrin (Tabarean and Narahashi, 1998). When the depolarizing pulses were separated by a 1- to 2-s interval, no change in the peak sodium current amplitude was observed either before (Fig. 2A) or after deltamethrin application (Fig. 1B). This current remained relatively stable during the 50 depolarizing steps (Fig. 1B). Similar results were obtained for TTX-R currents (data not shown). However, if the 50 depolarizing steps were applied without a 1-s interval (the minimum time between episodes being limited only by the speed of the D/A converter, and the apparent delay being of the order of several milliseconds), a significant decay (∼20%) of the peak current was observed before deltamethrin treatment, reflecting that some channels entered inactivated states (Fig. 2B). After deltamethrin treatment the “leak” current (the amplitude of the current before the depolarizing step) and the tail current increased. Figure 1A shows that the leak current prior to a depolarizing step corresponds well with the amplitude of the tail current, which follows the preceding depolarizing step.
This proves that the variation in leak current is caused by persistent sodium current.

To estimate the amplitude of the peak current elicited by a depolarizing step, the leak current before a depolarizing step was scaled to the difference in driving force for sodium ions (at \(-110\) and \(0\) mV) and then subtracted from the current trace. Figure 2C shows currents elicited by step depolarizations to \(0\) mV from a holding potential of \(-110\) mV during a 50-steps series. The current traces (not corrected) were “aligned” to the level of the current at the end of the depolarizing pulse to give the best qualitative description of the data: the peak current decreased while the leak current (i.e., tail current) amplitude increased. Also, the time to peak was slightly increased (0.6 ms compared with 0.47 ms in the control) and decay of the current was slower, suggesting the contribution of some deltamethrin-modified channels.

The amplitude of the tail current was much larger after the 50th step relative to the amplitude of the tail current after the first step. However, if another series of 50 steps was applied again several seconds after the end of the previous series, the tail current amplitude after the first step was the same as or only slightly larger than that corresponding to the first step of the previous series of 50 steps (and much smaller than the tail current corresponding to the 50th step of the previous series). As previously reported (Tabarean and Narahashi, 1998) the effect of deltamethrin developed slowly, taking minutes after the application of the drug. Application of a series of depolarizing steps did not increase the speed of onset of the drug action (data not shown).

Similar experiments as those presented for TTX-S channels were performed for TTX-R channels and yielded qualitatively similar results. Figure 3, A and B, compare the peak currents (before and after deltamethrin treatment) and the amplitude of the slow tail current for a series of 50 depolarizing steps (from \(-110\) to \(0\) mV, no interepisode interval) for TTX-S and TTX-R currents. The data show that the peak current decay is similar in control conditions and after deltamethrin treatment, and that there is no correlation between the time course of this decrease and the increase in tail current. For TTX-R channels there was also a decrease in the tail current after the 10th step, and the peak decrease was more pronounced than that in TTX-S channels both before and after deltamethrin treatment. These effects are probably caused by the fact that longer depolarizing steps were used for the TTX-R channels (14 ms compared with 4 ms for TTX-S channels) because TTX-R channels have slower kinetics. A similar decrease in the tail current was observed for TTX-S channels, but a larger number of steps was required (data not shown). The decay of the tail current amplitude probably reflects the inactivation of deltamethrin-modified channels. It corresponds well with the decay of the deltamethrin-modified currents elicited by long depolarizing pulses, which has an exponential time course with a time constant of the order of hundreds of milliseconds (Tabarean and Narahashi, 1998).

For the type I pyrethroid tetramethrin, some tail current accumulation could be observed only during the first one to five episodes in a series (data not shown). Because the tail current was much faster than that induced by deltamethrin,
a shorter interpulse interval (less than 50 ms) was long enough to prevent tail current accumulation.

**Effects of Pyrethroids on Sodium Channels with Fast Inactivation Removed by Papain.** To study the effects of pyrethroids on the activation kinetics of sodium channels without complication due to the inactivation mechanism, experiments were performed using the cells in which inactivation had been removed. Intracellular perfusion with papain (0.5 mg/ml) removed the fast inactivation of both TTX-S and TTX-R channels. Within minutes after starting the whole-cell recording, a noninactivating current followed the transient current. This effect developed slowly in time. The effect of papain was accompanied by a negative shift by ~10 mV in the voltage dependence of activation (data not shown). After all or a large fraction of the channels was modified by papain, tetramethrin or deltamethrin was applied in the extracellular solution. Figure 4 shows the effect of 1 µM tetramethrin on TTX-S and TTX-R channels with fast inactivation removed. Tetramethrin caused a negative shift in the activation characteristics of the channels: larger currents than the control were activated for step depolarizations to potentials close to the activation potential for both types of currents (~60 and ~50 mV for TTX-S and ~40 and ~30 mV for TTX-R). The rate of decay of the current during depolarization was greatly accelerated by tetramethrin at these test potentials. However, for larger depolarizations both of these effects were much reduced. The effect of tetramethrin had a fast onset and washed out within 3 min after being removed from the bath solution.

The effects of deltamethrin on sodium currents were similar to those observed for normal channels (with fast inactivation intact). The deltamethrin-modified currents had a much slower onset, were activated at potentials 20 to 30 mV more negative than the control, and decayed slowly in both TTX-S and TTX-R currents (Fig. 5).

**Kinetic Description of Time Course of Deltamethrin-Modified TTX-S and TTX-R Currents.** We have previously reported that deltamethrin-modified sodium channels activated much more slowly than normal, unmodified channels, and that the currents displayed a very slow rise toward a peak followed by a similarly slow decay (Tabarean and...
modified sodium channels.

Activation was reflected in larger

rents elicited for test steps to potentials near the threshold of

control currents. As expected, the slower kinetics of the cur-

rents elicited by depolarizations near the threshold of acti-

F

TABLE 1
The time constants of activation ($\tau_{a}$) of normal and deltamethrin-modified sodium channels

<table>
<thead>
<tr>
<th>Channels</th>
<th>$\tau_{a}$ at Threshold (ms)</th>
<th>$\tau_{a}$ of Fast Component at Maximal Activation (ms)</th>
<th>Number of Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTX-S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.5–4</td>
<td>0.18–0.2</td>
<td>3</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>200–700</td>
<td>5–10</td>
<td>6</td>
</tr>
<tr>
<td>TTX-R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5–10</td>
<td>1.5–4</td>
<td>4</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>500–1000</td>
<td>15–50</td>
<td>8</td>
</tr>
</tbody>
</table>

Narahashi, 1998). The kinetics of deltamethrin-modified so-

dium current was analyzed by fitting to the kinetic eqs. 1 and

Equation 1 describes the time course of normal voltage-

gated sodium currents. We used this equation to characterize

the kinetics of the sodium currents. Equation 1 fitted well the

control currents. As expected, the slower kinetics of the cur-
ents elicited for test steps to potentials near the threshold of

activation was reflected in larger $\tau_{a}$ values than those ob-

tained for the currents elicited by larger depolarizations (Ta-

ble 1).

For deltamethrin-modified (both TTX-S and TTX-R) cur-

cents elicited by depolarizations near the threshold of acti-

vation, subunitary $n$ coefficients of eq. 1 were required to fit

adequately the data (Fig. 6, A and D) and the $\tau_{a}$ values

required were in the order of hundreds of milliseconds (Table

1). For more depolarized potentials the fit with eq. 1 yielded

smaller $\tau_{a}$ values (Table 1) and larger $n$ values (in the range

1–2.6). However, eq. 1 could not fit adequately the current

transients. The clear discrepancy appeared in the region of the

peak: the curve fit always yielded a “sharper” peak than the
current trace (dotted line in Fig. 6B, C, E, and F). Instead,

perfect fits could be obtained by introducing a second term in

the activation component (eq. 2; see Materials and Methods)

(Fig. 6B, C, E, and F: the fitted curves cannot be distin-

guished from the current traces).

Fig. 6. Characterization of the kinetics of activation of deltamethrin-

modified TTX-R (A–C) and TTX-S (D–F) sodium currents elicited

by depolarizations from $-110$ mV to the indicated potentials. A, TTX-R

current trace (solid line) at $-50$ mV was fitted with eq. 1 with

$n = 0.7$ and $\tau_{a} = 870$ ms (dotted line), $n = 1$ and $\tau_{a} = 432$ ms (dashed line), or $n = 3$

and $\tau_{a} = 149$ ms (dashed and dotted line). B, TTX-R current traces (solid line) at $-40$ mV could not be fitted adequately with eq. 1 (dotted line: $n = .6$ and $\tau_{a} = 87$ ms) but could be fitted very well with eq. 2 (dashed line, which overlaps with the current trace: $n = 2.2$ and $\tau_{a} = 9$ ms, $nI = 1.1$

and $\tau_{a,I} = 208$ ms). C, TTX-R current trace (solid line) at $-30$ mV could not be fitted adequately with eq. 1 (dotted line: $n = .6$ and $\tau_{a} = 87$ ms) but could be fitted very well with eq. 2 (dashed line, which overlaps with the current trace: $n = 2.2$ and $\tau_{a} = 7.1$ ms, $nI = 1.3$ and $\tau_{a,I} = 212$ ms). D, TTX-R current trace (solid line) at $-20$ mV could not be fitted adequately with eq. 1 (dotted line: $n = .6$ and $\tau_{a} = 30$ ms) but could be fitted very well with eq. 2 (dashed line, which overlaps with the current trace: $n = 2.2$ and $\tau_{a} = 7.1$ ms, $nI = 1.3$ and $\tau_{a,I} = 212$ ms). E, TTX-R current trace (solid line) at $-10$ mV could not be fitted adequately with eq. 1 (dotted line: $n = .6$ and $\tau_{a} = 30$ ms) but could be fitted very well with eq. 2 (dashed line, which overlaps with the current trace: $n = 2.2$ and $\tau_{a} = 7.1$ ms, $nI = 1.3$ and $\tau_{a,I} = 212$ ms). F, TTX-S current trace (solid line) at $-40$ mV could be fitted more adequately with eq. 2 (dashed line, which overlaps with the current traces: $n = 4$, $\tau_{a} = 10$ ms, $nI = 1.2$ and $\tau_{a,I} = 285$ ms) than with eq. 1 (dotted line: $n = 2.7$ and $\tau_{a} = 14.4$ ms).
It is interesting to note that, although the values did differ from cell to cell, $\tau_{m}$ and $\tau_{tail}$ were always severalfold different (one being in the range of tens or hundreds of milliseconds, whereas the other in the range of milliseconds or tens of milliseconds), and the component with a smaller $\tau_{m}$ had a larger $n$. Also, the faster component always had a $\tau_{m}$ of an order of magnitude larger than the $\tau_{m}$ of the control. Thus, although the faster component appeared only at more depolarized potentials (where normal channels are activated as well), the current was not carried by the normal channels but by deltamethrin-modified channels (Table 1). It should be noted that the values obtained by fitting the time course of currents after fast inactivation had been removed with papain (before being treated with deltamethrin) yielded results in the same range as the currents that were not modified by papain.

Conductance-Voltage Relationship of Deltamethrin-Modified Channels. Effects of deltamethrin on voltage dependence of sodium channel activation were analyzed using preparations in which fast inactivation had been removed by papain. Figure 7 shows the conductance-voltage relationship for control sodium channels and deltamethrin-modified channels (after removal of fast inactivation). The conductance was measured both at the end of the depolarizing pulse (open triangles) and from the tail current amplitude upon repolarization to $-110$ mV (filled triangles). Deltamethrin shifted the conductance-voltage relationship by 20 mV in the hyperpolarizing direction. The conductance increased exponentially in the range of negative potentials for both TTX-S and TTX-R deltamethrin-modified channels. The slope of the linear region of the log-conductance-voltage relationship was 0.23 for TTX-S channels, and 0.18 for TTX-R channels. Applying these values to eq. 3 (see Materials and Methods) yielded an apparent gating charge of 6.1 e$^-$ for TTX-S channels and 4.8 e$^-$ for TTX-R channels. In the calculation, it is assumed that single-channel conductance is constant at least in this potential range, and that the conductance is directly proportional to the open probability ($p_o$), and thus eq. 3 can be applied for the conductance data.

Voltage Dependence of Deactivation Kinetics Is Altered by Tetramethrin and Deltamethrin. Upon returning to hyperpolarized membrane potentials, the activated sodium channels rapidly deactivate. The rate of deactivation became faster at more negative membrane potentials for normal TTX-R channels (Fig. 8A). Figure 8, B and C, shows the deactivation time constants of control TTX-S and TTX-R channels, respectively, obtained by fitting the tail currents with a single exponential function. The deactivation kinetics was markedly slowed by pyrethroids. In the presence of tetramethrin (Fig. 9) or deltamethrin (Fig. 10) this voltage dependence of the deactivation kinetics remained steep for both TTX-S and TTX-R currents but the time constants of decay ($\tau_{tail}$) were much larger than those of the control: in the order of milliseconds for tetramethrin or hundreds of milliseconds for deltamethrin (at $-110$ mV). Figures 9, B and C, and 10, B and C, present the time constants of decay of the tail currents of tetramethrin- and deltamethrin-modified channels, respectively. The pyrethroids also induced a more gradual voltage dependence: the voltage dependence was well fitted by an exponential function for modified channels, whereas for the control the voltage dependence was steeper (on a logarithmic scale it was not linear but exponential). It is interesting to note that the slope of the linear fit to the voltage dependence of $\tau_{tail}$ (Figs. 9, B and C, and 10, B and C) was the same for both TTX-S and TTX-R channels modified by the same pyrethroid: $\sim 0.028$ for deltamethrin (data from three cells for TTX-S channels and three cells for TTX-R channels) and $\sim 0.016$ for tetramethrin (data from three cells for TTX-S currents and three cells for TTX-R currents).

Recordings from a Cell-Attached Patch Suggest That Deltamethrin Has a Much Lower Mobility in the Membrane than Tetramethrin. To compare the mobility of tetramethrin and deltamethrin in the membrane, experiments were performed using the following protocol. Sodium currents were recorded from cell-attached patches. The pipette solution was the one used as external solution (thus, accidental detachment of the patch from the cell could be easily monitored as a large change in the reversal potential and amplitude of the sodium current recorded from the patch). The patch was hyperpolarized by 50 mV from the cell’s resting membrane potential by applying a 50-mV holding potential from which depolarizations by 70 to 130 mV were applied. In these experiments TTX-S and TTX-R currents were not separated. Tetramethrin or deltamethrin was applied in the bath. Within 3 min after applying 1 $\mu$M tetramethrin in the bath solution, the effect of tetramethrin was observed for...
the currents recorded from the patch: slow tail currents were recorded upon repolarization (Fig. 11, A and B). The effect of tetramethrin disappeared within 10 min after removing the drug from the bath solution. Deltamethrin (1 μM) applied similarly did not elicit any tail current even after 40 min of application. However, if cell-attached patches were obtained from cells pretreated with deltamethrin, the currents recorded displayed the very slow tail currents typical for deltamethrin-modified channels (Fig. 11C). These experiments indicated that deltamethrin had a much slower mobility in the membrane than tetramethrin. This also suggests that deltamethrin can modify the sodium channels in their resting state.

**Discussion**

This article represents further elucidation of the mechanism of sodium channel modification caused by pyrethroids. As an extension of our previous study (Tabarean and Narahashi, 1998), several new features have been unveiled. 1) This article shows for the first time the effects of delta- methrin and tetramethrin on sodium channels that have inactivation removed by papain. This allowed us to show that the pyrethroid effects are due mainly to modification of the activation process. 2) Apparent use-dependent effect of deltamethrin can be explained by accumulation of prolonged tail 

![Fig. 8](image-url). A, TTX-R control tail currents elicited upon repolarization to the indicated test potentials. B, voltage dependence of $\tau_{\text{tail}}$ (the time constant of the single exponential fit to the tail current decay) of normal TTX-S currents (pooled data from four cells). C, voltage dependence of $\tau_{\text{tail}}$ of normal TTX-R currents (pooled data from six cells).

![Fig. 9](image-url). A, TTX-S tail currents (in the presence of 1 μM tetramethrin) elicited upon repolarization to the indicated test potentials. B, voltage dependence of $\tau_{\text{tail}}$ (the time constant of the single exponential fit to the tail current decay) of tetramethrin-modified TTX-S currents (pooled data from three cells). C, voltage dependence of $\tau_{\text{tail}}$ of tetramethrin-modified TTX-R currents (pooled data from three cells).
currents during repetitive stimulation. 3) We provide a quantitative description of the deltamethrin-modified currents and propose a modified version of the Hodgkin-Huxley equation, which can adequately fit the data. 4) We present the voltage dependence of the tail currents: control, tetramethrin, and deltamethrin-modified. Although both tetramethrin and deltamethrin have different affinity for TTX-R and TTX-S channels, the tail currents induced by these drugs have the same voltage dependence for the two channel types (the slope is 0.016 for tetramethrin and 0.028 for deltamethrin).

During repetitive stimulation with no interpulse interval the tail current of pyrethroid-modified sodium channels increases while the peak current decreases (for both TTX-S and TTX-R sodium currents). At first sight this finding suggests that we are dealing with a use-dependent effect. However, the tail current amplitude increases because of an increase in the number of modified channels, whereas the fast peak current decreases because of fewer normal rapidly activating and inactivating channels. A higher affinity of deltamethrin for the activated channels than for the resting channels could explain such a use-dependent effect. However, our data show that a decrease in peak current with similar amplitude and

![Figure 10](image1.png)

**Fig. 10.** A, TTX-R tail currents (after 1 μM deltamethrin treatment) elicited upon repolarization to the indicated test potentials. B, voltage dependence of $\tau_{\text{tail}}$ (the time constant of the single exponential fit to the tail current decay) of deltamethrin-modified TTX-S currents (pooled data from three cells). C, voltage dependence of $\tau_{\text{tail}}$ of deltamethrin-modified TTX-R currents (pooled data from three cells).

![Figure 11](image2.png)

**Fig. 11.** A, sodium currents recorded from a cell-attached patch. B, sodium currents recorded from the same membrane patch as in A but after bath application of 1 μM tetramethrin. The currents had a slower decay, and upon repolarization typical tetramethrin-induced tail currents were produced (arrow, three of three patches). This shows that tetramethrin can diffuse rapidly within or through the membrane. A similar protocol with 1 μM deltamethrin failed to transform the currents recorded from a cell-attached patch (data not shown) (four of four patches). C, sodium currents recorded from a cell-attached patch from a cell pre-treated with 1 μM deltamethrin. Upon repolarization typical slow tail currents were observed (arrow). Similar currents were recorded in three other patches of pretreated cells.
time course can be observed when repetitive stimulation with no interpulse interval is applied even in the absence of pyrethroids. We have previously reported that deltamethrin-modified sodium channels (both TTX-S and TTX-R) in DRG neurons activate and inactivate much more slowly than normal channels (Tabarean and Narahashi, 1998). Only a part of the modified channels will be activated during a short (4–10 ms) depolarizing step, but the activated channels will close slowly (by either deactivation or inactivation) and a part of them will remain open during subsequent depolarizations. Thus, the increase in the amplitude of the tail current during repetitive stimulation probably represents accumulation of activated modified channels, rather than progressive modification of channels by deltamethrin.

The effects described above are not observed when an interpulse interval of 1 to 2 s is applied. This can be explained by the fact that during this interval (at –110 mV) the modified channels can deactivate and the normal channels recover from inactivation. Thus, each voltage step finds the channels in the same (statistical) state and consequently yields the same currents. Another possibility is that the drug unbinds from the use-dependent site on the channel within this short period (1–2 s) and thus a possible use-dependent effect can be observed only at a higher frequency of stimulation. However, if the presumed use-dependent modification took place at the same binding site (which would be more accessible to deltamethrin when the channel is activated), the unbinding rate of the drug should be the same and consequently the tail current amplitude after 50 steps should remain stable instead of recovering to the initial level. There remains the possibility that use-dependent modification occurs at a different site. However, it is highly unlikely that modification at a different site would affect the channels’ gating in the same way: the tail currents (for all the 50 steps in a series) have the same time course of decay (data not shown). Although we cannot rule out a use-dependent component of pyrethroid modification, our data show that the increase in tail current during repetitive stimulation (in parallel with a decrease in the peak of the transient sodium current) can be explained by accumulation of the modified channels in the activated state. The much lesser accumulation observed for tetramethrin can be explained by the fact that the activation of tetramethrin-modified channel is much faster than the activation of deltamethrin-modified channels. Most of the tetramethrin-modified channels are activated during the first depolarizing pulse.

Both tetramethrin and deltamethrin shifted the activation voltage of papain-modified channels in the hyperpolarizing direction, indicating that this shift is caused by the direct action of the drug on the activation process and not via modification of fast inactivation. The two pyrethroids also induced other effects observed for channels with fast inactivation intact: slow tail currents and slowed rise time of the modified currents. However, for tetramethrin the rise time of the currents was increased only at the threshold of activation voltage, whereas for deltamethrin this slow rise time was larger and occurred over a wider voltage range. This is in agreement with the general pattern of deltamethrin (a type II pyrethroid) causing more efficacious effects than tetramethrin (a type I pyrethroid). It should be noted that the currents induced by tetramethrin at potentials near the threshold of activation had a characteristic shape: a large increase in current followed by a relatively fast decay (faster than the papain-modified current). This effect was not observed for deltamethrin and constitutes the only clear discrepancy between the effects caused by the two pyrethroids.

A previous study in neuroblastoma cells has found that deltamethrin strongly prolongs the open time of single sodium channels and frequently induces subconductance states, suggesting that this drug stabilizes sodium channel states (Chinn and Narahashi, 1986). We have previously shown (and the data presented above provide further evidence) that deltamethrin slows the activation and deactivation processes of sodium channels (Tabarean and Narahashi, 1998). This change in the kinetics of these processes will result in more stable open and closed states: the channels will open more slowly (the resting state appearing more stable) and close more slowly (the open state appearing more stable). The slowing of the kinetics of activation and inactivation can explain the negative shift in the voltage dependence of activation of deltamethrin-modified sodium channels. “Stabilization” of activated states increases the probability of opening at negative potentials where the normal channels undergo incomplete activation (normal channels deactivate faster at more negative potentials). This explanation is particularly appealing if activation is thought of as a concerted conformational change that takes place in the different domains of the channel. Stabilization by deltamethrin will increase the probability of finding more of the “activation gates” of a channel in an activated state simultaneously, and thus increase the probability of the channel reaching a fully activated state and open at negative potentials (where the activated states are short-lived for normal channels).

A similar mechanism may explain the effects of tetramethrin: slowing of the activation and deactivation processes of the channels will cause a negative shift in the voltage dependence of activation. The slower activation is obvious for tetramethrin-modified currents only at the near threshold voltage of activation, possibly because the negative shift in gating caused by this drug (Tatebayashi and Narahashi, 1994; present study) may make this effect less obvious. There remains, however, the discrepancy between deltamethrin and tetramethrin modification: the latter induces currents that decay faster than the (papain-modified) control currents at potentials close to the threshold of activation. At more positive potentials (where the difference in amplitude between modified-channels and control also decreases) this effect was much less pronounced (Fig. 4), as if tetramethrin-modified channels enter a normal “gating” mode. This also suggests that the additionally activated channels are the ones that decay faster. Thus, the tetramethrin-modified channels appear to have two gating modes: one, manifest at potentials near the threshold of activation only, yields fast decaying currents; and a second one, similar to the gating mode of normal channels (apart from slower deactivation reflected in the tail currents). It is puzzling that for potentials near the threshold of activation tetramethrin increases the probability of a channel reaching the fully activated state (open state) but this state is short-lived, as if tetramethrin is destabilizing the open state. However, as noted above these fast decaying currents reflect an “additional” gating mode. Thus, the lifetime of the tetramethrin-induced open states should not be compared with the one of normal open states,
but with the lifetime of the incompletely activated states (at the same test potential) of normal channels. Obviously, this is a difficult task because these states are nonconducting, but, intuitively, it seems plausible that these states are shorter than the open time of the normal channels. It is interesting to note that when fast inactivation is not removed by papain these fast decaying currents are not recorded in the presence of tetramethrin (Tatebayashi and Narahashi, 1994; present study). Inactivation may be fast enough to cause the closing (by the inactivation gate) of the modified channels before they open. A similar effect was not found for deltamethrin (probably because all the deltamethrin-induced open states are of longer duration than the control ones).

It is important to note that the very slow rise time of the deltamethrin-modified currents comes in contradiction with a use-dependent effect. If the channels were modified after being activated then the rise time of the modified currents would be expected to become much faster at potentials where the normal channels reach full activation (and open) than at subthreshold potentials (where only modified currents are opening). As can be seen in Fig. 5 this is not the case for either TTX-S or TTX-R currents.

We used the Hodgkin-Huxley equation to characterize the kinetics of the deltamethrin-modified currents and compare them with the control. This approach provided a quantitative measure of the change in the kinetics of activation (Table 1). Most importantly, for potentials more positive than the threshold of activation, the data could be fitted adequately only by a sum of two Hodgkin-Huxley functions having different $\tau_m$ and $n$ values. This finding clearly suggests that the deltamethrin-modified channels present some heterogeneity in the mechanism of activation. Although a mechanistic interpretation of the Hodgkin-Huxley model (e.g., three independent gating particles) may be simplistic, it seems plausible that modification by deltamethrin unveils discrete steps in the activation mechanism.

Taking advantage of the negative shift in gating caused by deltamethrin modification, we were able to apply an equation that estimates the gating charge of sodium channels (Sigworth, 1995). The values obtained (6.1 and 4.8 e\(^{-}\) for TTX-S and TTX-R channels, respectively) were much smaller than those reported for normal sodium channels (Hirschberg et al., 1995) and potassium channels (Zagotta et al., 1994) in which those reported for normal sodium channels (Hirschberg et al., 1995) and potassium channels (Zagotta et al., 1994) in which the values were $\sim 12$ e\(^{-}\), suggesting that the gating charge of deltamethrin-modified sodium channels is immobilized. A similar idea has been suggested by studies of the effect of fenvalerate, a type II pyrethroid, on gating currents in crayfish axons (Salgado and Narahashi, 1993).

It is well established that pyrethroids slow the deactivation process of sodium channels and consequently in their presence slow tail currents are observed (Narahashi, 1992, 1996). The voltage dependence of the tail current decay for both tetramethrin and deltamethrin was steep and had the similar slope for both TTX-S and TTX-R channels (0.016 for tetramethrin and 0.028 for deltamethrin). Because the two pyrethroids display a higher affinity for TTX-R than for TTX-S channels (Tatebayashi and Narahashi, 1994; Tabarean and Narahashi, 1998), this suggests that the tail current decay probably does not reflect the unbinding of the pyrethroid from the sodium channel, but a slowed return of the activation gate when the pyrethroid is bound to the channel. We have suggested previously that deltamethrin exhibits the slow diffusion of the drug within the membrane toward the channel.

In conclusion, the apparent use dependence of deltamethrin modification of sodium channels is due primarily to the accumulation of prolonged tail currents during repetitive stimulation, and the activation mechanism of sodium channels is the major target of pyrethroids.

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**References**


Goni T and Hille B (1987) Gating of Na channels: inactivation modifies discrimi-


