Effect of the pyrethroid insecticide fenvalerate on sodium current of rat dorsal root ganglion neuron

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Fenvalerate is a widely used pyrethroid insecticide. The report presents our findings on the effect of fenvalerate on isolated whole-cell sodium currents in single rat dorsal root ganglionic neurons in culture, studied with patch-clamp technique. Fenvalerate decreased the amplitude of whole-cell sodium current and slowed the inactivation and tail current kinetics.

FENVALERATE is a commonly used photostable insecticide and belongs to the Pyrethroid family in its insecticidal behaviour. It is an ester of 2-(4-chlorophenyl)-3-methyl-butyric acid without a cyclopropane ring. It exists as a racemic mixture of four optical isomers of which the (2S, αS) is the most biologically active followed by the (2S, αR) isomer[1,2].

Earlier studies[3-5] have shown effects of fenvalerate on the nerve membrane activity and ionic currents. The studies are, however, primarily confined to invertebrate and amphibian systems. Very little is known about the effect of fenvalerate on mammalian ionic channels which determine the action potential waveform of an excitable cell. We report here our findings on the effect of fenvalerate on isolated sodium currents of rat dorsal root ganglion (DRG) neurons.

Isolated sodium channel currents (I_{Na}) were studied in the whole-cell configuration of the patch-clamp technique[6,7]. Single DRG neurons were obtained following dispersion of ganglia aseptically dissected from the thoracic and sacral spinal region of 7-day-old Wistar rats in 0.1% trypsin, Ca^{2+}–Mg^{2+}-free phosphate buffered saline (Hi-media, India). The dispersed neurons were plated on poly-L-lysine (Sigma) coated glass coverslips placed in 35 mm sterile culture dishes (Tarsons, India) containing DMEM (Hi-media) with 10% new-born calf serum (Sigma).

Currents were recorded using fire polished omega-dot glass micropipette electrodes (electrode resistance 15 megohm) (Figure 1) and a List EPC-7 patch-clamp amplifier. The pipette solution contained (in mM): 125 CsCl, 5 NaCl, 1 $CaCl_2$, 10 EGTA, 5 HEPES, pH 7.4 with Trizma base; while the bath solution contained (in mM): 135 NaCl, 10 tetraethylammonium chloride (TEACl), 5 HEPES, pH 7.4 with Trizma base[8]. Experiments were done at room temperature (24° C). Voltage pulses were generated using a Neurodata PG4000A stimulator. Current signals were filtered (8 pole Bessel, 2 kHz) using a locally built filter. The voltage and filtered current signals were led through a CED 1401 interface (Cambridge Electronic Design, UK), and analysis done using the Strathclyde VCAN software (J. Dempster, University of Strathclyde, Glasgow) on a PC-AT 286 (PCL, India).

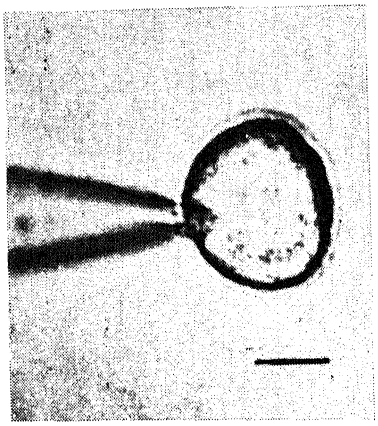

Figure 1. Photomicrograph of rat dorsal root ganglion neuron 24 h in culture with a fire polished patch-pipette electrode in cell attached condition, with the neuron bathed in the external recording solution (bar = 10 μm).

Under voltage-clamp, voltage activated isolated sodium currents (I_{Na}) were observed following dialysis of the cell with K^+ free, Cs^+ solution, while the bath contained Ca^{2+}–K^+–free Na^+ solution. TEACl (10 mM) was included in the external solution to block the outward delayed rectifier K^+ current.

Figure 2 *A* (*a*) shows samples of isolated I_{Na} records elicited at step depolarizations indicated (upper set of traces) from a holding potential of −75 mV in the control. The third trace shows a typical isolated inward I_{Na} record with rapid activation indicated by a downward deflection of the current trace from baseline, followed by slow relaxation of the current during the period of pulse application. The fast and slow capacitance transients in the current trace were adequately compensated before the recording. The cell membrane capacitance was 47 pF read from the C_{slow} compensation knob of the amplifier.

Figure 2 *A* (*b*) shows I_{Na} records to similar test pulse amplitudes at 5 min following bolus application of fenvalerate to achieve a final bath concentration of 50 μM. A comparison of the traces in Figures 1 *A* (*a*) and (*b*) shows a decrease in I_{Na} amplitudes in the presence of fenvalerate.

Figure 2 *B* is a plot of peak inward Na^+ current amplitudes (*I*) at different membrane potentials (*V*), in control and in the presence of 50 μM fenvalerate, whose sample I_{Na} records are shown in Figure 2 *A*. The *I*–*V* plot in the control situation indicated the peak activation of I_{Na} to occur at −25 mV, and there was no drift in the peak activation value in the presence of fenvalerate. Also, fenvalerate decreased the sodium

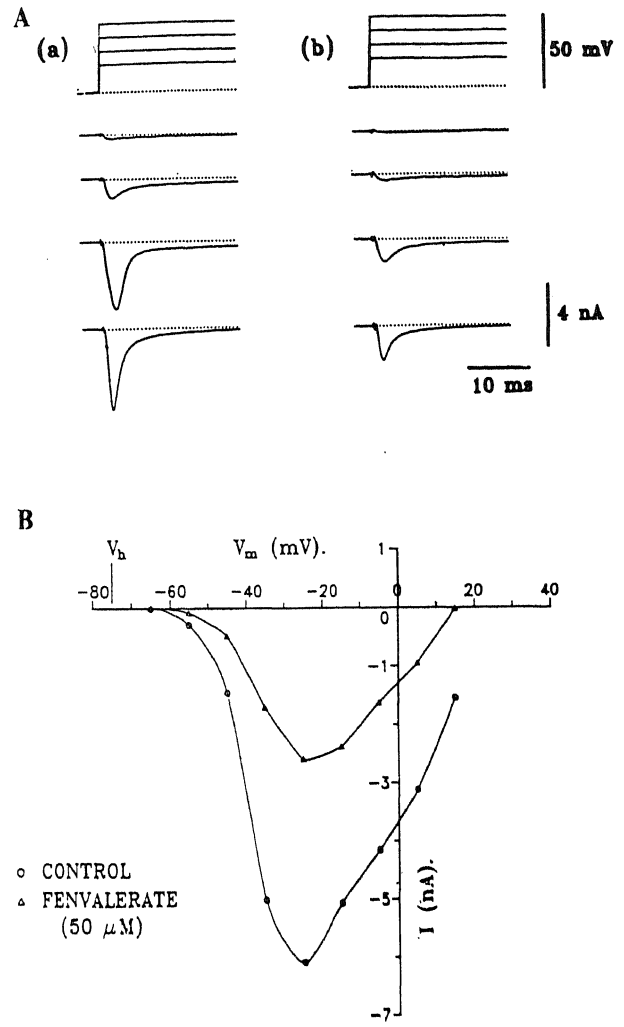

Figure 2. Effect of fenvalerate on I_{Na} amplitude. ***A***, Whole cell inward I_{Na} elicited by step depolarizations to −55, −45, −35 and −25 mV from a holding potential (V_h) of −75 mV in control (a) and in presence of 50 μM of fenvalerate (b); ***B***, Current (I) vs. voltage (V) plot of peak I_{Na} amplitudes for data shown in *A*.

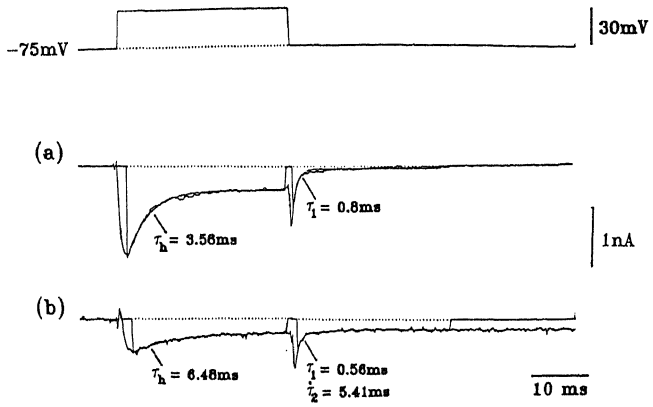

Figure 3. Kinetic analysis of I_{Na} inactivation and tail current relaxations. The plots are of inward currents elicited by depolarizing the membrane to −45 mV from a holding potential of −75 mV in control (*a*) and in presence of 50 μM fenvalerate (*b*). The smooth line over the current record indicates the exponential curve contained within the analysis area. An iterative nonlinear Levenberg Marquadt algorithm (SSQMIN, K. Brown, Univ. of Cincinnati) was used to perform the fit defined by equations (1) and (2) given in the text. τ_h, inactivation time constant; τ_1 and τ_2 tail current relaxation time constants.

current amplitude at the different membrane potentials tested.

Figure 3 shows plots of I_{Na} in control and in presence of 50 μM fenvalerate from the same cell shown in Figure 2, following a step depolarization to −45 mV from a holding potential of −75 mV. Shown in the records are Na^+ tail current relaxations on returning the test pulse to holding potential level. The inactivating component of I_{Na} and the tail current relaxations were fitted with either a single exponential function of the form:

$$I_{Na}(t) = I'_{Na} \exp(-t/\tau_h), \quad (1)$$

or a double exponential function of the form:

$$I_{Na}(t) = I'_{Na} \exp(-t/\tau_1) + I''_{Na} \exp(-t/\tau_2), \quad (2)$$

where I'_{Na} or I''_{Na} are amplitudes, t is time, and τ_1 and τ_2 are time constants of fast and slow processes respectively.

The time constant of inactivation (τ_h) determined from the exponential fit (equation (1)) was 3.56 ms in control, while in the presence of 50 μM fenvalerate it was 6.48 ms, indicating a slowing of the inactivation kinetics.

The tail current relaxation in control was fit by a single exponential function as in equation (1) with a time constant (τ_1) of 0.8 ms. However, in the presence of fenvalerate the current relaxation was fit by a double exponential function (equation (2)) with two time constant values (τ_1 and τ_2) of 0.56 and 5.4 ms respectively, indicative of an inherent fast (τ_1) and slow (τ_2) kinetic process. For exponential fit of tail current relaxations, 2.5 ms of the current trace following the step depolarization was discarded to avoid contamination by capacitative current component.

From Figures 2 and 3 it is clear that fenvalerate acts on I_{Na} of DRG neuron to: (a) decrease the current amplitude, (b) slow the inactivation process at least at some membrane potentials and (c) affect the tail current relaxation to induce an additional slow kinetic process. Similar observations were made in a total of four different DRG neurons obtained from different rats.

The whole-cell I_{Na} recorded in Figures 2 and 3 represents the summated current due to opening of a number of individual channel proteins that undergo conformational changes among at least three functionally distinct states during voltage activation, viz. $R \langle - - \rangle O \langle - - \rangle I$, where R is the resting state, O the open state, and I the inactivated state. The inactivating component of the whole-cell I_{Na} record appears due to progressive decrease in the probability of opening of

single Na^+ channels during the period of pulse application, following pulse onset. The amplitude of the tail current is proportional to the Na^+ current at the time of repolarization and is only seen if repolarization occurs before the inactivation process is complete, and its analysis is particularly used in insecticide research since the tail current characteristics give information about channels in the open state. The tail current in fenvalerate-treated condition is also proportional to the number of open channels, but the decay of the current is much slower. In Figure 3*b* it is seen that there is an additional slow component of tail current that does not decay during the time scale shown. This slow component is most likely due to modified open channels which return to the resting state slower by a factor of about 10 as opposed to normal channels. It is also likely that fenvalerate induces some inactivated channels to open spontaneously and become modified, resulting in the appearance of the slow tail current component following fenvalerate addition, as has been suggested earlier for the pyrethroid tetramethrin in squid axons[9]. While both fenvalerate and tetramethrin slow the inactivation and the tail current characteristics, fenvalerate decreases the amplitude of the current elicited during a depolarization pulse, and tetramethrin does not. The difference can partly be explained by the structural differences between fenvalerate and tetramethrin; while fenvalerate is a cyano pyrethroid compound, tetramethrin is a non-cyano pyrethroid.

There is also reason to believe that fenvalerate acts differently on the Na channels of animals in the evolutionary scale. Thus, while fenvalerate decreases the Na current characteristics during depolarization, viz. current amplitude and inactivation time constant of mammalian DRG neurons suggested by our experiments, it only affects the tail current kinetics of amphibian Na channels without affecting the sodium current elicited during the depolarization pulse, demonstrated earlier in myelinated nerve fibres of the clawed frog, *Xenopus laevis*[4]. This difference can partly be explained by inherent subtle differences in the Na channel structures, although it is true that basic structural and functional aspects of votage-gated ionic channels are strongly conserved over evolution.

The decrease in peak I_{Na} amplitude with fenvalerate addition can be attributed to either closure of a subpopulation of Na^+ channels in the resting state and/or channel modification to decrease single channel conductance. Further, the effects described above could be due to fenvalerate's action on a 'pyrethroid receptor' on the Na^+ channel[3]. These are aspects which are in the process of detailed investigation.

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Characterization of cellulose and hemicellulose degrading *Bacillus* sp. from termite infested soil

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A *Bacillus* strain having a broad spectrum of enzymes responsible for degradation of cellulosic and hemicellulosic components of agricultural wastes was isolated from the termite infested soil. The optimum temperature and pH for growth of the bacterium were 37°C and 7.2 respectively. The isolate was capable of fast growth on soluble products, xylan ($\mu max = 0.45\ h^{-1}$) and carboxymethyl cellulose ($\mu max = 0.20\ h^{-1}$) rather than on the rice husk ($\mu max = 0.15\ h^{-1}$), the insoluble substrate. The organism was characterized morphologically and biochemically. Similarities with *Bacillus licheniformis* are very evident.

TERMITES are prominent members of arid ecosystems. They are considered to be supreme converters of organic matters in soil from the tropics to the desert; they stir and mix and with the aid of bacteria, protozoa and fungi recycle cellulosic materials[1]. The importance of bacterial component of the termite's intestinal microbiota in cellulose digestion has been extensively reviewed[2], however, the role of microorganisms inhibiting termite infested soil is scanty. It is therefore of interest to ascertain the characteristics of bacteria isolated from this specialized ecological niche located in