CALCIUM CHANNEL CURRENTS IN NEURONES FROM LOCUST (SCHISTOCERCA GREGARIA) THORACIC GANGLIA

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Summary
1. Using the patch-clamp technique, Ca$^{2+}$ channel currents were recorded from neurones freshly isolated from the thoracic ganglia of the desert locust Schistocerca gregaria.
2. In solutions containing 10mmol l$^{-1}$ Ba$^{2+}$ we observed high-voltage-activated whole-cell inward currents with sustained and transient components, both of which had similar steady-state inactivation properties.
3. Substitution of Ca$^{2+}$ for Ba$^{2+}$ was found to reduce whole-cell currents, whereas removal of monovalent cations had no effect.
4. Cd$^{2+}$ (1mmol l$^{-1}$) completely blocked the whole-cell current, but at 10μmol l$^{-1}$ preferentially inhibited the sustained component without affecting the transient component.
5. Verapamil (1μmol l$^{-1}$) inhibited both current components but appeared to be more selective for the sustained component, whereas nitrendipine (1μmol l$^{-1}$) had no effect on either component.
6. A single-channel recording suggested that the transient component was carried by a low-conductance channel.
7. Certain compounds with insecticidal action (ryanodine, S-bioallethrin, deltamethrin and avermectin) did not affect calcium channel currents in these cells.
8. These data suggest that there are two types of Ca$^{2+}$ channels present in locust neurones. These channel types have properties differing from the T-, L- and N-type channels found in vertebrates and, furthermore, were not targets for the insecticides we tested.

Introduction
Little is known about voltage-sensitive calcium channels in insect neurones. The presence of calcium-dependent action potentials has been demonstrated in peripheral
neurosecretory neurones of the stick insect *Carausius morosus* (Orchard, 1976) and in the thoracic ganglion of *Schistocerca gregaria* (Goodman and Heitler, 1979), and whole-cell calcium currents have been observed in embryonic cultures of *Drosophila melanogaster* (Byerly and Leung, 1988) and cockroach (Christensen *et al.* 1988) neurones. In addition, Ba^{2+}-permeable Ca^{2+} channels from *Drosophila* brain membranes have been reconstituted into lipid bilayers from which unitary currents were then recorded (Pelzer *et al.* 1989). Biochemical investigations indicate the presence of binding sites for organic calcium channel antagonists in the same tissues (Pauron *et al.* 1987; Greenberg *et al.* 1989).

In vertebrate tissues, calcium channels have been classified by their electrophysiological and biochemical properties. A popular classification divides these channels into T-, L- and N-type channels (Nowycky *et al.* 1985; Fox *et al.* 1987). The transient T-type current is activated by small depolarisations, while the sustained L-type currents are activated by large depolarisations and blocked by organic calcium channel antagonists. The N-type currents are also activated by large depolarisations but can be either transient or sustained (Plummer *et al.* 1989).

In this study we have used the whole-cell patch-clamp technique to investigate the calcium channel currents in neuronal cell bodies freshly dissociated from the thoracic ganglia of the locust *Schistocerca gregaria*. We have attempted to characterise the electrophysiology and pharmacology of these channels. In particular, we have studied activation/inactivation properties and sensitivity of the channels to organic and inorganic calcium channel antagonists in order to compare the properties of these channels with those found in vertebrates.

Calcium channels in insects represent an unexploited potential target site for new insecticides. We have therefore investigated the action on calcium channels in locust neurones of a variety of insecticidal compounds, some of which are thought to interact with vertebrate calcium channels (Narahashi, 1986; Sutko *et al.* 1985; Smith *et al.* 1986a,b). A preliminary abstract describing some of this work has been presented to the Physiological Society (Lees *et al.* 1989).

**Materials and methods**

Cells were prepared from thoracic ganglia of the desert locust *Schistocerca gregaria* (Forskål). Sections of ventral body wall containing the three thoracic ganglia were removed from 4–5 adult locusts of either sex and placed in dissecting solution (see below). The thoracic ganglia were separated, and digested in 1mgml^{-1} trypsin (bovine pancreatic trypsin, BDH) for 10–15min at 30°C to facilitate dispersion of individual cell bodies. This was followed by washing in 2mgml^{-1} bovine serum albumin (Sigma) for approximately 1min, to inactivate the trypsin, and further washing in the dissecting solution. The fine membranous sheaths surrounding the ganglia were removed and individual nerve cell bodies were isolated by repeatedly passing the ganglia through a narrow-bore (1–1.5mm) glass Pasteur pipette. The cell suspension was filtered through a 50μm nylon mesh to remove the larger debris, and the filtrate was then repeatedly (4–5 times) spun down (200g for 2min) and washed in dissecting solution containing...
penicillin (200 i.u. ml$^{-1}$) and streptomycin (200 i.u. ml$^{-1}$) to reduce the risk of infection. Cell suspensions were prepared on the day of the experiment and stored at 4°C. For electrophysiological recordings, cells were plated onto 35mm tissue culture dishes, left for 4–5 min, after which time they were found to adhere to the bottom of the dish, and then continuously perfused (5–10 ml min$^{-1}$) with recording solution (see below).

**Solutions**

The compositions of the solutions used are given in Table 1. All solutions were filtered through a 0.2 μm pore filter before use. The osmolarity of each solution was adjusted to the stated level with sucrose, and the pH was adjusted to 7.4 with either Tris or CsOH.

Unless otherwise stated, cells were bathed in the recording solution of Table 1, so the whole-cell currents measured were Ba$^{2+}$ currents or calcium channel currents rather than Ca$^{2+}$ currents *per se*. Ba$^{2+}$ was used as the charge-carrying ion, first because it is more permeant than Ca$^{2+}$ through the calcium channels in these cells (see Results), thereby giving a better signal to noise ratio and, second, because Ca$^{2+}$ entering the cell could give rise to Ca$^{2+}$-dependent inactivation of the channels under investigation. Ba$^{2+}$ also has the advantage that it blocks contaminating K$^{+}$ currents, which were further blocked by the presence of tetroethylammonium (TEA$^+$), both inside and outside the cells, and Cs$^{+}$ in the pipette solution. Tetrodotoxin (TTX) was also present in the bathing solution to block voltage-dependent Na$^{+}$ channels.

Verapamil (Sigma) and nitrendipine (a gift from Bayer) were dissolved in ethanol to give stock solutions of 1mg ml$^{-1}$ before being further diluted in recording solution to the required concentration. Avermectin B1 (a gift from Merck, Sharpe and Dohme) and S-bioallethrin and deltamethrin (both 99% pure and synthesised at Wellcome Research Laboratories, Berkhamsted) were dissolved in dimethylsulphoxide at 1mg ml$^{-1}$ as stock solution. Final concentrations of organic solvents in recording solutions were always less than 0.01% v/v and an equal quantity of solvent was added to control solutions before recording. Ryanodine (98.5% pure, obtained from plant material, Wellcome Research Laboratories) and CdCl$_2$ (BDH) were dissolved in recording solution to give a stock solution of 1mg ml$^{-1}$.

**Whole-cell recordings**

Calcium channel currents were measured according to the methods of Hamill *et al.* (1981). Pipettes (resistance 4–8 MΩ) were pulled from thin-walled borosilicate glass capillaries (Clark Electromedical Instruments), coated in Sylgard resin and fire-polished in a microforge immediately before use.

For whole-cell current measurement, cells were clamped using an Axoclamp-2 single-electrode voltage-clamp amplifier at a switching frequency of 8–15 kHz. A series of 30–35 voltage pulses (duration 200ms at 0.4 Hz, 10mV step) from a holding potential of −80mV was used to calculate leakage and capacitance. From the same holding potential, current–voltage (I–V) curves were then constructed by applying depolarising voltage pulses (duration 200ms at 0.1 Hz) over a range of test potentials in 10mV increments (or occasionally 5mV increments). In some experiments, the above procedure was repeated...
<table>
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<th>NaCl</th>
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<th>Choline chloride</th>
<th>KCl</th>
<th>MgCl₂</th>
<th>CaCl₂</th>
<th>BaCl₂</th>
<th>HEPES</th>
<th>TEABr</th>
<th>TTX</th>
<th>EGTA</th>
<th>pH</th>
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<td>2</td>
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<td>(with Br⁻ as charge carrier)</td>
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<td>7.4</td>
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Values are concentrations in mmol L⁻¹ except for osmolarity and pH. The osmolarity of solutions was adjusted to the required level with sucrose.

TEABr, tetramethylammonium bromide; TTX, tetrodotoxin.
at different holding potentials. We waited 90s after changing the holding potential to allow time for any steady-state inactivation of channels to become complete.

In experiments which involved the application of drugs, *I*–*V* curves were first obtained in control solution at a holding potential of $-80\text{mV}$ as above. Repetitive pulses (duration 200ms at 0.05–0.1Hz) were then applied from the same holding potential to a test potential of $-10\text{mV}$ during perfusion (5–10mlmin$^{-1}$) with control solution, and subsequently during perfusion with the test solution. When the $\text{Ca}^{2+}$ channel current reached a steady level during the test perfusion, another *I*–*V* curve was obtained as described above. In other cases, drug action was evaluated by comparing currents in two groups of cells bathed in recording solution containing either the compound under test or the vehicle.

After each experiment, the capacitance of the cell was measured as follows. Using discontinuous current-clamp mode, the cell was continuously depolarised to a potential between $+40$ and $+60\text{mV}$ by steady d.c. current passage of around 0.1–0.5nA, a procedure that inactivates calcium channels and eliminates regenerative responses. Current pulses of 0.03–0.1nA (duration 200–400ms at 0.4Hz) leading to approximately 10mV voltage steps were then applied. Cell capacitance was then calculated from the time course of the potential changes as described below.

**Single-channel recordings**

Single-channel recordings were made (using a List EPC-7 amplifier) from cells bathed in dissecting solution but with the penicillin and streptomycin omitted and using the pipette solution shown in Table 1. Resting membrane potential was measured either before or during an experiment by impaling the cell with an intracellular microelectrode. The potential across the membrane patch was then set to $-80\text{mV}$ (i.e. cytoplasm surface negative with respect to external surface) and the patch was repetitively depolarised (200ms duration, 0.1Hz) to a range of test potentials.

**Analysis**

All data were stored on magnetic tape (Racal Store 4DS) and digitised (3–5kHz) using a Computer Automation computer and CED 701 interface controlled by programs written in Fortran.

For whole-cell currents, linear leak and capacitance were subtracted by averaging 30–35 10mV steps (see above) and scaling to the appropriate test potential. These subtracted currents were then measured for peak inward current ($I_{\text{pk}}$), the current averaged over the last 10ms of the step, as a measure of sustained current ($I_{\text{sus}}$), and the difference between these two values ($I_{\text{trans}}$), as a measure of the transient component of current. The corresponding voltage step was also measured. The *I*–*V* curves for peak, sustained and transient currents were obtained in this way.

The whole-cell capacitance was obtained from the current clamp data (described above) as follows. For each voltage pulse, the voltage ($V$) over the last 10ms (when the voltage had stabilised) was measured, and the initial rising phase was fitted (by least-squares regression) to an exponential of time constant $\tau$. The magnitude of the current pulse ($I$) was also measured, allowing calculation of input resistance ($R$) and, hence, cell
capacitance ($C$) from $\tau/R$ assuming spherical cells (as was roughly the case by selection).

Values for $R$ and $C$ were averaged over 15–20 pulses for each cell.

Data from whole-cell experiments are presented either as absolute values for current or after normalisation by dividing by the cell capacitance. The latter method assumes that calcium channel current density is constant, so that variations in current between cells are proportional to cell surface area and hence capacitance, as was found to be the case (see Results).

Single-channel data were filtered at 1kHz with a Bessel filter before digitisation as above. Captured data were subtracted by computer for capacitance and leak using the mean of 5–30 test steps that showed no channel activity. Subtracted data were plotted and then measured for open times, closed times, amplitude and latency.

Data are presented as mean ± standard error of the mean (S.E.M.), averaged over cells in control or test solutions. Values were compared using a Student’s two-tailed $t$-test or a paired $t$-test, as appropriate, with the level for significance set at $P<0.05$.

**Results**

*Properties of whole-cell calcium channel currents in locust neurones*

Whole-cell patch-clamp recordings from locust thoracic ganglion neurones (Fig. 1A) revealed large inward currents activated by depolarisation of cells held at $-80$ mV with Ba$^{2+}$ as charge carrier and in the presence of Na$^+$ and K$^+$ (recording solution in Table 1). Fig. 1B shows the $I$–$V$ relationships for both the peak and sustained currents in the same cell, and the difference between these measurements ($I_{\text{trans}}$), which therefore represents the amount of current that inactivates during the step. In this cell, an inward current was first seen when the cell was depolarised to test potentials greater than $-40$ mV. At this test potential and at $-30$ mV and $-25$ mV, the current was sustained throughout the test pulse. With stronger depolarisations, an inactivating current was present together with the sustained current. The difference between the voltage of activation for the sustained and transient components of current is clearly seen when the $I$–$V$ relationships for $I_{\text{sus}}$ and $I_{\text{trans}}$ are compared. Similar characteristics were seen in 57 other cells. Three other cells, however, apparently had only a transient current. Peak, sustained and transient currents are all maximal at the same potential.

Sustained currents were usually activated by smaller potential steps than were transient currents, suggesting the presence of two channel types. However, the similar potential for maximal activation of sustained and transient currents clearly indicates that these insect channels differ from their vertebrate counterparts, in which the transient T-type current is
Ca$^{2+}$ channels in locust neurones

**Fig. 1**

Panel A: Graph showing voltage ($V_{test}$) vs. current at different voltages. The voltage range is from -50 to -80 mV.

Panel B: Graph showing current ($I$) vs. voltage ($V_{test}$) at different voltages.

Panel C: Graph showing peak inward current ($\text{Peak inward current (nA)}$) vs. cell capacitance (pF). The data points are plotted with a trend line.
activated by smaller potential steps than the sustained L-type current and has a more negative potential for maximal activation (Fox et al. 1987).

The peak inward current was approximately linearly related to cell capacitance (Fig. 1C), and the gradient of the fit had a slope of 57.1 AF\(^{-1}\). Assuming that the membrane has a capacitance of 1 \(\mu\)F cm\(^{-2}\) (Katz, 1966), this represents a peak calcium channel current density in these cells of 0.57 pA \(\mu\)m\(^{-2}\).

**Effect of holding potential on whole-cell calcium channel currents**

To investigate any differential effect of holding potential on the sustained and transient phases of the calcium channel current, cells were held at a range of holding potentials from which test depolarisations were applied. The mean \(I-V\) curves for 14 cells at various holding potentials are shown in Fig. 2 for the peak (Fig. 2A), sustained (Fig. 2B) and transient (Fig. 2C) currents.

As mentioned above for an individual cell, when held at −80 mV, activation of \(I_{pk}\) started to occur at approximately −40 mV; the current reached a maximum at around −10 mV (Fig. 2A). At holding potentials of −60 mV to −30 mV, \(I_{pk}\) was significantly reduced in amplitude, but the activation voltage and potential for maximal current were largely unchanged. No inward current occurred during test pulses from the −20 mV holding potential. Apparent reversal potentials became more negative as the holding potential became more positive, but this change may be due to incomplete block of background outward currents rather than to a change in reversal potential. The effect is most obvious at the −20 mV holding potential, where the calcium channel current appears to be completely inactivated and a small outward current can be clearly seen (not shown). Thus, it may well be that there was no change in reversal potential.

\(I_{sus}\) and \(I_{trans}\) (Fig. 2B,C) behaved similarly to \(I_{pk}\), except that they were, of course, smaller. This suggests that both the transient and sustained components of current have similar steady-state inactivation characteristics.

To investigate the relative extent of inactivation of peak, sustained and transient currents at different holding potentials, steady-state inactivation curves for each were obtained (Fig. 2D). There were no significant differences between the level of steady-state inactivation of any of these currents at each holding potential.

In summary, the sustained and transient components of current in these cells are both inactivated by 50% at a holding potential of −55 mV and are fully inactivated when a holding potential of −30 mV is used. Thus, the use of a more depolarised holding potential does not allow the separation of the transient and sustained components of current.

**Ions involved in channel permeation**

To investigate whether sodium and/or potassium ions contributed to the evoked currents we replaced these ions with choline (Table 1). There were no significant differences in the magnitude of whole-cell inward currents or in the reversal potential between sodium/potassium-free solution (10 mmol l\(^{-1}\) Ba\(^{2+}\) as charge carrier) and the recording solution (containing 150 mmol l\(^{-1}\) Na\(^{+}\), 5 mmol l\(^{-1}\) K\(^{+}\) and 10 mmol l\(^{-1}\) Ba\(^{2+}\)) that was used for all other experiments (Table 2), indicating little, if any, sodium or
Ca\textsuperscript{2+} channels in locust neurones

Fig. 2. I–V curves at different holding potentials. Cells were held successively at each holding potential (–80mV, ○; –60mV, ●; –40mV, ◀; –30mV, ▼; –20mV, □) and I–V curves were constructed for peak (I_{pk}, A), sustained (I_{sust}, B) and transient (I_{trans}, C) currents. Values shown are means±S.E.M. of the inward currents averaged over 14 cells at each holding potential. (D) Steady-state inactivation curve for peak (○), sustained (∆) and transient (□) currents. Currents are expressed as a percentage of the corresponding peak, sustained and transient currents at a holding potential of –80mV and for a test potential of –10mV, for the same cells as in A, B and C. No significant differences were found between peak, sustained and transient values at each holding potential (paired t-test). A single curve was fitted by the least-squares method using the Boltzmann distribution: \( I/I_{max} = 1/[1 - \exp(V_h - V_{1/2})/k] \) where \( V_{1/2} = -55.3\text{mV} \), \( V_h \) is holding potential and \( k = 7.4\text{mV} \).

Replacing barium by calcium in sodium/potassium-free solutions (Table 2) did not change the reversal potential, but inward whole-cell currents were significantly reduced. This indicates that the Ca\textsuperscript{2+} channels underlying the inward current in these cells are, like their high-threshold vertebrate counterparts (Kostyuk et al. 1989), more permeable to Ba\textsuperscript{2+} than to Ca\textsuperscript{2+}.

Effect of cadmium on calcium channel currents

To investigate further the nature of the channels contributing to the whole-cell inward currents, the inorganic Ca\textsuperscript{2+}-channel-blocking agent Cd\textsuperscript{2+} was applied to cells.
Fig. 3 shows that 1mmol l$^{-1}$ Cd$^{2+}$ completely blocks the peak inward currents. This effect was partially reversed by washing the cell in control solution. Similar effects were seen in two other cells. Thus, it is clear that Ca$^{2+}$ channels are involved in both components of the inward current.

Lower concentrations of Cd$^{2+}$ selectively inhibit L- and N-type (but not T-type) channels in vertebrates (Nowycky et al. 1985; Narahashi et al. 1987), so we investigated the channel types present in the locust using 10 mmol l$^{-1}$ Cd$^{2+}$. Only a partial block of the calcium channel current occurred (Fig. 4A). Fig. 4B shows that the sustained but not the transient component was blocked. This was confirmed by study of the $I$–$V$ curves for the same cell (Fig. 4C): there was a marked reduction in the sustained ($I_{\text{sus}}$) component (but without a change in voltage-dependence) in the presence of 10 mmol l$^{-1}$ Cd$^{2+}$ but no effect on the transient component ($I_{\text{trans}}$). Similar effects were seen for other cells tested; for instance, during steps to $-10$ mV, $I_{\text{sus}}$ was reduced by 70.5±6.6% ($N=3$) whereas $I_{\text{trans}}$ was not significantly affected.

These results demonstrate that Ca$^{2+}$ channel currents underlie the inward current and further suggest that two components of the calcium channel current exist in these cells.

**Effect of verapamil on calcium channel currents**

Organic calcium channel antagonists act on L-type Ca$^{2+}$ channels in vertebrates (see Introduction). The action of verapamil (a phenylalkylamine) was therefore tested on the whole-cell calcium channel currents in cells from locust ganglia in an attempt to classify the channel types pharmacologically.

Verapamil (1 μmol l$^{-1}$) caused partial block of the peak inward current (Fig. 5A). Fig. 5B shows that both sustained and transient components were blocked. Fig. 5C shows $I$–$V$ curves for the same cell; there were again reductions in both $I_{\text{sus}}$ and (to a lesser extent) $I_{\text{trans}}$. Similar results were obtained for other cells: at $-10$ mV test potential, $I_{\text{sus}}$ was reduced by 85±16% ($P<0.005, N=5$) and $I_{\text{trans}}$ by 60±8.4% ($P<0.01, N=5$). The inhibition of $I_{\text{sus}}$ was accompanied by a significant ($P<0.01, N=5$) shift in the potential for...
maximum current of the $I-V$ curve (from $-5.0\pm1.6$ mV for controls to $-14.2\pm0.9$ mV in the presence of verapamil), whereas no such shift was seen for $I_{\text{trans}}$ ($-6.8\pm1.4$ mV for controls, $-4.6\pm3.5$ mV for verapamil).

To summarise, verapamil (1 $\mu$mol l$^{-1}$) caused reductions in both sustained and transient currents. The effect was greater on the sustained component and was selectively accompanied by a shift to the left of the $I-V$ curve for $I_{\text{sust}}$.

Effect of nitrendipine on calcium channel currents

The dihydropyridine Ca$^{2+}$ channel antagonist, nitrendipine, which also acts on L-type channels in vertebrates, was applied to neurone cell bodies from locust ganglia to characterise the channel types further. Fig. 6 shows mean $I-V$ curves for six cells before and during perfusion with nitrendipine (1 $\mu$mol l$^{-1}$) at a holding potential of $-80$ mV. There were no significant effects of nitrendipine on either peak (not shown), transient (Fig. 6A) or sustained (Fig. 6B) currents (paired $t$-test).

A holding potential of $-80$ mV tends to favour agonist-type behaviour for dihydropyridines (Brown et al. 1986; Bean, 1984), so further experiments were carried out at a less hyperpolarised holding potential ($-40$ mV). However, when mean $I-V$
Fig. 4
Curves were obtained for 13 cells in normal solution and compared with those for 15 cells in nitrendipine (1 μmol l⁻¹), again no significant differences were seen (data not shown). Therefore, the lack of action of nitrendipine was not due to a voltage-dependent effect.

Single-channel recordings

Single-channel studies in cell-attached patches were carried out to characterise the channels in this preparation. In the 232 cells where a high-quality seal was formed (seal resistance >10 GΩ) only one patch was found to contain inward channel activity. Examples of the inward current are shown in Fig. 7A. The channel conductance (Fig. 8A) was 6.9 pS and the extrapolated reversal potential was +82 mV. Channel open times were fitted by single exponentials (Fig. 8B) and increased at depolarised potentials (Fig. 8C). The activation threshold was approximately −30 mV (Fig. 8D), which is consistent with the high thresholds of activation seen for the whole-cell recordings.

Ensemble-averaged currents were obtained by averaging traces in which channel activity was seen for each test potential (Fig. 7B); they showed a transient nature up to 0 mV, indicating that the channel observed in this patch would give rise to a transient, inactivating component of current in whole-cell conditions.

In summary, although our single-channel data are very limited, a transient low-conductance channel activated by high voltage does appear to be present in these cells.

Effect of insecticidal agents on calcium channel currents

Ryanodine interacts indirectly with Ca²⁺ channels in crab muscle (Goblet and Mounier, 1981) and muscle of moth larvae (Pearson, 1990). We investigated the interaction of ryanodine with locust neuronal Ca²⁺ channels.

Whole-cell calcium channel current was measured in cells bathed in either control solution (21 cells) or in solution containing ryanodine (10 μmol l⁻¹ for 5–30 min, 18 cells). There was no effect of ryanodine on either the transient (Fig. 9A) or sustained (Fig. 9B) component of current at a holding potential of −80 mV. Thus, ryanodine (10 μmol l⁻¹) appears to have no direct effect on Ca²⁺ channels in this preparation.

Pyrethroids are an important class of insecticide whose main site of action is thought to be voltage-sensitive Na⁺ channels, where they prolong open times (e.g. Narahashi, 1986). Type I pyrethroids have also been reported to block T-type and, to a lesser extent, L-type Ca²⁺ channels in the N1E-115 cell line (Narahashi, 1986). The effects of a type I and a type II pyrethroid on locust Ca²⁺ channels were therefore studied.
Fig. 5

1 μmoll⁻¹ verapamil

Difference

Control

2nA

100ms

1 min
Fig. 10A shows $I-V$ curves for peak currents in 12 control cells and 15 cells treated with the type I pyrethroid S-bioallethrin (1 $\mu$mol l$^{-1}$ for 5–30min, holding potential 280mV). Fig. 10B shows similar curves for cells treated with the type II pyrethroid deltamethrin (5 $\mu$mol l$^{-1}$ for 5–30min, 12 control cells, 13 cells treated with deltamethrin). Neither of these pyrethroids affected the peak, sustained or transient (data not shown) calcium channel currents.

Avermectin is a powerful anthelmintic agent and appears to act on chloride channels (which in some cases are sensitive to $\gamma$-aminobutyric acid, e.g. Mellin et al. 1983). The possibility that avermectin may modulate or activate Ca$^{2+}$ channels has been suggested from studies in locust neurones (Lacey, 1987), so we have investigated this further.

Cells ($N=6$) were held at −80mV and avermectin B1 (1nmol l$^{-1}$) was perfused for a minimum of 5min. The $I-V$ curves obtained before and after treatment with avermectin are shown in Fig. 11. Avermectin was without effect on either transient or sustained components of current. Higher concentrations of avermectin (10nmol l$^{-1}$, 100nmol l$^{-1}$) caused large increases in the holding current, which made leak subtraction and effective
clamping of the cells impractical. Thus, although avermectin had no effect at low concentration, effects at higher concentrations could not be ruled out.

**Discussion**

The experiments described here were designed to study Ca\(^{2+}\) channel currents in the somata of neuronal cells freshly dissociated from the thoracic ganglia of the locust, *Schistocerca gregaria*. Large inward currents were seen when cells were depolarised in a solution containing Ba\(^{2+}\) as the charge-carrying ion. Evidence that these currents were carried through Ca\(^{2+}\) channels was provided by a number of different experiments: (1) all experiments were carried out under conditions which should prevent currents carried by ions other than calcium or barium (see Materials and methods); (2) replacing Na\(^{+}\) and K\(^{+}\) in the bathing solution with choline did not significantly affect the inward current, indicating that these monovalent ions do not traverse the channel; (3) this inward current (which was also carried by Ca\(^{2+}\)) was blocked by the calcium channel blocking agents Cd\(^{2+}\) and verapamil. Taken together, these observations suggest that the inward currents are carried by the passage of Ba\(^{2+}\) through voltage-dependent Ca\(^{2+}\) channels. The inward Ca\(^{2+}\) current amplitudes in these cells were approximately proportional to the cell
Ca\textsuperscript{2+} channels in locust neurones

There was slight contamination of the calcium channel current by a small outward current that was apparent when cells were held at a potential of \(-20\text{mV}\) (see Fig. 2). Since all Na\textsuperscript{+} and K\textsuperscript{+} currents might reasonably be expected to be blocked by the solutions used both inside and outside the cells, it seems likely that this current was carried by Cl\textsuperscript{−}. However, the presence of this current is unlikely to have a significant effect on the results reported here because it is minimal at a test potential of 0mV and, if blocked by the agents used in this study, would be expected to cause an apparent increase in the inward current.

The Ca\textsuperscript{2+} channel currents seen in these cells appeared to have both transient and sustained components. These seem to be caused by different channel types: different activation thresholds were seen, and differential sensitivities to low concentrations of cadmium and verapamil were observed. Furthermore, single-channel studies pointed to the existence of transient low-conductance channels. The transient and sustained current components seen in whole-cell experiments could not be separated by imposing more...
depolarised holding potentials on the cells, indicating that both channel types have similar steady-state inactivation properties. To prove the presence of two channel types, more detailed single-channel studies would be necessary. The lack of success of the single-

![Graph](image)

Fig. 9. Effect of ryanodine (10 μmol·l⁻¹) on I–V curves. Mean I–V relationships for the transient (I_{trans}, A) and sustained (I_{sust}, B) currents are shown. Cells were bathed in either control solution (□, N=21) or solution containing 10 μmol·l⁻¹ ryanodine (○, N=18). Currents were normalised by dividing by the individual cell capacitance. Values shown are the means ± s.e.m. There were no significant differences between control values and ryanodine-treated values at each test potential (Student's t-test).

![Graph](image)

Fig. 10. Effect of pyrethroids on peak current I–V curves. (A) Mean I–V relationships for peak current in cells bathed in control solution (○, N=12) or solution containing S-bioallethrin (1 μmol·l⁻¹, □, N=15). (B) Mean I–V relationships for peak current in cells bathed in either control solution (●, N=12) or deltamethrin (5 μmol·l⁻¹, ■, N=13). Currents in both A and B were normalised by dividing by the individual cell capacitance. Values shown are the means ± s.e.m. No significant differences between control values and pyrethroid-treated values at each test potential were found for either compound (Student’s t-test).
channel experiments was unexpected in view of the high current density calculated from whole-cell experiments. Assuming a pipette tip diameter of 0.5 μm (Hamill et al. 1981), an area under the patch of approximately 1 μm² might be expected. Thus, from the current density measurements we should expect to see at least one channel in every 2–3 patches. A possible explanation for the absence of single-channel currents in the majority of the patches may lie in the glass used for fabrication of the patch pipette: some glasses can release divalent cations in sufficient quantities to affect channel function (Furman and Tanaka, 1988) and the same type of glass as that used here appears to prevent the observation of single Ca²⁺ channel currents in rat dorsal root ganglion neurones (H. A. Pearson and A. C. Dolphin, unpublished observations).

The channel types present in locust neurones cannot be identified with any of the T-, L- and N-type currents seen in vertebrate neurones (see Introduction). The sustained component present in locust neurones bears some resemblances to L-type channels. It is activated by high voltage, sensitive to verapamil (which blocks and shifts the I–V curve) and sensitive to low concentrations of cadmium. However, unlike L-type channels, steady-state inactivation of the sustained component in these cells occurs at more negative holding potentials than expected, and this component is insensitive to the dihydropyridine nitrendipine (and is therefore similar to T- or N-type channels). The transient component in locust neurones has some features in common with N-type channels: it is activated by high voltage, undergoes steady-state inactivation at relatively negative holding potentials and is insensitive to nitrendipine. However, this component is sensitive to verapamil (like L-type channels), insensitive to low concentrations of cadmium and appears to have a low conductance (like T-type channels).

The pharmacology of the calcium channels in locust neurones is consistent with biochemical studies of Drosophila brain membranes (Pauron et al. 1987; Greenberg et al. 1987).
1989), which bind phenylalkylamines with very high affinity but only bind
dihydropyridines with very low affinity. Furthermore, on reconstitution of these brain
membranes into lipid bilayers (Pelzer et al. 1989), the channels were highly sensitive to
phenylalkylamines but insensitive to dihydropyridines. However, other channel types
were also observed which were sensitive to dihydropyridines but not to
phenylalkylamines or which were insensitive to both classes of compound (Pelzer et al.
1989). Benzothiazepines have no effect on the Ca\textsuperscript{2+} current in cultured Drosophila
embryo neurones, but a weak block could be seen with high concentrations of
phenylalkylamines (Byerly and Leung, 1988).

We also studied the actions of some compounds with insecticidal activity (ryanodine,
types I and II pyrethroids and avermectin), which might potentially have acted on calcium
channels. However, none of these agents had any effects on the calcium channels in this
locust neuronal preparation.

The inability of either avermectin or ryanodine to affect the locust calcium channel
currents at the concentrations used is consistent with their known main actions on
chloride channels (Mellin et al. 1983; Duce and Scott, 1985) and the Ca\textsuperscript{2+} release
channels of sarcoplasmic reticulum (Sutko et al. 1985).

The lack of action of pyrethroids on locust neurone calcium channels was perhaps
surprising in view of their blocking action on mammalian calcium channels (Narahashi,
1986). However, the concentrations required to produce a block in mammalian cells were
much higher than those used in the present experiments. Our concentrations are sufficient
to produce profound neurophysiological effects in target insects (e.g. Salgado et al.
1983).

To summarise, we present evidence that Ca\textsuperscript{2+} channels are present in locust neurones
and that there appear to be two types of channel present, one transient and the other
sustained. These channel types had properties differing physiologically and
pharmacologically from the T-, L- and N- types of channel found in vertebrate cells.
These differences could be a potential target site for new insecticides; however, none of
the insecticidal agents we tested had actions on the channels.

References

Bean, B. P. (1984). Nitrendipine block of cardiac calcium channel channels: High-affinity binding to the
Brown, A. M., Kunze, D. L. and Yatani, A. (1986). Dual effects of dihydropyridines on whole cell and
unitary calcium currents in single ventricular cells of guinea-pig. J. Physiol., Lond. 379, 495–514.
neurons cultured from embryonic cockroach (Periplaneta americana) brains. J. exp. Biol. 135,
193–214.
Pharmac. 85, 395–401.
distinguishing three types of calcium currents in chick sensory neurones. J. Physiol., Lond. 394,
149–172.
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