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IONIC CURRENTS IN IDENTIFIED SWIMMERET MOTOR NEURONES OF THE CRAYFISH *PACIFASTACUS LENIUSCULUS*

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Summary

Ionic currents from freshly isolated and identified swimmeret motor neurones were characterized using a whole-cell patch-clamp technique. Two outward currents could be distinguished. A transient outward current was elicited by delivering depolarizing voltage steps from a holding potential of −80 mV. This current was inactivated by holding the cells at a potential of −40 mV and was also blocked completely by 4-aminopyridine. A second current had a sustained time course and continued to be activated at a holding potential of −40 mV. This current was partially blocked by tetraethylammonium. These outward currents resembled two previously described potassium currents: the K^+ A-current and the delayed K^+ rectifier current respectively.

Two inward currents were also detected. A fast transient current was blocked by tetrodotoxin and inactivated at holding potential of −40 mV, suggesting that this is an inward Na^+ current. A second inward current had a sustained time course and was affected neither by tetrodotoxin nor by holding the cell at a potential of −40 mV. This current was substantially enhanced by the addition of Ba^{2+} to the bath or when equimolar Ba^{2+} replaced Ca^{2+} as the charge carrier. Furthermore, this current was significantly suppressed by nifedipine. All these points suggest that this is an L-type Ca^{2+} current.

Bath application of nifedipine into an isolated swimmeret preparation affected both the frequency of the swimmeret rhythm and the duration of power-stroke activity, suggesting an important role for the inward Ca^{2+} current in maintaining a regular swimmeret rhythmic activity in crayfish.

Key words: crayfish, swimmeret system, motor neurones, voltage-clamp, *Pacifastacus leniusculus*.

Introduction

Swimmerets in decapod crustaceans are the paired ventral abdominal appendages which beat during rhythmic behaviours such as swimming and burrow ventilation. For several decades, extensive studies have been carried out on the neural control of the swimmeret motor system in crustaceans. In most cases, the swimmeret system spontaneously displays the characteristic forward-propagating metachronal rhythm. The swimmeret motor patterns recorded in the isolated swimmeret system were quantitatively indistinguishable from those produced by intact animals beating their swimmerets (Mulloney *et al.* 1990). Each swimmeret is driven by alternating bursts of impulses of the antagonistic power- and return-stroke motor neurones. The swimmeret motor rhythm can be either induced or inhibited by stimulation of certain abdominal interneurones (Wiersma and Ikeda, 1964; Chrachri *et al.* 1994) or through pharmacological stimulation by the neuropeptide proctolin and the biogenic amine octopamine (Mulloney *et al.* 1987).

There is little information, however, on membrane currents associated with the generation of swimmeret motor activity. Voltage-clamp studies have been used to study ionic currents in crustacean neurosecretory cells (Cooke *et al.* 1989; Onetti *et al.* 1990) and more recently in thoracic neurones of lobster (Jackel *et al.* 1994). Because of the difficulty of working with dissociated and identified swimmeret neurones, no attempt has yet been made to try to investigate the membrane ionic currents of neurones belonging to the swimmeret motor system using the whole-cell configuration of the patch-clamp technique.

In the present study, swimmeret motor neurones were labelled retrogradely with the fluorescent dye Lucifer Yellow and dissociated following enzymatic digestion with protease. This method has previously been used by Lipton and Tauck (1987) to label rat retinal ganglion cells. Membrane ionic currents from freshly isolated and identified swimmeret motor neurones were examined using whole-cell recordings (Hamill *et al.* 1981).

This study reports the pharmacological as well as the voltage separation of two outward currents and two inward currents in the swimmeret motor neurones. These time- and voltage-dependent currents will be discussed in this report, as will the effect of a Ca^{2+} current blocker, nifedipine, on the swimmeret rhythm recorded in an isolated ventral nerve cord similar to that used by Chrachri and Neil (1993).

Materials and methods

Crayfish (*Pacifastacus leniusculus* Dana) of both sexes were used in this study. Animals were obtained from commercial suppliers and held in aquaria that contained aerated tap water. The abdominal central nervous system containing the abdominal ganglia (A1–A6) was removed from the animal under cold anaesthesia (Chrachri and Neil, 1993).

Dye backfilling

Lucifer Yellow (Stewart, 1978) was used to label swimmeret motor neurones from both the posterior branch, containing power-stroke (PS) motor neurones, and the anterior branch, containing return-stroke (RS) motor neurones. A Vaseline well was made near the first abdominal nerve and the distal end of this nerve was placed in the well, to which a small drop of 5 % Lucifer Yellow was added. The nerve end was then cut and the well covered with Vaseline. The preparation was placed overnight in the refrigerator at 4 ˚C and the next day the swimmeret motor neurones were visualised with a fluorescence microscope (Fig. 1A).

Identification of the swimmeret motor neurones

In crayfish, each swimmeret is innervated through one first abdominal nerve (N1). This nerve is divided initially into anterior and posterior branches containing, respectively, axons of RS and PS motor neurones. Lucifer Yellow (this report) as well as cobalt backfills (Mulloney *et al.* 1990) show that all the motor neurones innervating each swimmeret have cell bodies located in the same ganglion (Fig. 1A). Mulloney *et al.* (1990) have estimated that each swimmeret is innervated by 33 RS motor neurones and 36 PS motor neurones.

Cell dissociation

Desheathed abdominal ganglia were incubated for 1.5–2 h in 8 mg ml^{-1} protease (Nagrase, Sigma) in normal crayfish saline (van Harreveld, 1936). Swimmeret motor neurones labelled with Lucifer Yellow were then dissociated by gentle trituration with siliconized fire-polished pipettes (Leifer *et al.* 1984; Lipton and Tauck, 1987). After careful washing with normal saline, the isolated swimmeret motor neurones were plated on glass coverslips (Fig. 1B,C). All procedures were performed at room temperature (22–25 ˚C).

Recording apparatus

For both extracellular and intracellular recordings of the swimmeret motor pattern in the isolated preparation, the crayfish abdominal nerve cord was pinned out in a Sylgard-lined dish and bathed continuously with oxygenated crayfish saline. Extracellular recordings were obtained using platinum

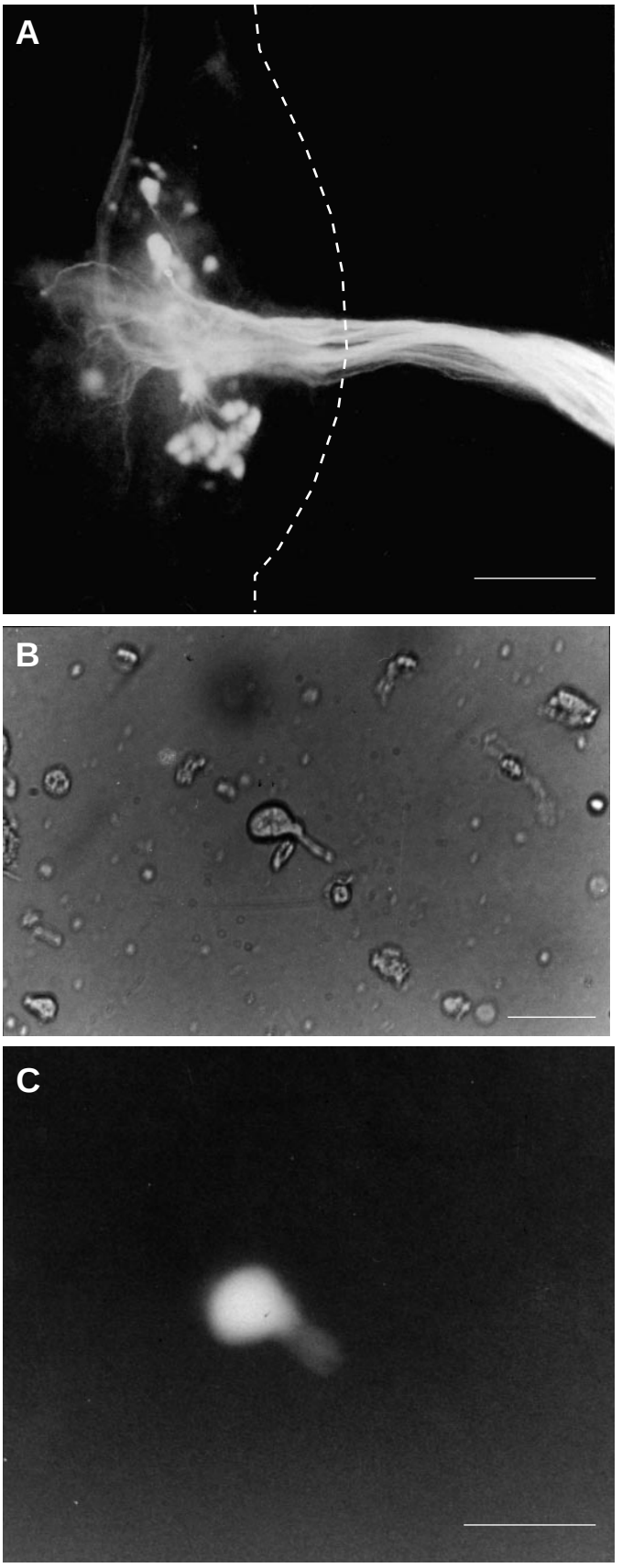

Fig. 1. Dye backfill and cell dissociation. (A) Whole mount of crayfish abdominal ganglion A4 with swimmeret motor neurones stained retrogradely with Lucifer Yellow. The dashed line outlines the ganglion. Scale bar, 200 μm. (B) Photomicrograph of a freshly dissociated and labelled swimmeret motor neurone. Scale bar, 50 μm. (C) Same field as B viewed with a fluorescence microscope. Scale bar, 50 μm. Eighteen animals were used for the present study, and an average of 32 labelled swimmeret motor neurones were dissociated from each swimmeret hemiganglion.

wire electrodes placed in contact with the appropriate motor nerves and insulated from the bath with Vaseline. These electrodes were positioned in contact with the posterior branch of the first abdominal nerve, which contains the axons of power-stroke motor neurones. Intracellular neuropile recordings were made with glass microelectrodes filled with 3 mol l^{-1} KCl and with tip resistances of 20–40 MΩ. A conventional bridge circuit was used for recording and passing current pulses with a single microelectrode.

Ionic currents in isolated swimmeret motor neurones were investigated under voltage-clamp using the patch-clamp technique in the whole-cell configuration (Hamill *et al.* 1981). The experiments were performed using a laboratory-made patch-clamp amplifier with a feedback resistance of 1 GΩ in the headstage current–voltage (*I–V*) converter. Whole-cell pipettes, made from glass capillary tube (1.5 mm i.d., Clark Electromedical), had resistances of 1–2 MΩ when filled with potassium or caesium aspartate. Series resistance was electronically compensated (usually to >90 %). Thus, voltage errors of only a few millivolts occurred at peak current levels, and this error was not corrected for. An agar bridge connected the bath to ground, and a silver wire connected the electrode to the headstage of the amplifier. Junction potentials between ground and the input were nulled by an offset sufficient to make the output current zero when the electrode was in the bath. Junction potential drift over the course of an experiment was usually less than a few millivolts, and no correction was made for this error when the data were analyzed. Pulse generation and data acquisition were performed with a 12-bit D/A and A/D converter (Scientific Solution Inc. Labmaster/DMA) and an IBM/AT-compatible computer using pClamp software (Axon Instruments). After the gigaseal had been formed, the pipette capacitance was compensated. Whole-cell mode was established by suction, and the capacitance of the cell membrane was calculated from the area under a graph of the transient capacitative current produced by a 10 mV rectangular input step from a holding potential of −60 mV. Data from motor neurones with poor space clamping, rapid run-down or with unstable currents were not included.

Solutions and drugs

Normal crayfish saline contained (in mmol l^{-1}): 198, NaCl; 5.4, KCl; 13.5, $CaCl_2$; 2.6, $MgCl_2$; 10, Hepes buffer at pH 7.4. Other solutions were made by equimolar substitution of this basic formula. Ca^{2+} was replaced with Ba^{2+}, giving the solution referred to as Ca^{2+}-free crayfish saline. Na^+ was substituted with choline, giving the solution referred to as Na^+-free crayfish saline.

Patch pipettes were filled with a solution containing (in mmol l^{-1}): 217, D-aspartate; 0.25, $CaCl_2$; 2, $MgCl_2$; 5, EGTA; 10, Hepes, titrated with KOH to a pH of 7.4, to study outward currents or CsOH to a pH of 7.4 to block outward currents and therefore to study inward currents.

Pharmacological agents (Sigma) were used to block ionic currents selectively. These included tetrodotoxin (TTX), tetraethylammonium (TEA^+), 4-aminopyridine (4-AP), caesium (Cs^+), cobalt (Co^{2+}) and nifedipine (dissolved in absolute ethanol to make 5 mmol l^{-1} stock solutions and stored at 5 °C in the dark). Experiments with nifedipine were carried out in dim light to prevent photo-oxidation.

Statistics

The software package MINITAB was used for statistical analysis. The beginning of successive power-stroke (PS) bursts (obtained from extracellular recordings, see Fig. 2 first trace) was used as a reference to calculate the frequency of the swimmeret rhythm. The frequency is expressed as a function of time, each point corresponding to the mean value ± 1 S.D. calculated from three different experiments (60 PS bursts from each). Similarly, the PS burst duration was calculated from 60 PS bursts before, and 60 bursts 20–30 min after, bath application of nifedipine.

Results

Spontaneous swimmeret motor pattern

In the isolated ventral nerve cord, the first abdominal nerve of each segment 2–5 usually displays spontaneous rhythmic activity consisting of an alternating discharge between power-stroke activity, recorded in its posterior branch, and return-stroke activity, recorded in the anterior branch (Davis, 1969; Heitler, 1978; Chrachri and Neil, 1993). An example of swimmeret rhythmic activity is shown in Fig. 2, in which intracellular recordings from two PS motor neurones (arbitrarily named PS1.MN and PS2.MN) show strong synchronous membrane potential oscillations which contribute to the rhythmic motor activity in the motor nerve (first trace). Such spontaneous, continuous motor patterns are quantitatively indistinguishable from those produced by intact animals beating their swimmerets (Mulloney *et al.* 1990).

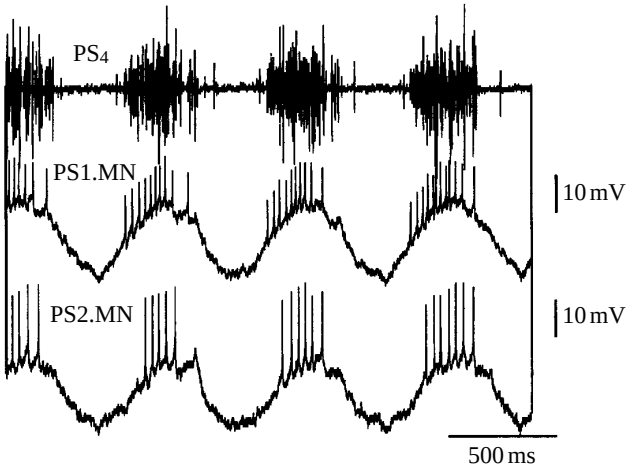

Fig. 2. Spontaneous swimmeret motor activity recorded extracellularly from the posterior branch of the first abdominal nerve of segment 4 (PS_4). This nerve contains the axons of power-stroke motor neurones (PS). The lower traces are intracellular recordings from two PS motor neurones (PS1.MN and PS2.MN).

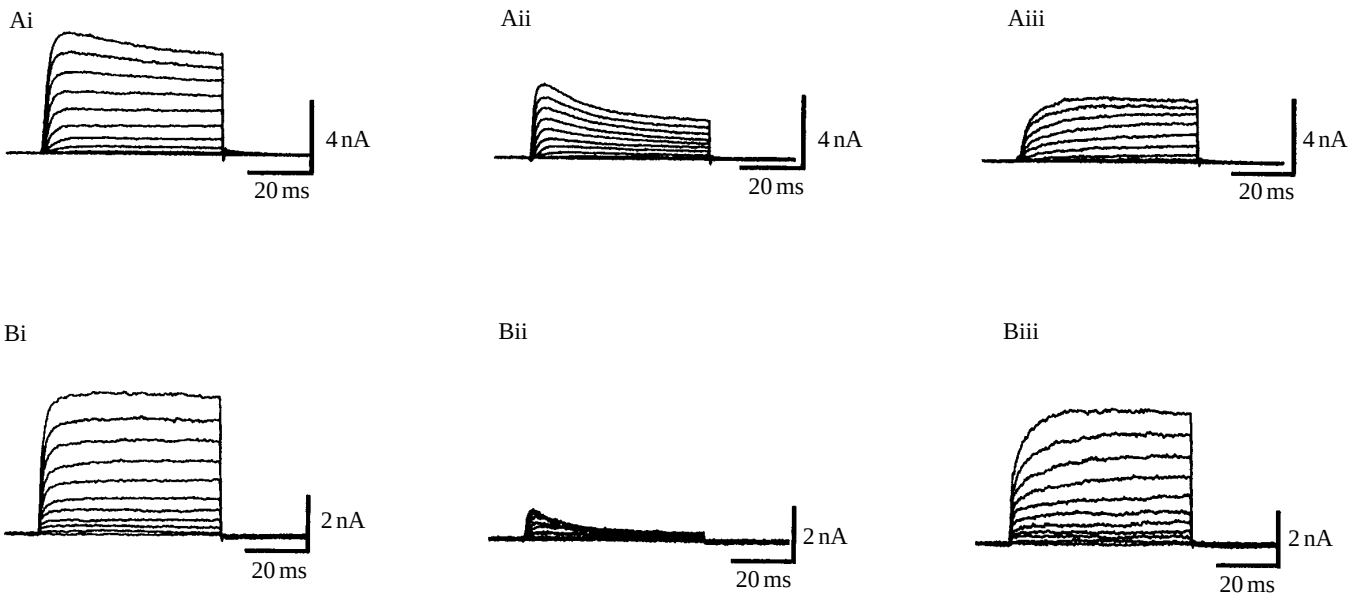

Fig. 3. Tetraethylammonium (TEA^+) and Ba^{2+} affected the outward K^+ current recorded from freshly isolated swimmeret motor neurones. (Ai) Control recordings; current records were obtained in response to membrane depolarizations to voltages ranging from −50 mV to +50 mV from a holding potential of −60 mV. (Aii) Bath application of 30 mmol l^{-1} TEA^+ reduced substantially the size of the outward current. (Aiii) The TEA^+-sensitive outward current obtained by subtracting the TEA^+-insensitive current (Aii) from the total outward current (Ai). (Bi) In the control, current records were obtained from another freshly dissociated swimmeret motor neurone in response to membrane depolarizations to voltages ranging from −50 mV to +50 mV from a holding potential of −60 mV. (Bii) Bath application of 3 mmol l^{-1} Ba^{2+} reduced the size of this outward current. (Biii) The Ba^{2+}-sensitive outward current obtained by subtracting the Ba^{2+}-insensitive current (Bii) from the total outward current (Bi).

Outward currents

Outward currents were studied using potassium aspartate solution in the pipette. These currents grew larger as the extracellular K^+ concentration was elevated and smaller as the extracellular K^+ concentration was reduced (not shown, four motor neurones). Furthermore, these outward currents were substantially suppressed (up to 50 %) by the K^+ current blockers TEA^+ (Fig. 3Aii, seven motor neurones) and Ba^{2+} (Fig. 3Bii, five motor neurones). Therefore, these outward currents were presumably carried by K^+ or at least included a large component of K^+ current.

In most cases, experiments were carried out under conditions in which the inward Na^+ current was blocked by bath application of 1 μmol l^{-1} TTX.

82 % of swimmeret motor neurones (14 out of 17) showed two outward currents: a transient fast-inactivating current and a sustained current (Fig. 4Aiii). In the remaining 18 % (three swimmeret motor neurones), only the sustained outward current was recorded. Two methods were used to isolate the transient outward current from the sustained outward current. The first method took advantage of the sensitivity of the transient current to 4-AP (Thompson, 1977). In the control, starting from a holding potential of −80 mV, membrane depolarizations to voltages ranging from −50 mV to +50 mV (with 10 mV increments) induced two outward currents (Fig. 4Ai) consisting of a transient outward current and a sustained outward current. 10 min after bath application of 4 mmol l^{-1} 4-AP, the transient outward current was suppressed, leaving only the sustained outward current (Fig. 4Aii). The transient outward current illustrated in Fig. 4Aiii was obtained by subtracting the 4-AP-insensitive outward current (Fig. 4Aii) from the total outward current (Fig. 4Ai). The second method (Connor and Stevens, 1971*b*) set the holding potential to −40 mV, which inactivated practically all of the transient outward current (Fig. 4B). Subtraction of the −40 mV-insensitive outward current from the total outward current revealed the transient outward current (Fig. 4B).

The sustained outward current, which can be separated by blocking the transient outward current with 4-AP (Fig. 4Aii) or by holding the potential at −40 mV (Fig. 4B, sustained), was reduced after bath application of 30 mmol l^{-1} TEA^+ (Fig. 3Aii). As has also been shown in the neurosecretory neurones of crayfish (Onetti *et al.* 1990), simultaneous bath application of both 4-AP (4 mmol l^{-1}) and TEA^+ (30 mmol l^{-1}) blocked both of these K^+ currents in identified swimmeret motor neurones (data not shown).

Inward currents

Inward currents were studied using caesium aspartate solution in the pipette to block the outward K^+ currents described above. Depolarizing pulses of 100 ms duration, from a holding potential of −60 mV, were used to set the membrane potential at voltages ranging from −50 mV to +20 mV with 10 mV increments. Two inward currents were observed in swimmeret motor neurones (Fig. 5A). A fast transient current (asterisk, *N*=25 swimmeret motor neurones), which is

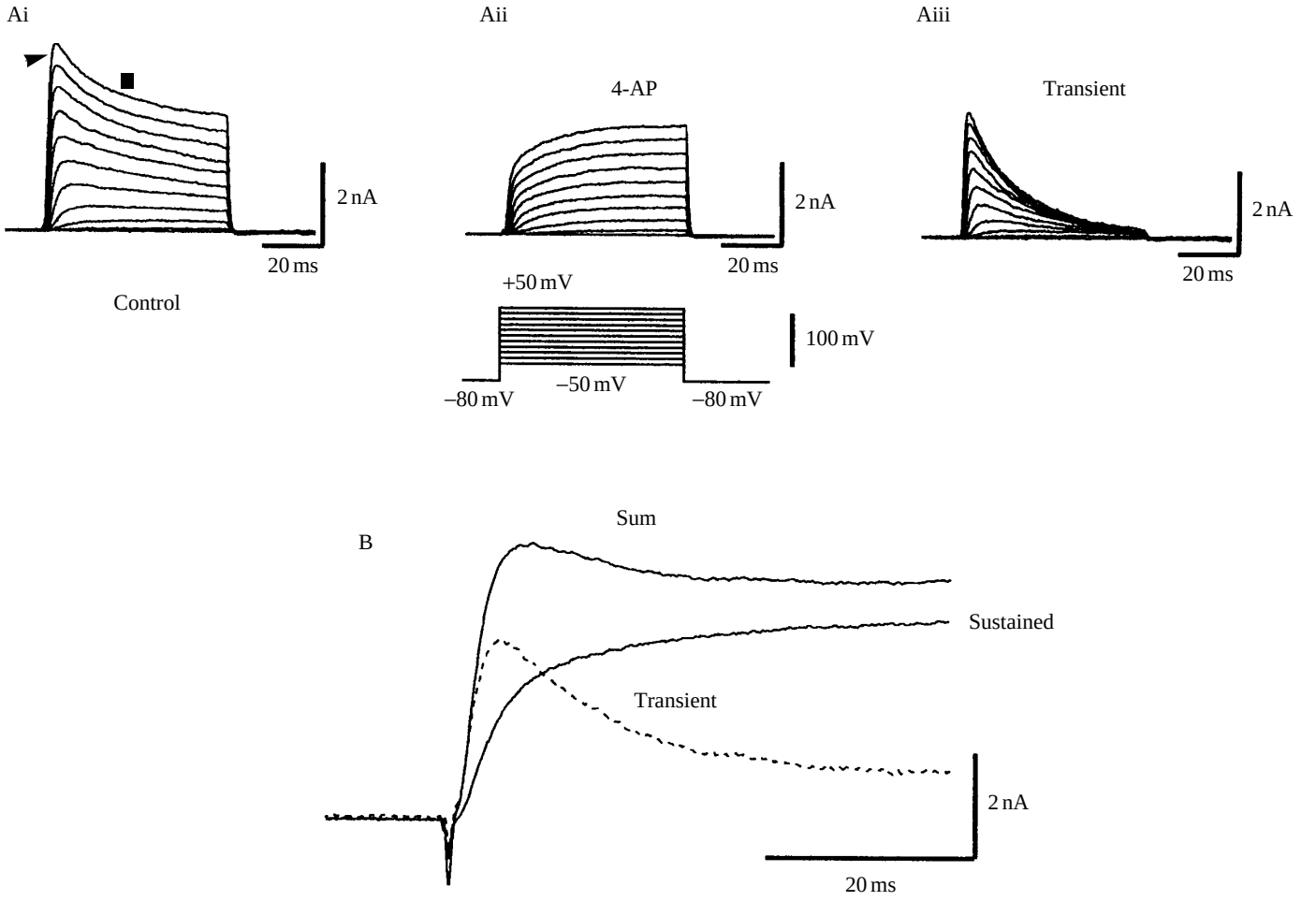

Fig. 4. Separation of the fast transient potassium outward current (A-current) in the swimmeret motor neurones. (Ai) Outward current in response to membrane depolarizations to voltages ranging from −50 mV to +50 mV from a holding potential of −80 mV. The total outward current was composed of a transient outward current (arrowhead) and a sustained outward current (filled square). (Aii) Bath application of 4 mmol l^{-1} 4-aminopyridine (4-AP) suppressed the transient outward current, leaving only the sustained outward current. (Aiii) The transient outward K^+ current (I_A) was then obtained by subtracting the 4-AP-insensitive outward current (Aii) from the total outward current (Ai). (B) The trace labelled sum shows the total outward current recorded for the step from −80 mV (holding potential) to +50 mV. If the holding potential was then set to −40 mV and the step to +50 mV, the A-current was blocked (trace labelled 'sustained'). Subtraction of the trace 'sustained' from the trace 'sum' gave the A-current 'transient'.

presumably responsible for spike generation, disappeared 5 min after the addition of 1 μmol l^{-1} TTX to the bath, leaving only a sustained inward current (Fig. 5B).

Three lines of evidence suggest that this fast transient inward current is a Na^+ current (I_{Na}). First, it was blocked by TTX (Fig. 5B); second, it was also blocked by Na^+-free saline (data not shown); and third, it was inactivated when the swimmeret motor neurones were held at a potential of −40 mV (Connor and Stevens, 1971*a*).

The sustained inward current (*N*=35 swimmeret motor neurones) was not affected by TTX and was increased in amplitude 5 min after equimolar Ba^{2+} replaced Ca^{2+} as the charge carrier (Fig. 5C). It could be blocked 5 min after external application of 2 mmol l^{-1} Co^{2+} (Fig. 6) or Cd^{2+} (not shown). Furthermore, this current was significantly reduced in a dose-dependent manner by nifedipine (Fig. 7D), suggesting that it is an L-type Ca^{2+} current (I_{Ca}). The effect of nifedipine on the Ca^{2+} current is illustrated in Fig. 7. In control conditions, depolarizing pulses from a holding potential of −60 mV to potentials ranging from −50 mV to +30 mV revealed a sustained inward current (Fig. 7A). This Ca^{2+} current was blocked 10 min after bath application of 15 μmol l^{-1} nifedipine (Fig. 7B). The effect of nifedipine is also illustrated by the current–voltage relationships before (open circles) and after (filled circles) the application of 15 μmol l^{-1} nifedipine (Fig. 7C). In addition, nifedipine blocked this Ca^{2+} current in a dose-dependent manner (Fig. 7D). Threshold concentration for the blocking effect of nifedipine was 2 μmol l^{-1}.

Effect of nifedipine on the swimmeret rhythm

Bath application of TTX blocks spike generation, but does not affect the oscillating membrane properties of swimmeret motor neurones in an isolated preparation. These results suggest

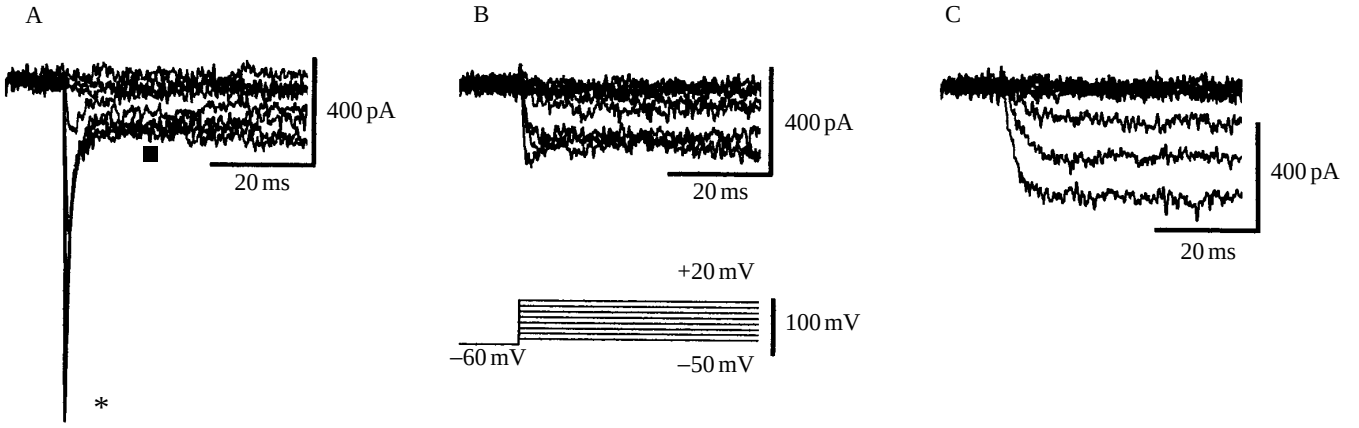

Fig. 5. Inward currents recorded from freshly dissociated swimmeret motor neurones. (A) In the control, inward current records were obtained in response to membrane depolarizations to voltages ranging from −50 mV to +20 mV from a holding potential of −60 mV. Two components of inward current were elicited: a fast transient Na^+ current (asterisk) and a sustained calcium current (filled square). (B) 5 min after the addition of 1 μmol l^{-1} tetrodotoxin (TTX) to the bath, the Na^+ current disappeared, leaving only the sustained Ca^{2+} current. (C) The addition of 2 mmol l^{-1} Ba^{2+} to the bath increased the amplitude of this Ca^{2+} current.

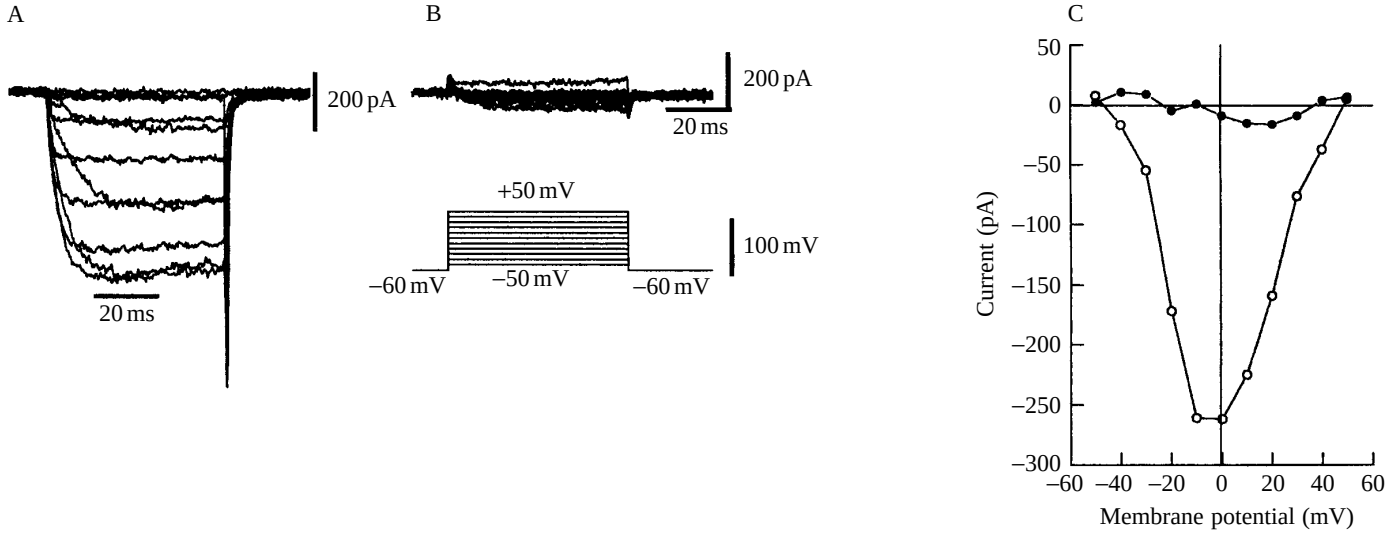

Fig. 6. Effect of Co^{2+} on the sustained Ca^{2+} current. (A) In the control, Ca^{2+} current records were obtained in response to membrane depolarizations to voltages ranging from −50 mV to +50 mV from a holding potential of −60 mV. (B) Addition of 2 mmol l^{-1} Co^{2+} solution to the bath completely blocked the Ca^{2+} current. (C) Current–voltage relationships of the Ca^{2+} currents before (open circles) and 10 min after (filled circles) bath application of 2 mmol l^{-1} Co^{2+}.

that the slow regenerative depolarizations in these motor neurones are due to Ca^{2+} (Murchison *et al.* 1993). As shown above, the swimmeret motor neurones possess an L-type Ca^{2+} current which could be blocked by nifedipine (Fig. 7). The question arises whether nifedipine has any effect on the swimmeret rhythm. To test this, an isolated swimmeret preparation similar to that of Chrachri and Neil (1993) was used (*N*=4 preparations). In the experiment illustrated in Fig. 8, recordings were made both extracellularly from the posterior branch of the first abdominal nerve (which carries the axons of PS motor neurones) and intracellularly from an unidentified swimmeret interneurone (INT_{SW}). In the control, the swimmeret system displayed the usual spontaneous rhythmic activity (Fig. 8A). 15 min after bath application of 20 μmol l^{-1} nifedipine, the swimmeret rhythm was markedly altered and the amplitude of the interneurone's spikes was apparently reduced (Fig. 8B). The swimmeret system recovered its full rhythmicity 30 min after washing (Fig. 8C). Application of nifedipine affected both the frequency of the swimmeret rhythm and the duration of power-stroke bursts, which became very variable (Fig. 9). A preparation displaying a normal swimmeret rhythm had an average PS burst frequency of 1.6±0.2 Hz (open circles, Fig. 9A) and PS burst duration of about 0.3±0.1 s (open bar, Fig. 9B). Bath application of nifedipine reduced significantly the mean PS burst frequency to 1.1±0.7 Hz (P<0.05, paired *t*-test, *N*=177 swimmeret cycles) (filled circles, Fig. 9A), and the

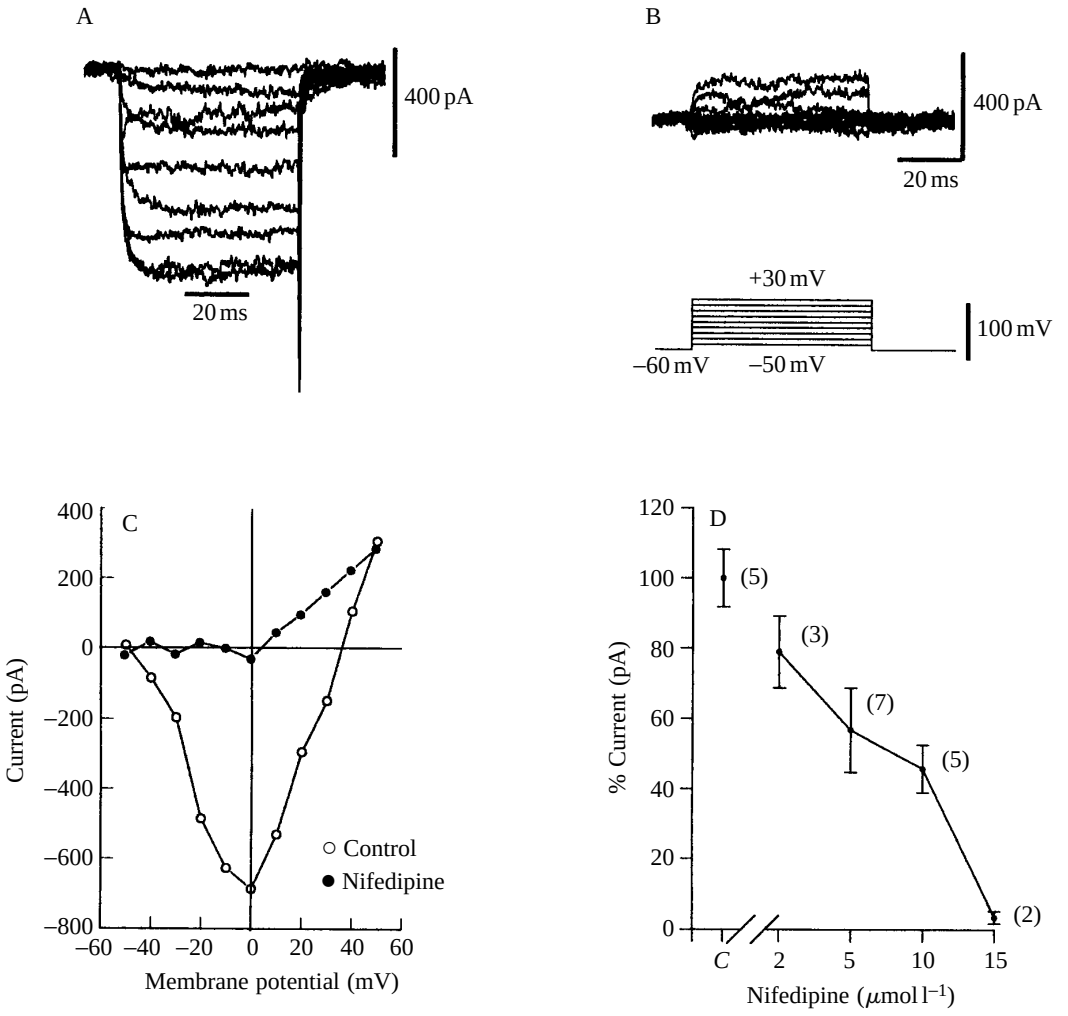

Fig. 7. Effect of nifedipine on the sustained calcium current. (A,B) Current recorded in response to membrane depolarizations to voltages ranging from −50 mV to +30 mV from a holding potential of −60 mV before (A) and after (B) bath application of 15 μmol l^{-1} nifedipine. (C) Current–voltage relationships of the Ca^{2+} currents shown in A before (open circles) and after (filled circles) bath application of 15 μmol l^{-1} nifedipine. (D) Dose–response curve for the effect of nifedipine on the Ca^{2+} currents. Each point corresponds to a mean value ± 1 S.D. calculated from a number of different experiments. The number of swimmeret motor neurones in which each concentration was tested is shown in parentheses beside the mean value. *C*, control.

PS burst duration increased significantly to 0.8±0.4 s (P<0.05, paired t-test, N=180 PS bursts) (filled bar, Fig. 9B).

Discussion

The present study describes the general membrane properties of freshly dissociated swimmeret motor neurones of the crayfish. There appear to be four voltage-sensitive currents present in these neurones. A fast transient inward Na^+ current, two outward K^+ currents and a sustained inward Ca^{2+} current.

Na^+ current

The fast inward current was activated immediately after the onset of the command pulse, reached a peak in few milliseconds and inactivating within 4–5 ms. As in many other excitable tissues (Armstrong *et al.* 1973; Bullock and Schauf, 1979; Lo and Shrager, 1981; Neher, 1971; Lasater, 1986), this fast inward current is carried by Na^+, because it was blocked when motor neurones were perfused with Na^+-free crayfish saline. It was also blocked by TTX and inactivated by holding the cells at −40 mV. In all cases where the axon was present on the dissociated swimmeret motor neurones, a fast inward Na^+ current was recorded. By contrast, in swimmeret motor neurones without an axon, no fast inward current was recorded, suggesting that this inward Na^+ current is of axonal origin. An axonal site for spike generation is a common feature of crustacean neurones (Kuffler and Eyzaguirre, 1955; Hartline, 1967; Selverston *et al.* 1976; Onetti *et al.* 1990).

K^+ currents

When the swimmeret motor neurones were depolarized to voltages more positive than −30 mV from a holding potential of −80 mV, an outward K^+ current developed. The overall current was composed of two components. These two time- and voltage-dependent K^+ currents were distinguishable both

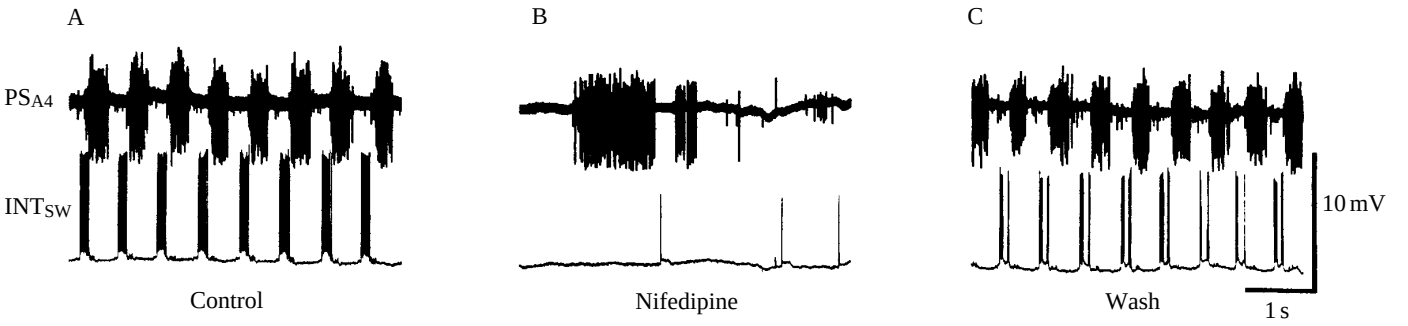

Fig. 8. Effect of nifedipine on the swimmeret rhythm in an isolated crayfish preparation. (A) In the control, the swimmeret system displayed spontaneous rhythmic activity. The extracellular record (PS_{A4}) was obtained from the posterior branch of the first abdominal nerve from abdominal ganglion A4. The intracellular record (INT_{SW}) was from a swimmeret interneurone impaled in abdominal ganglion A4. (B) Bath application of 20 μmol l^{-1} nifedipine disrupted the swimmeret rhythm. (C) 30 min after return to normal saline, the swimmeret rhythm reappeared.

by their pharmacological sensitivity to 4-AP or TEA^+ and by their voltage-dependence of inactivation (Connor and Stevens, 1971*a*,*b*; Thompson, 1977). On the basis of their characteristics, they correspond to the K^+ A-current (I_A) and the K^+ delayed rectifier (I_K) (Connor and Stevens, 1971*a*,*b*). Similar results have been reported in the neurosecretory cell somata in the X-organ of the crayfish (Onetti *et al.* 1990), in lobster cardiac ganglion (Tazaki and Cooke, 1986) and in other invertebrate neurones (Thompson, 1977; Adams *et al.* 1980; Johansen and Kleinhaus, 1986).

Ca^{2+} current

A vast amount of work has been carried out on the voltage-gated Ca^{2+} channels in many different types of nerve cells (Liu and Lasater, 1994). In the swimmeret motor neurones of crayfish, no transient low-voltage-activated Ca^{2+} currents (T-type; Nowycky *et al.* 1985) were recorded. However, the high-voltage-activated Ca^{2+} current (Carbone and Lux, 1984) was detected. The attribution of this sustained inward current to Ca^{2+} is based on its dependence on the external Ca^{2+} concentration, its independence of the external Na^+ concentration, its persistence in the presence of TTX (Fig. 5B) and its inhibition by Co^{2+} (Fig. 6C) and Cd^{2+}. These results confirm those obtained in cardiac ganglion neurones of the lobster (Tazaki and Cooke, 1986). This inward current has a higher permeability to Ba^{2+} than to Ca^{2+}, as demonstrated in Fig. 5C, so it satisfies the criteria for identification of Ca^{2+}-selective channels suggested by Hagiwara and Byerly (1981).

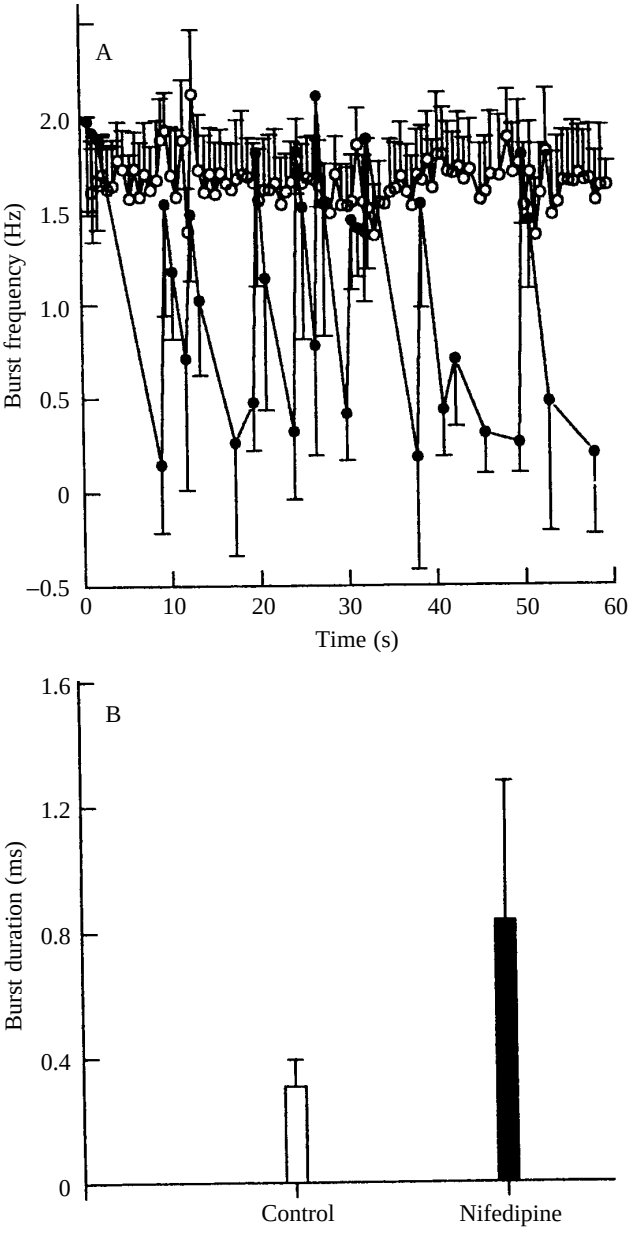

Fig. 9. Effect of nifedipine on the frequency and duration of power-stroke bursts of the swimmeret rhythm. (A) The frequency of power-stroke swimmeret motor activity expressed as a function of time before (open circles) and after (filled circles) bath application of 20 μmol l^{-1} nifedipine. Each point corresponds to the mean value ± 1 S.D. calculated from three different experiments (60 PS bursts from each). (B) The duration of power-stroke activity before (open bar) and after (filled bar) bath application of nifedipine. Each bar is the mean value + 1 S.D. calculated from three different experiments (for calculations, see Materials and methods).

Furthermore, it is also blocked in a dose-dependent manner by nifedipine (Fig. 7D). The presence of Ca^{2+} currents in crustacean neurones has previously been demonstrated in cardiac ganglion neurones of lobster and neurosecretory cells of crayfish (Tazaki and Cooke, 1986; Onetti *et al.* 1990).

A large body of information has been collected concerning the existence of the TTX-insensitive Na^+ currents that are an important feature of bursting pacemaker neurones, notably in *Aplysia californica* (Colmers *et al.* 1982; Adams and Benson, 1985) and in crustacean neurosecretory cells (Onetti *et al.* 1990). These currents were not detected in crayfish swimmeret motor neurones, possibly because they might need to be unmasked by application of neuromodulators such as the neuropeptide proctolin, which is capable of activating and modulating the swimmeret rhythm (Mulloney *et al.* 1987).

The four intrinsic membrane currents described here can be combined to explain the spiking behaviour recorded with microelectrodes in the swimmeret motor neurones (Fig. 2). The spike is due to the activation of I_{Na}. Following inactivation of I_{Na}, motor neurones are rapidly repolarized as a result of the activation of I_A followed by I_K. Together, these two outward K^+ currents are capable of repolarizing the membrane sufficiently to reactivate I_{Na}. Further spikes follow as I_A inactivates and the motor neurone slowly depolarizes. The slow regenerative depolarization recorded with microelectrodes from isolated crayfish preparations (Fig. 2) or even after TTX perfusion (see Murchison *et al.* 1993) may be caused by the inward current that is carried by Ca^{2+} and blocked by Co^{2+} and Cd^{2+}. Further evidence for the role that this inward Ca^{2+} current could play in the slow regenerative depolarization is demonstrated by the effect of nifedipine on the isolated swimmeret preparation (Figs 8, 9). Nifedipine, which selectively affected this inward Ca^{2+} current (Fig. 7), disrupted the swimmeret rhythm by affecting the burst duration and the frequency of PS rhythmic activity, suggesting that I_{Ca} is important in maintaining a regular swimmeret motor rhythmic activity.

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