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Long-lasting potassium channel inactivation in myoepithelial fibres is related to characteristics of swimming in diphyid siphonophores

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Summary

Diphyid siphonophores swim using bursts of propulsive jets, which are produced by contractions of a monolayer of subumbrellar myoepithelial fibres lining the nectophore. This swimming behaviour is characterised by successive increases in the force generating the jets during the initial jets of the burst. Action potentials that generate the contractions propagate throughout the myoepithelial layer: both their amplitude and duration successively increase during the first part of the burst. To investigate the ionic mechanism of this action potential augmentation, single myoepithelial cells were enzymatically dissociated and whole-cell voltage clamped. Na^+, Ca^{2+} and K^+ currents were recorded under different internal and external salt compositions. The Na^+ current was blocked by a relatively high concentration (4 μmol l^{-1} or higher) of tetrodotoxin (TTX), indicating that the Na^+ channel belongs to a group of TTX-resistant Na^+ channels. The Ca^{2+} current was blocked by nifedipine (10 μmol l^{-1}) and Co^{2+} (5 mmol l^{-1}), indicating that the Ca^{2+} channel is L-type. The K^+ current possessed a unique property of long-lasting inactivation. The K^+ current fully inactivated during a depolarisation to +30 mV with a time-constant of ~9 ms, and the time constant of recovery from inactivation at –70 mV was 13.2 s. This long-lasting inactivation of the K^+ channel was the major factor in the augmentation of both action potentials and contractions of the myoepithelial sheet during the initial part of the burst.

Key words: diphyid, behaviour, striated muscle, K^+ channel, inactivation, *Diphyes chamissonis*.

Introduction

Diphyid siphonophores are small rocket-shaped cnidarians (Fig. 1). The polygastric stage of the animals consists of one or two nectophores, which trail a long fishing stem with batteries of nematocysts. There are numerous species, but physiological work has mainly been done upon the relatively abundant *Chelophyes appendiculata*. Like all diphyids, *Chelophyes* swims rapidly by propulsive jets; if undisturbed in large aquaria, it shows occasional apparently spontaneous short bursts of jet pulses at frequencies up to ~8 Hz (Mackie and Carré, 1983). Vibration or light touch evoke similar series of jet pulses, during which *Chelophyes* withdraws the fishing stem and attains maximum instantaneous speeds of 20 body lengths per second (20 BL s^{-1}; Bone and Trueman, 1982).

The propulsive jets are produced by contractions of a thin (1–2 μm) monolayer of subumbrellar myoepithelial fibres lining the chambers of the nectophores. The cross-striated myoepithelial fibres are coupled by numerous gap junctions, and all muscle fibres seem to be similar, so that the subumbrellar sheet contracts as a unit. The fibres have no sarcoplasmic reticulum (SR) as an intracellular Ca^{2+} store, although they possess an abundant invaginated tubular system [morphologically analogous to a transverse-tubular (T-tubular) system in skeletal muscle; Mackie and Carré, 1983]. Hence, the fibres contract using only the influx of extracellular Ca^{2+} through voltage-gated Ca^{2+} channels, and relaxation is solely due to Ca^{2+} excretion by Na^+/Ca^{2+} exchange across the membrane (Bone et al., 1999). The type of Ca^{2+} channel involved in excitation–contraction coupling is unclear.

A curious feature of their swimming behaviour is that the force generating the propulsive jets is weak at first and then successively increases during the initial jets. Previous studies of the electrical properties of the myoepithelium showed that action potentials that propagate through the muscle sheet were carried by both Na^+ and Ca^{2+} and possessed unusual features: during a short burst of action potentials, the initial action potentials were of conventional rapidly rising and rapidly falling form, but subsequent action potentials showed both a greatly increased overshoot and the formation of a plateau or hump on the falling phase, leading to greatly prolonged action potentials (Chain et al.,

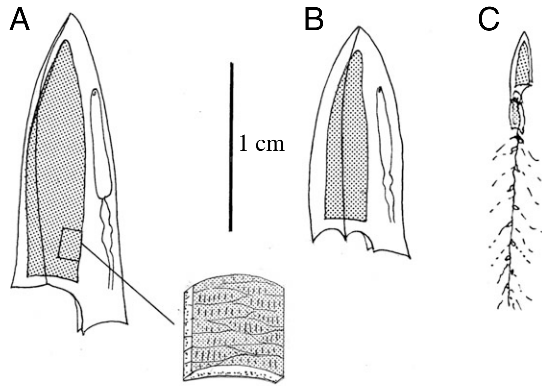

Fig. 1. Schematic illustrations of the anterior nectophore of *Chelophyes appendiculata* Eschscholtz (A) and that of *Diphyes chamissonis* Huxley (B). The scale bar indicates 1 cm. The subumbrellar myoepithelium sheet lining the nectophore is shaded, and a portion is shown enlarged below. (C) The entire colony of *C. appendiculata* shows fishing stem and tentacles only partially extended.

1981; Bone, 1981; Bone et al., 1999). It has therefore been suggested that this augmentation of the action potential evokes successive increases in myoepithelial tension by increased Ca^{2+} influx during the action potentials (Bone, 1981; Bone et al., 1999). However, in order to investigate the ionic mechanisms of the action potential augmentation, potential recordings are not sufficient and it is necessary to develop an experimental system enabling ionic currents passing through voltage-gated ionic channels to be recorded under controlled membrane voltage. In the present study, we developed a protocol whereby single myoepithelial fibres were isolated, and we independently recorded Na^+, Ca^{2+} and K^+ currents by subjecting the fibre to whole-cell voltage clamp. We describe here the experimental results showing that the action potential augmentation is produced by voltage-dependent long-lasting inactivation of K^+ channels, not by a successive increase in activities of Na^+ or Ca^{2+} channels. The whole-cell clamp experiments on single fibres also revealed the pharmacological properties of the ion channels that could not be detected in *in vivo* preparations.

Materials and methods

Materials

Whole-cell voltage clamp experiments were conducted on single muscle fibres enzymatically isolated from the myoepithelial layer of *Diphyes chamissonis* Huxley at the Ine Marine Laboratory of National Institute for Physiological Sciences, Ine, Kyoto, Japan. The animals were collected with a plankton net in Wakasa Bay, off the coast of Ine. Action potential recordings were taken using *Chelophyes appendiculata* Eschscholtz at the Station Zoologique, Villefranche sur Mer, France. The animals were collected in the Rade de Villefranche.

Solutions and chemicals

Artificial seawater (ASW) consisted of (in mmol l^{-1}) 450 NaCl, 9 KCl, 10 $CaCl_2$, 50 $MgCl_2$ and 15 Na-Hepes buffer (pH 7.8). An ASW containing a lower (4 mmol l^{-1}) concentration of Ca^{2+} (4Ca-ASW) was also prepared to avoid damage to the cell in whole-cell voltage clamp experiments. Nominally Ca^{2+}-free ASW (N-Ca-free-ASW) was made by replacing $CaCl_2$ in ASW with $MgCl_2$. Ca^{2+}-free ASW (Ca-free-ASW) was made by replacing $CaCl_2$ in ASW with Na-EGTA. The pipette (intracellular) solution for whole-cell voltage clamp experiments consisted of (in mmol l^{-1}) 450 Cs-D-aspartate, 15 $MgCl_2$, 15 EGTA, 15 Cs-MOPS buffer (pH 7.2). This solution is referred to as Cs-asp. For K^+ current measurements, Cs-D-aspartate and Cs-MOPS were replaced with K-D-aspartate and K-MOPS, respectively (K-asp). Stock solutions of 100 mmol l^{-1} $CoCl_2$, 1 mmol l^{-1} tetrodotoxin (TTX; Sankyo, Tokyo, Japan) dissolved in distilled water and 10 mmol l^{-1} nifedipine (Sigma, St Louis, MO, USA) dissolved in ethanol were kept in the dark at 4°C. The experiments were carried out at a room temperature of 20–22°C, which was close to the sea temperature.

Isolation of single locomotor muscle fibres

The anterior nectophore of *D. chamissonis* was cut open and incubated in a dissociation medium (Ca-free-ASW containing 5 mg ml^{-1} of trypsin; Boehringer, Mannheim, Germany) at 25°C. When the myoepithelium was detached from the nectophore, it was taken and incubated in a new dissociation medium until the connection between muscle fibres became loose. Then the myoepithelium was gently agitated in an experimental chamber filled with an external solution for each experiment. Single muscle fibres that attached to the bottom of the chamber were used for experiments. The isolated fibres gradually became spherical and lost the structure of cross-striation. We therefore finished an experiment shortly (45 min) after fresh preparations were made.

Whole-cell current recordings

An experimental chamber was placed on the mechanical stage of an inverted microscope (TMD, Nikon, Tokyo, Japan), and whole-cell voltage clamp was carried out with a patch clamp amplifier (EPC-7, List, Darmstadt, Germany). Electrode resistance was 1–2 MΩ, and the series resistance of 1 MΩ was compensated. Pulse generation and data acquisition were performed with a 12-bit D/A and A/D converter (Labmaster/DMA, Scientific Solution, Solon, OH, USA) and an IBM/AT compatible computer using pCLAMP software (Axon Instruments, Chicago, IL, USA). The cell membrane capacitance was calculated from the area under a transient capacitive current produced by a 10 mV step depolarisation from a holding potential of –70 mV. Linear currents were subtracted digitally using a P/4 pulse protocol.

Recordings of action potentials and contractions from the myoepithelium sheet of C. appendiculata

The animals were pinned to the Sylgard™ base of a small dish, using *Opuntia* spines. The anterior nectophore was

partially cut open to permit access of microelectrodes to the myoepithelial fibres. Conventional 3 mol l^{-1} KCl-filled microelectrodes (15–30 MΩ) with long flexible shanks were led *via* a laboratory-made follower amplifier. The preparations were stimulated at the basal nerve rings using fine polyethylene suction electrodes fed by a stimulator (S48, Grass, Quincy, MA, USA) *via* an isolating unit. A single stimulus usually evoked a short burst of action potentials and associated contractions in the myoepithelium. Contractions were detected with a fine probe attached to a strain gauge (N801; SensoNor, Horteu, Norway). Both electrical and mechanical signals were simultaneously recorded with a PCM recorder (PC204; Sony, Tokyo, Japan).

Results

Form of the action potentials in Chelophyes

Although cells of the myoepithelial sheet in *Chelophyes* are only around 1–2 µm thick, stable penetrations of microelectrodes are possible for long periods, even during bursts of contractions, as previously reported (Bone et al., 1999). Fig. 2 shows progressive increases in both the amplitude and duration of muscle action potentials during a swimming burst, which correspond to increase in the tension developed by the muscle of *Chelophyes.* The action potentials and contractions in intact or cut preparations were insensitive to TTX (Chain et al., 1981) and Co^{2+} (Bone, 1981). But Co^{2+} applied to both faces of the myoepithelial cells (in the bath and injected into the mesogloea) blocked the contractions (Bone et al., 1999). Although we have not made similar recordings from *Diphyes*, analysis of the membrane currents shows that similar changes take place in *Diphyes* action potentials.

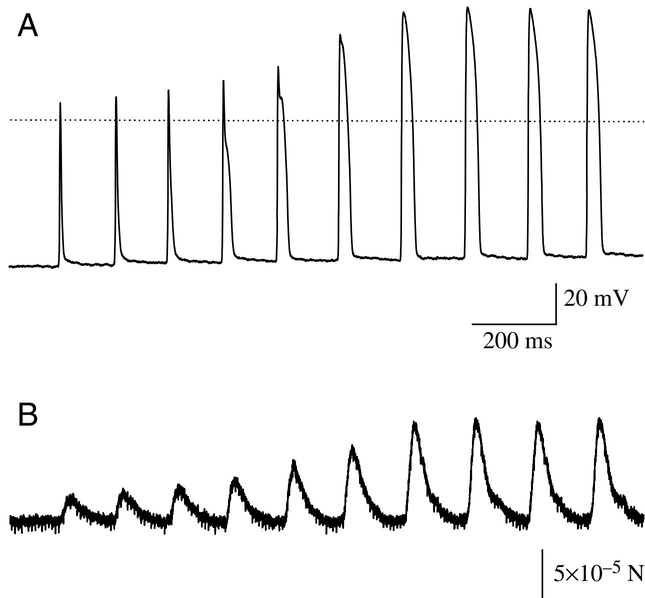

Fig. 2. Burst of action potentials and associated contractions in the myoepithelium sheet. (A) Series of spontaneous action potentials from subumbrellar myoepithelium evoked by a single stimulus, showing a successive increase in both the amplitude and duration. The dotted line indicates 0 mV. (B) Trace of strain gauge output, showing that the force of contractions of the myoepithelium sheet became stronger as both amplitude and duration of the action potentials increased. The external solution was ASW.

Membrane ion currents in Diphyes

Na^+, Ca^{2+} and K^+ currents under whole-cell voltage clamp

Isolated single myoepithelial fibres were whole-cell voltage clamped, and membrane currents were recorded under different internal and external salt compositions. The mean value of the cell membrane capacitance was 147.9±101.2 pF (mean ± S.D., N=77). To detect Na^+ currents, fibres bathed in N-Ca-free-ASW were dialysed with Cs-asp. Note that internal Cs^+ blocks currents through K^+ channels. Short (10 ms) depolarising voltage pulses from –50 mV to +50 mV in 10 mV increments were applied to the membrane from a holding potential of –70 mV (pulse protocol is shown in Fig. 3A). Transient inward currents appeared at voltages more positive than –20 mV (Fig. 3).

The whole-cell clamp experiments revealed that the Na^+ current could be blocked by TTX at relatively high concentrations, in contrast to earlier work on intact animals (Chain et al., 1981). When the external N-Ca-free-ASW contained TTX at 4 µmol l^{-1} or higher, a Na^+ current was not observed in 53 of 54 cells examined. But at 1 µmol l^{-1} TTX, a Na^+ current remained in all nine cells examined. Therefore, the Na^+ channel was neither TTX-insensitive nor highly sensitive; it belongs to a group of 'TTX-resistant Na^+ channels' according to the classification of Anderson (1987).

To detect Ca^{2+} currents, fibres were bathed in 4Ca-ASW containing 4–10 µmol l^{-1} TTX and dialysed with Cs-asp.

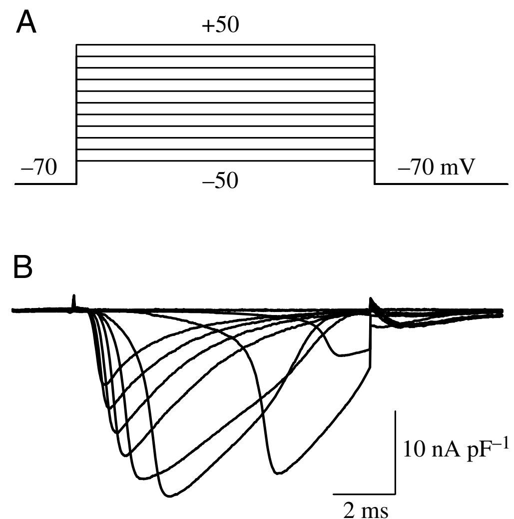

Fig. 3. Na^+ currents at different voltages. (A) Pulse protocol of the whole-cell voltage clamp. Step depolarisations from –50 mV to +50 mV in 10 mV steps every 5 s were applied from a holding potential of –70 mV. (B) Whole-cell membrane currents in response to the step depolarisations. Eleven traces are superimposed. The bath solution was N-Ca-free-ASW and the pipette solution was Cs-asp. Cell membrane capacitance (C_m)=124.4 pF.

Depolarising pulses of –50 to +50 mV with 25 ms duration evoked long-lasting inward currents (Fig. 4A). No distinct spontaneous run-down of the current was observed. The *I–V* relationship of the peak currents obtained from eight experiments (Fig. 4B) shows that the inward current appeared at voltages higher than –40 mV and peaked at +10 mV. Whole-cell voltage clamp experiments revealed that the currents were sensitive to nifedipine, a dihydropyridine derivative (*N*=5). Fig. 4C superimposes five current records at +10 mV pulses obtained from the same fibre before (1, 2) and after (3–5) external application of 10 μmol l^{-1} nifedipine; records 1 and 2 were obtained 3 and 6 min after the whole-cell configuration was made, and records 3–5 were obtained 2, 4 and 6.5 min after the nifedipine application. The inward currents were also blocked by 5 mmol l^{-1} Co^{2+} applied to the bath (*N*=6; data not shown). The results show that these currents were carried by Ca^{2+} through L-type Ca^{2+} channels.

When fibres were dialysed with K-asp instead of Cs-asp in N-Ca-free-ASW containing 4–10 μmol l^{-1} TTX, outward currents appeared associated with depolarisations to more than –10 mV (Fig. 5). Because this current component did not appear when the internal cation was Cs^{+}, these currents are thought to be carried by K^{+} through K^{+} channels. The outward

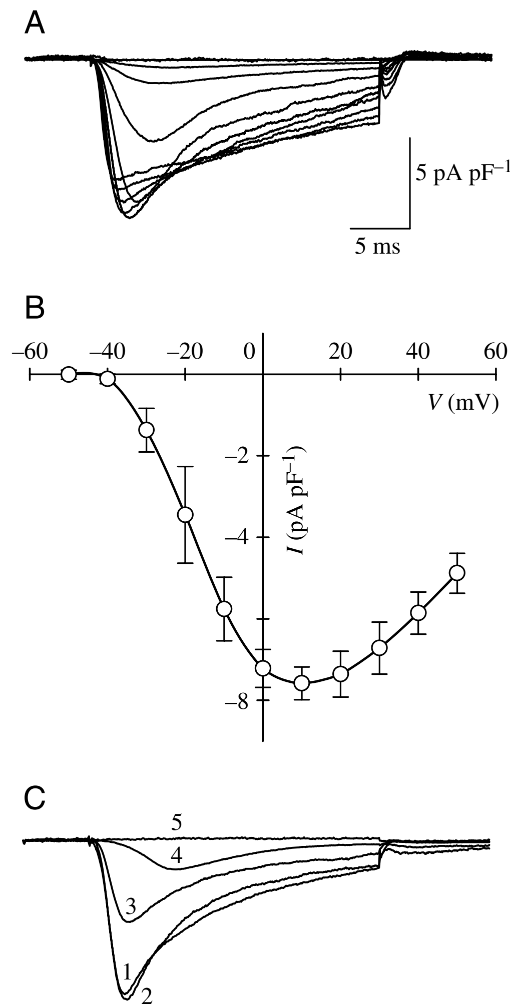

Fig. 4. Ca^{2+} currents at different voltages and the effect of nifedipine. (A) Whole-cell membrane currents in response to the step depolarisations (same pulse protocol as in Fig. 3A except that the pulse duration was increased to 25 ms). Eleven traces are superimposed. The bath solution was 4Ca-ASW containing 4 μmol l^{-1} TTX, and the pipette solution was Cs-asp. Cell membrane capacitance (C_m)=109.0 pF. (B) Current–voltage (*I–V*) relationship of the peak currents obtained from eight experiments. Values are means ± S.D. (C) Superposition of five current records at +10 mV pulses before (1, 2) and after (3–5) application of 10 μmol l^{-1} nifedipine. Records 1 and 2 were obtained 3 and 6 min after the whole-cell configuration was made, and records 3, 4 and 5 were obtained 2, 4 and 6.5 min after the nifedipine application. Nifedipine was applied after record 2 was taken. C_m=138.2 pF.

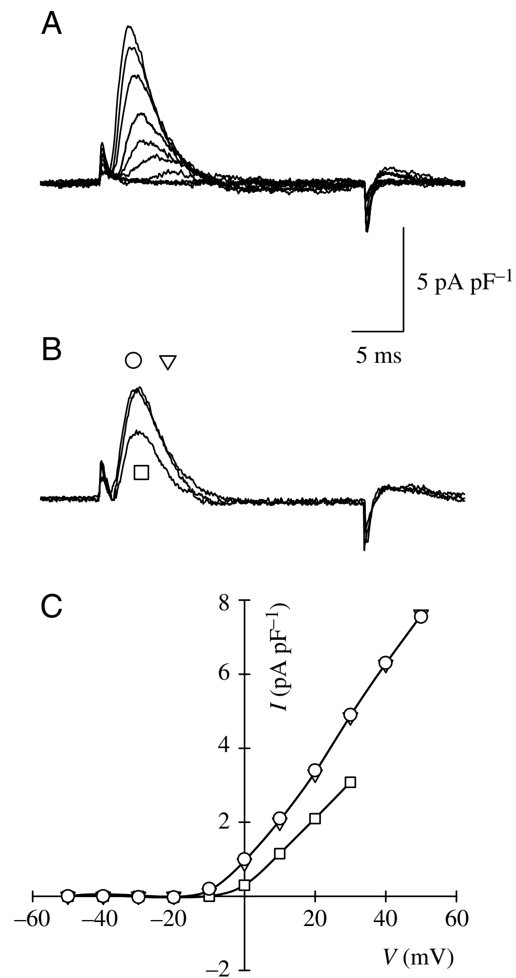

Fig. 5. K^{+} currents at different voltages. (A) Whole-cell membrane currents in response to the step depolarisations (same pulse protocol as in Fig. 3A except that the pulse duration was increased to 25 ms). Eleven traces are superimposed. The bath solution was N-Ca-free-ASW containing 4 μmol l^{-1} TTX and the pipette solution was K-asp. Cell membrane capacitance (C_m)=132.6 pF. (B) Membrane currents associated with depolarisations to +30 mV from three different holding potentials of –40 mV (square), –70 mV (circle) and –90 mV (triangle). (C) Current–voltage (*I–V*) relationships of the peak currents at three different holding potentials of –40 mV (open squares), –70 mV (open circles) and –90 mV (open triangles).

current appeared ~1 ms after each depolarisation, reached a peak in 3–10 ms, depending on the voltage, and greatly inactivated during each depolarisation (Fig. 5A). Note that recovery of this current from inactivation was very slow (time constant of recovery was ~13 s at –70 mV; see Fig. 8). Therefore, under the present pulse protocol (5 s interval between depolarisations), the peak value was smaller than that it would have attained after full recovery from inactivation (only 30% at +30 mV). Two other sets of records were taken from the same fibre at different holding potentials of –90 and –40 mV. Fig. 5B compares three traces associated with depolarisations to +30 mV from holding potentials of –40 mV, –70 mV and –90 mV. There was no significant difference in the current time courses and their voltage dependencies between the holding potentials of –70 and –90 mV (see also Fig. 5C). This means that there is no difference in the rate of recovery of the K^+ channel from inactivation between the two voltages. On the other hand, the K^+ currents were more inactivated at the holding potential of –40 mV (Fig. 5B,C). Externally applied EGTA (10 mmol l^{-1}, *N*=2), Co^{2+} (up to 6 mmol l^{-1}, *N*=3) or nifedipine (5 μmol l^{-1}, *N*=1) to the external N-Ca-free ASW had no effect on these properties of the K^+ current.

Changes in the Na^+, Ca^{2+} and K^+ currents during repetitive depolarisations

There are several possible causes for the augmentation of action potentials during repetitive firings; i.e. (1) an increase in the activities of Na^+ and Ca^{2+} channels during repetitive depolarisations, (2) a decrease in the activity of K^+ channels or (3) both the cases of (1) and (2) together. We measured changes in the Na^+, Ca^{2+} and K^+ currents during repetitive depolarisations under whole-cell voltage clamp. Ten depolarising pulses of 100 mV from the holding potential of –70 mV were given at a 200 ms interval (5 Hz; see Fig. 6A). As can be seen from Fig. 6A,B, there was no augmentation of Na^+ or Ca^{2+} current during the repetitive depolarisations. Instead, both currents slightly decreased, and the final amplitude of Na^+ and Ca^{2+} currents at their peak (relative to the initial values) became approximately 86% and 93%, respectively. On the other hand, the K^+ current completely inactivated during the first depolarisation with a time constant of ~9 ms (Fig. 6C) and did not recover from inactivation during the repetitive depolarisations.

Effect of external Ca^{2+} on the K^+ current

To test if a Ca^{2+}-activated K^+ current component is present and contributes to the action potential augmentation, fibres were dialysed with K-asp and bathed in 3Ca-ASW containing 4 μmol l^{-1} TTX. Two depolarising pulses with the same amplitude and duration were applied at a 200 ms interval. Fig. 7A shows an example of records associated with depolarisations to +30 mV from the holding potential of –70 mV. The current associated with the first depolarisation consisted of two components: an inward Ca^{2+} current and an outward K^+ current that appeared ~1 ms after depolarisation.

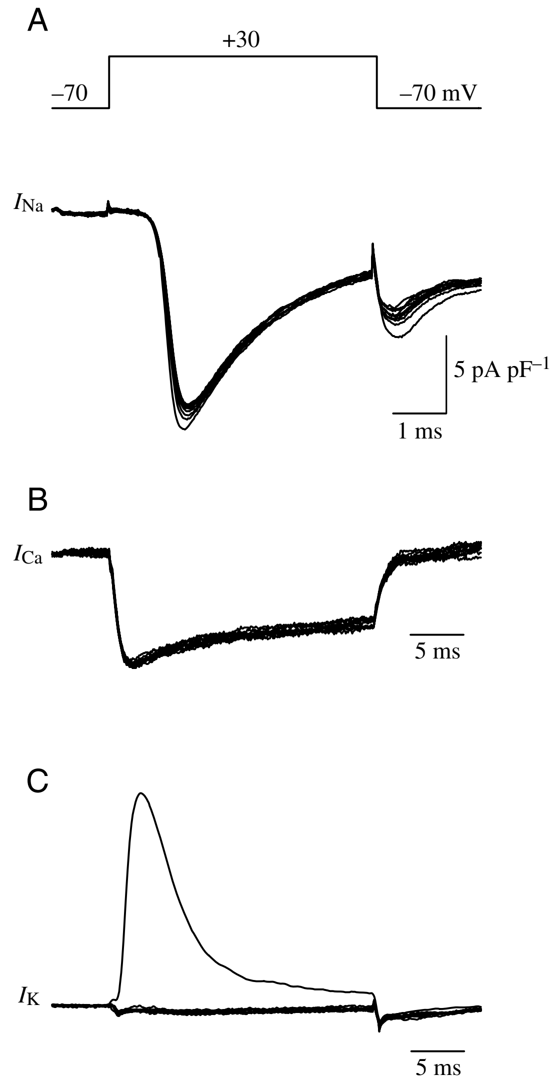

Fig. 6. Changes in Na^+, Ca^{2+} and K^+ currents during 10 repetitive depolarising pulses with a 200 ms interval. (A) Upper panel: pulse protocol of the repetitive stimulations. Step depolarisations from the holding potential of –70 mV to +30 mV. Lower panel: superposition of 10 traces of Na^+ currents in response to step depolarisations. The bath solution was N-Ca-free-ASW, and the pipette solution was Cs-asp. Cell membrane capacitance (C_m)=68.0 pF. (B) Superposition of 10 traces of Ca^{2+} currents. The pulse duration was 25 ms. The bath solution was 4Ca-ASW containing 4 μmol l^{-1} TTX and the pipette solution was Cs-asp. C_m=64.9 pF. (C) Superposition of 10 traces of K^+ currents. The pulse duration was 25 ms. Note that K^+ current was observed only during the first pulse. The bath solution was N-Ca-free-ASW containing 4 μmol l^{-1} TTX, and the pipette solution was K-asp. C_m=187.9 pF.

This time lag was not Ca^{2+} dependent, as this was observed in all K^+ current records taken without external Ca^{2+}. The outward K^+ current disappeared in the current associated with the second depolarisation. Subtraction of the second current record from the first current record exhibited a current shape very similar to those recorded without external Ca^{2+} (Fig. 7B). The results strongly suggest that the Ca^{2+} influx did not

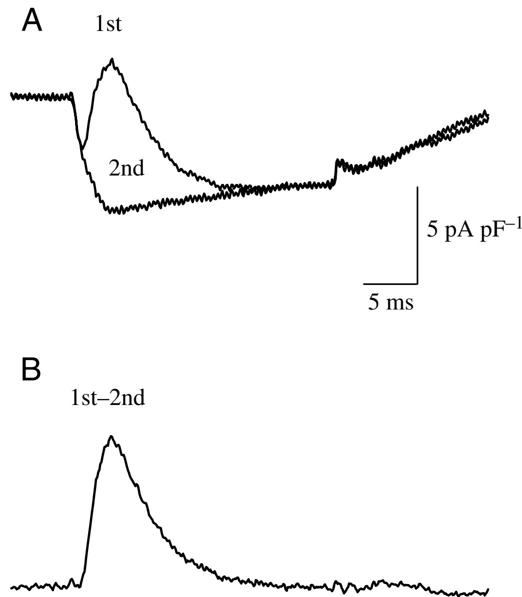

Fig. 7. Effect of Ca^{2+} current on K^+ current. (A) Superposition of two current traces associated with depolarisations to +30 mV with a 200 ms interval. The pulse duration was 25 ms. (B) K^+ current component obtained by subtracting the second current record from the first current record in A. The bath solution was 3Ca-ASW containing 4 μmol l^{-1} TTX, and the pipette solution was K-asp. Cell membrane capacitance (C_m)=110.4 pF.

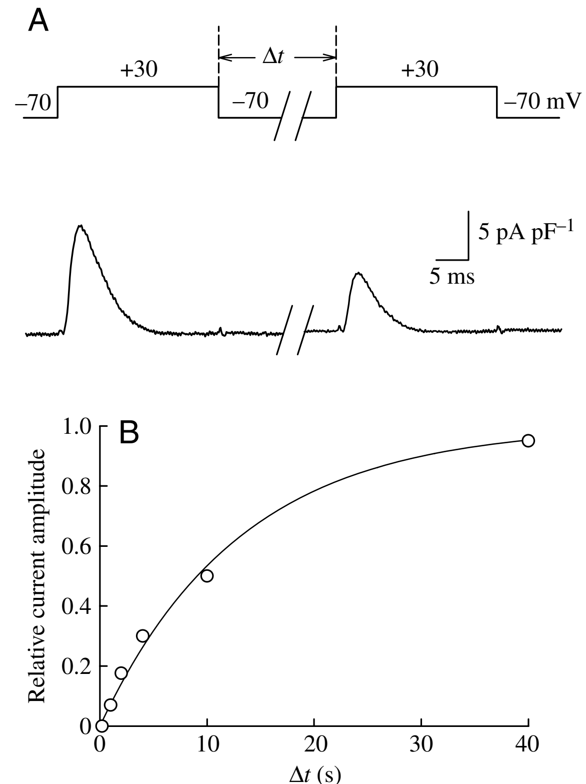

Fig. 8. Time course of recovery of K^+ current from inactivation. (A) Upper panel: protocol of the double pulses stimulation. Two-step depolarisations from the holding potential of –70 mV to +30 mV with the interval of Δt were applied. Lower panel: K^+ currents in response to double-pulse stimulation (Δt=10 s). Amplitude of the 2nd K^+ current (right) was ~50% of the 1st current (left). (B) Relationship between the recovery of K^+ current amplitude and Δt. The curve was fitted with an equation of experimental decay, $I(\Delta t)/I_0=1-\exp(-\Delta t/\tau)$, where $I(\Delta t)/I_0$ is the relative amplitude of K^+ current after an interval time of Δt (s), and τ is the time constant in s for K^+ current recovery. The value of τ is 13.2 s. The bath solution was N-Ca-free-ASW containing 4 μmol l^{-1} TTX, and the pipette solution was K-asp. Cell membrane capacitance (C_m)=143.2 pF.

produce any additional K^+ current component having long-lasting inactivation. It is also noted that the time to peak of the Ca^{2+} current after depolarisation is similar to that of the K^+ current, suggesting that the Ca^{2+} spike would be more influenced by the K^+ channel inactivation.

Recovery of the K^+ current from inactivation

The time constant of recovery of the K^+ current from inactivation was determined by changing the interval (Δt) of two depolarising pulses from –70 to + 30 mV (pulses protocol is shown in the upper panel of Fig. 8A). The lower panel of Fig. 8A shows the currents at Δt=10 s. The peak amplitude of the second current was ~50% of the initial one (Fig. 8A). Fig. 8B plots the peak value of K^+ current amplitude (I_K) during the second pulse relative to that during the first pulse against Δt, and the plots were fitted with a single exponential function, $1-\exp(-\Delta t/\tau)$. The time constant (τ) of the recovery from inactivation was calculated to be 13.2 s.

K^+ current inactivation during repetitive short depolarisations

We investigated whether the properties of the K^+ channel can explain the augmentation of the initial few action potentials during a burst using the potential records obtained from *Chelophyes appendiculata*. Fig. 9A superimposes the initial six action potentials of the burst shown in Fig. 2. It is seen from the traces that the augmentation was mainly brought about by the growth of the slower Ca^{2+} spike. As the duration of the action potential, especially the overshooting time, increased and as the action potential peak became higher, the subsequent action potential was further augmented. The overshooting times of the first to sixth action potentials were 1.5, 2.0, 2.5, 3.4, 14.9 and 20.4 ms, respectively, and the peak potentials were 9.3, 12.6, 16.3, 21.6, 29.3 and 47.1 mV, respectively, in this specific case. To test if K^+ channel inactivation during the action potentials could produce the action potential augmentation, 10 short (5 ms) depolarising pulses to +10 mV were applied at 200 ms intervals, and the decay of K^+ currents during the short depolarisations was measured. Fig. 9B superimposes K^+ current records produced by the ten repetitive depolarisations. The K^+ current did not fully inactivate during the first depolarisation; it decreased successively during the repetitive depolarisations. Fig. 9C plots relative peak values to the first K^+ current peak during the repetitive depolarisations. Each point was obtained by averaging seven data points, and

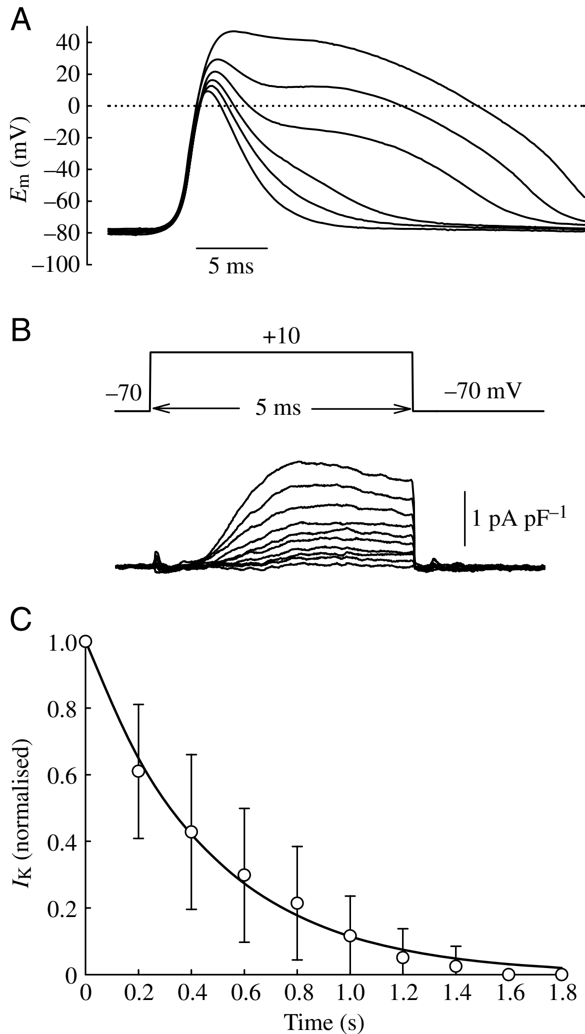

Fig. 9. Relationship between action potential augmentation and K^+ channel inactivation. (A) Superposition of initial six action potentials in Fig. 3 in *Chelophyes appendiculata*. (B) Superposition of 10 K^+ currents associated with repetitive short (5 ms) depolarising pulses to +10 mV at 200 ms intervals in *Diphyes chamissonis*. The bath solution was N-Ca-free-ASW containing 10 μmol l^{-1} TTX, and the pipette solution was K-asp. Cell membrane capacitance (C_m)=201.2 pF. (C) Decay of peak K^+ current values during repetitive depolarisations relative to the 1st current obtained from five experiments. Values are means ± S.D. The curve was drawn by fitting the points with a single exponential function. The time constant was calculated to be 0.46 s.

the error bar indicates the magnitude of S.D. The curve was drawn by fitting the averaged values with an exponential decay function. The time constant was 0.46 s. The depolarising pulse seemed to mimic the overshooting voltage of the fourth action potential. Therefore, this depolarisation would inactivate the K^+ channels more strongly than the first to third action potentials and less strongly than the fifth and sixth action potentials. For example, the initial action potential had much shorter overshooting time than the pulse duration. Therefore, the rate of inactivation produced by the initial action potential would be much smaller than that produced by the initial voltage pulse; hence, the second action potential was not much augmented. This may explain the characteristic property of the action potential augmentation. Because no other factor that produces the augmentation was found, we concluded that the action potential augmentation of diphyid siphonophores was due to K^+ channel inactivation during a burst of action potentials. It is also noted in Fig. 9A that the resting potential did not change during the action potential augmentation, indicating that this K^+ channel did not contribute to the resting potential generation.

Discussion

Ion channels in isolated fibres

The whole-cell voltage clamp experiments revealed the presence of an Na^+ channel, an L-type Ca^{2+} channel and a K^+ channel in the myoepithelial fibres of diphyid siphonophores. Direct exposure of the cell membrane to the bathing solution revealed the pharmacological properties of the ion channels, which could not be detected in *in situ* preparations. The Na^+ channel was shown to belong to a TTX-resistant Na^+ channel group, and the Ca^{2+} channel was sensitive to Co^{2+} and nifedipine. All the channels possess their own voltage and time-dependent activation–inactivation kinetic properties. The K^+ channel has the unique kinetic property of long-lasting inactivation. The voltage-dependent properties of the K^+ channel for the activation and the steady-state inactivation expected from the present results (Fig. 5) are similar to those of *Shaker* K^+ channels in jellyfish *Polyorchis penicillatus* (Grigoriev et al., 1997), suggesting that the siphonophore muscle K^+ channel belongs to the same family.

Muscle electrical responses

As Spencer and Satterlie (1981) pointed out, action potentials in the subumbrella swimming muscle sheet of the medusa *Polyorchis* not only propagate through the tissue to evoke contraction but also carry information about the required duration of contraction. The action potentials of the muscle sheet in large individuals last longer than those in small individuals, hence contraction assumed (reasonably) to result from Ca^{2+} entry during the action potential lasts longer in the larger individuals. A similar situation evidently occurs in diphyids, where whatever the size of the specimen, the first few action potentials of a burst differ from those succeeding (Chain et al., 1981). These latter authors used drugs and substitution experiments to show that the action potentials were carried both by Ca^{2+} and Na^+, recognising that further studies with whole-cell voltage clamp techniques were required. Until now, this latter approach has only been used in cnidarian muscle to examine isolated *Polyorchis* muscle cells (Lin and Spencer, 2001). Here, although previous experiments (Spencer and Satterlie, 1981) had suggested that Na^+ was required to support action potentials, the only inward current observed was a T-type Ca^{2+} current.

Excitation–contraction coupling in diphyids is apparently

solely dependent upon Ca^{2+} entry across the sarcolemma (Bone et al., 1999) and intracellular stores are absent. Presumably, a Na^{+}/Ca^{2+} exchange mechanism to extrude Ca^{2+} is found across the sarcolemma, similar to that in the small tunicate *Doliolum* (Bone et al., 1997), which also has no internal Ca^{2+} stores within the muscle cell (Inoue et al., 2002).

The striking feature of the diphyid action potential is its change in amplitude and form during repetitive stimulation. Our records show that this is brought about in an unique way, by long-lasting inactivation of the K^{+} channel. During a train of action potentials, the first few do not inactivate the K^{+} channel completely so that the first few action potentials gradually change to the form of the subsequent potentials during the train. (Note that this K^{+} channel plays a leading role in the action potential termination of only the initial few action potentials. It is not clear how the action potentials are regularly terminated after this K^{+} channel has been fully inactivated. This ionic mechanism remains unsolved.) Simultaneous records of action potential form during stimulated and 'spontaneous' series of contractions show that, just as in *Polyorchis*, contractions increase as the action potentials increase in duration (Bone, 1981). In these experiments, muscle contractions were measured with a strain gauge linked to the edge of the cut nectophore, hence no propulsive jets were produced and changes in the hydrodynamics of the propulsive jet or of flow around the intact free-swimming animal are not relevant. Our interest has been solely in the way in which membrane currents underlie the striking change in action potential form during successive potentials. It is, however, not unnatural to ask how such changes affect the swimming ability of diphyids.

In diphyids, rapid swimming almost always involves several powerful jet pulses in succession, as it does in the hydromedusan *Aglantha*, which is capable of very rapid swimming (Donaldson et al., 1980). There is, however, a striking difference between the two. The first jet pulse of *Aglantha* is the most effective in driving it forward, since subsequent pulses begin before the bell has completely refilled and hence are less effective, driving it forward only 40–60% of the distance given by the first pulse. By contrast, the first few pulses in diphyids are the least effective (Bone and Trueman, 1982), since their contractions are the least strong, because during the first few shorter action potentials less Ca^{2+} crosses the sarcolemma than it does during later potentials of the swimming burst.

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References

Anderson, P. A. V. (1987). Properties and pharmacology of a TTX-insensitive Na^{+} current in neurones of the jellyfish *Cyanea capillata*. *J. Exp. Biol.* **133**, 231-248.

Bone, Q. (1981). The relation between the form of the action potential and contractions in the subumbrellar myoepithelium of *Chelophyes* (Coelenterata: Siphonophora). *J. Comp. Physiol.* **144**, 555-558.

Bone, Q. and Trueman, E. R. (1982). Jet propulsion of the calycophoran siphonophores *Chelophyes* and *Abylopsis*. *J. Mar. Biol. Assn. UK* **62**, 263-276.

Bone, Q., Inoue, I. and Tsutsui, I. (1997). Contraction and relaxation in the absence of a sarcoplasmic reticulum: muscle fibres in the small pelagic tunicate *Doliolum*. *J. Muscle Res. Cell Motil.* **18**, 375-380.

Bone, Q., Carré, C., Tsutsui, I. and Inoue, I. (1999). Calycophoran siphonophore muscle fibres without any sarcoplasmic reticulum but with tubular invaginations morphologically analogous to a T-system. *J. Mar. Biol. Assn. UK* **79**, 1111-1116.

Chain, B. M., Bone, Q. and Anderson, P. A. V. (1981). Electrophysiology of a myoid epithelium in *Chelophyes* (Coelenterata: Siphonophora). *J. Comp. Physiol.* **143**, 329-338.

Donaldson, S., Mackie, G. O. and Roberts, A. O. (1980). Preliminary observations on escape swimming and giant neurons in *Aglantha digitale* (Hydromedusae: Trachylina). *Can. J. Zool.* **58**, 549-552.

Grigoriev, N. G., Spafford, J. D., Gallin, W. J. and Spencer, A. N. (1997). Voltage sensing in jellyfish *Shaker* K^{+} channels. *J. Exp. Biol.* **200**, 2919-2926.

Inoue, I., Tsutsui, I. and Bone, Q. (2002). Excitation-contraction coupling in isolated locomotor muscle fibres from the pelagic tunicate *Doliolum* which lack both sarcoplasmic reticulum and transverse tubular system. *J. Comp. Physiol. B* **172**, 541-546.

Lin, Y.-C. J. and Spencer, A. (2001). Localisation of intracellular calcium stores in the striated muscles of the jellyfish *Polyorchins penicillatus*: possible involvement in excitation-contraction coupling. *J. Exp. Biol.* **204**, 3727-3736.

Mackie, G. O. and Carré, D. (1983). Coordination in a diphyid siphonophore. *Mar. Behav. Physiol.* **9**, 139-170.

Spencer, A. and Satterlie, R. A. (1981). The action potential and contraction in subumbrellar swimming muscle of *Polyorchins penicellatus* (Hydroedusae). *J. Comp. Physiol.* **144**, 401-407.