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ISOLATED GIANT SMOOTH MUSCLE FIBRES IN *BEROE OVATA*: IONIC DEPENDENCE OF ACTION POTENTIALS REVEALS TWO DISTINCT TYPES OF FIBRE

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SUMMARY

1. The ionic dependence of action potentials evoked in giant smooth muscle fibres isolated by enzymatic digestion from the body wall of the marine invertebrate *Beroe ovata* (Ctenophora) has been investigated using conventional electrophysiological techniques.

2. Differences were observed in the two fibre types studied. The resting membrane potential was $-60 \pm 1{\cdot}35$ mV ($N = 25$) in longitudinal muscle fibres and $-66 \pm 1{\cdot}37$ mV ($N = 32$) in radial fibres. Action potentials had a short plateau in longitudinal fibres but not in radial fibres.

3. The action potential overshoot of both fibre types was decreased in Ca^{2+}-free artificial sea water (ASW). In Na^+-deficient ASW, action potentials could not be generated in radial fibres and showed a reduced overshoot in longitudinal fibres.

4. Tetrodotoxin (10^{-5} mol l^{-1}) added to ASW or Ca^{2+}-free ASW did not affect the action potentials of either type of fibre.

5. Action potentials of both fibres were partially blocked by Co^{2+} (20–50 mmol l^{-1}) or Cd^{2+} (1–2 mmol l^{-1}). Action potentials of longitudinal fibres in Na^+-deficient ASW were abolished by Co^{2+} (20 mmol l^{-1}). In Ca^{2+}-free ASW, the action potential overshoots of both sets of fibres were restored following the addition of Sr^{2+} or Ba^{2+}. In longitudinal fibres, Sr^{2+} increased the duration of the action potential plateau. In both longitudinal and radial muscle fibres, Ba^{2+} prolonged the action potential.

6. In longitudinal fibres exposed to tetraethylammonium chloride (TEACl) or 4-aminopyridine (4AP), the action potential was slightly prolonged. In these fibres, TEA^+ or 4AP added to Ca^{2+}-free ASW induced only a long-lasting depolarizing plateau. In radial fibres, the action potential duration was slightly increased in the presence of TEA^+; it was unaffected by 4AP. In Ca^{2+}-free ASW, TEA^+ and 4AP induced an oscillating membrane response which appeared to be dependent on the intensity of the injected current pulse.

7. It is concluded that (a) there are significant differences between the action potentials of longitudinal and radial muscle fibres but that both are dependent on

Key words: Ctenophora, isolated giant smooth muscle, action potential.

Na^+ and Ca^{2+}, (b) in longitudinal fibres, a Ca^{2+}-activated K^+ conductance and a TEA^+-sensitive voltage-activated K^+ conductance contribute to the repolarizing phase of the action potential, the former being predominant, (c) in radial fibres, the repolarizing phase of action potentials probably involves different membrane K^+ conductances among which is a TEA^+-sensitive K^+ conductance.

INTRODUCTION

Previous studies of the organization of the muscles in the marine invertebrate *Beroe ovata* (Ctenophora) (Hernandez-Nicaise & Amsellem, 1980) have revealed three systems of giant smooth muscle fibres: longitudinal, radial and circular. In longitudinal and radial systems, each muscle fibre runs freely within the transparent extracellular matrix, the mesogloea. In contrast, the muscle fibres of the circular system are anastomosed and build a continuous muscular sheet underlying the pharyngeal integument.

In electrophysiological experiments on *in situ* fibres, electrical constants of longitudinal muscle fibres have been determined and the ionic dependence of action potentials of both longitudinal and radial muscle fibres has been investigated (Hernandez-Nicaise, Mackie & Meech, 1980). This first study was limited by technical difficulties arising from the presence of the mesogloea because its viscoelastic properties hindered the microelectrode penetration into all but the superficial muscles. However, the main problem with using this preparation arises from the contractions of the circular fibres which invariably dislodge the micropipette shortly after penetration. These difficulties have been overcome in this study of single longitudinal and radial smooth muscle fibres isolated from the body wall of *Beroe* by enzymatic digestion of mesogloea (Hernandez-Nicaise, Bilbaut, Malaval & Nicaise, 1982). Examination of these isolated fibres has revealed significant differences between the ionic dependence of the action potentials recorded from the two types of fibre. In another ctenophore, *Mnemiopsis*, giant smooth muscle fibres arranged in two longitudinally oriented bundles have been isolated (Hernandez-Nicaise, Nicaise & Anderson, 1981); their basic electrophysiological properties resemble those of the longitudinal muscles of *Beroe* (Anderson, 1984). A preliminary report of this work has been published in abstract form (Bilbaut & Hernandez-Nicaise, 1987).

MATERIALS AND METHODS

Beroe ovata is a planktonic invertebrate (Fig. 1). Specimens of *Beroe* were collected from surface waters in the bay of Villefranche-sur-Mer, France, where they appear in the spring, and were kept at 15°C in large containers filled with natural sea water.

Single cells were obtained by the method of Hernandez *et al.* (1982), slightly modified to improve calcium tolerance of the fibres, and to isolate preferentially either longitudinal or radial fibres.

Slices of the body wall, 3–4 cm long and 3 mm wide, were dissected out. The long axis of the strip was parallel to the longitudinal axis of the body (i.e. to the longitudinal fibres). The covering integument, together with the external layer of mesogloea and longitudinal muscles, was then cut out, but the pharynx integument was retained. To optimize the yield of radial fibres the strips were resliced transversely.

The dissections were carried out in sea water, and the pieces of body wall were transferred to artificial sea water (ASW). The reference ASW had the following composition (in mmol l^{-1}): NaCl, 500; KCl, 10; $MgCl_2$, 58; $CaCl_2$, 10; and was buffered to pH 8·2 with Tris-HCl. After a short rinse in nominally Ca^{2+}-free ASW (ASW in which Ca^{2+} had been omitted), the tissues were incubated at 30 °C for 20–30 min in 0·02 % trypsin (type III, Sigma) and 0·12 % hyaluronidase (type III, Sigma) in Ca^{2+}-free ASW. When a few muscle cells were observed in the medium, digestion was slowed down by transferring pieces of tissue to Ca^{2+}-free ASW. The pieces were dissociated by gentle agitation. The released muscle cells were transferred to a 'recovery' medium, i.e. an ASW containing 2 mmol l^{-1} Ca^{2+} and 0·2 % bovine serum albumin (BSA, Sigma, fraction V). From this stage on, silicone resin-coated glassware (Rhodorsil RTV 141, Rhône Poulenc) was used.

Most of the single fibres were fragments of variable length from cells that had been cut during either dissection or dissociation. We have shown that fibres that are cut reseal almost immediately and behave as intact fibres (Hernandez-Nicaise *et al.* 1980). Longitudinal fibres (Fig. 2A) were selected as the longest cells of the batch. The presence of pharyngeal branched endings was used to identify radial fibres (Fig. 2B). The cells were kept in the cooled recovery medium for at least 2 h before being fit for an experiment. Longitudinal fibres could be used at least for the ensuing 24 h. The condition of the radial fibres appeared to be much more labile and they had to be studied in the following 4 h.

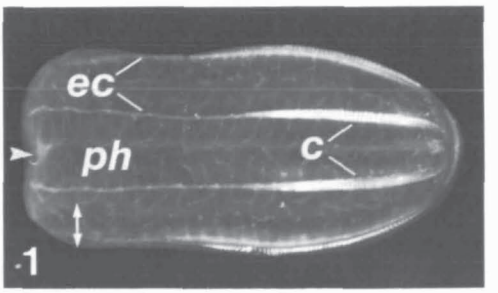

Fig. 1. Sagittal view of a living *Beroe ovata*. The animal is swimming mouth (arrowhead) forward. The whitish central zone corresponds to the large flattened pharynx (*ph*). Endodermic longitudinal channels (*ec*) run under and beyond the locomotory ciliated comb rows (*c*), and give rise to lateral branches through the mesogloea. The thickness of the body wall can be appreciated (↔) and corresponds to the length of radial fibres. Longitudinal fibres may run the full length of the animal. Magnification, ×0·75.

For experimental purposes, one or two isolated muscle fibres were transferred to glass dishes coated with a thin layer of Rhodorsil and containing 2·5 ml of ASW. Isolated fibres settled to the bottom of the dish and were maintained in position by the recording microelectrode which was usually inserted midway along their length. Changes of the bath solution could displace slightly the free extremities of the impaled fibre but did not interfere with the ongoing recording.

Intracellular current injection and transmembrane potential recording were performed with double-barrelled glass microelectrodes filled with 3 mol l^{-1} KCl. Each barrel had an initial resistance of 5–8 MΩ and their initial coupling resistance was about 100–200 kΩ. Following the penetration of the microelectrode into the fibre, the coupling resistance increased to five times its initial value. Depending on the injected current, a stray resistive voltage was introduced in the transmembrane potential recording. If required, this artefactual voltage was subtracted from membrane responses elicited by current pulses passed through the cell membrane. The recording side of the double-barrelled microelectrode was connected to a high-impedance amplifier and current injected into fibres through the other branch was monitored from a constant-current source. The reference electrode was an Agar–ASW bridge with a Ag/AgCl wire. Signals were displayed on a storage oscilloscope and resting membrane potential was continuously monitored on a pen recorder.

In electrophysiological experiments ASW and Ca^{2+}-free ASW were prepared as described above. The Ca^{2+} chelator EGTA added to nominally Ca^{2+}-free ASW was used at concentrations of 1–2 mmol l^{-1}. Na^{+}-deficient solutions were made by substituting 475 mmol l^{-1} NaCl with Tris-HCl or choline chloride, and contained 25 mmol l^{-1} NaCl. Na^{+}-free ASW was not used because it caused irreversible damage to isolated muscle fibres. Preliminary experiments showed that isolated fibres contracted strongly in Na^{+}-free ASW and most of them distintegrated after 20–30 s of exposure. Stock solutions of tetrodotoxin (TTX, 10^{-3} mol l^{-1}) and apamine (10^{-5} mol l^{-1}) were added to the test solution to give final concentrations of 10^{-5} and 10^{-6} mol l^{-1}, respectively. The divalent cations Co^{2+} (15–50 mmol l^{-1}), Cd^{2+} (2–10 mmol l^{-1}) and Mn^{2+} (20 mmol l^{-1}) were added to ASW, and Sr^{2+}

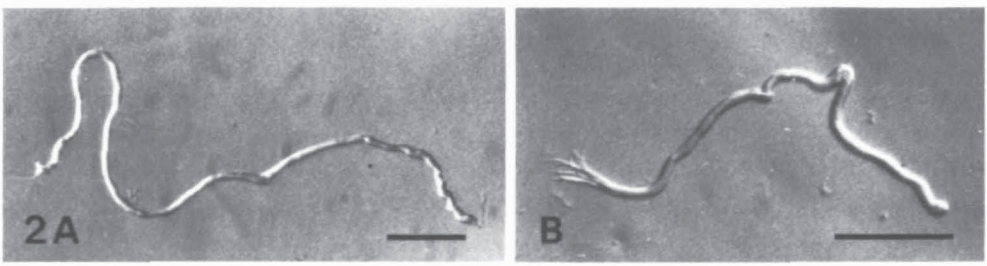

Fig. 2. Micrographs of living muscle fibres isolated by enzymatic digestion from the body wall of *Beroe*. (A) Longitudinal muscle fibre: both ends are resealed. (B) Radial muscle fibre: the fibre has retained its pharyngeal branched end (left), while the opposite branch has been cut away together with the covering epithelium during dissection. Scale bars, 250 μm.

(10 mmol l^{-1}) and Ba^{2+} (10 mmol l^{-1}) were added to Ca^{2+}-free ASW. ASW and Ca^{2+}-free ASW containing 20 or 50 mmol l^{-1} tetraethylammonium chloride (TEACl) were also used. 4-Aminopyridine (4AP) was used at 1–2 mmol l^{-1}; the pH of 4AP stock solutions was adjusted to 8·2 before each experiment. All artificial solutions were prepared immediately before use; solutions were exchanged at a flow rate of 10 ml min^{-1}.

Experiments were performed at room temperature (20°C).

RESULTS

Resting membrane potential

Resting membrane potentials were measured 3–4 min after one fibre had been transferred from 2 mmol l^{-1} Ca^{2+} ASW into normal 10 mmol l^{-1} Ca^{2+} ASW. Some fibres underwent an irreversible contraction as soon as they came in contact with the higher-Ca^{2+} solution and so they were discarded. Impaled fibres remained in good condition for 60 min or more.

The membrane potential was initially −50 mV but increased to a steady level 1–2 min after the penetration of the microelectrode into the fibre. In longitudinal fibres, steady membrane potential varied between −49 and −70 mV, with a mean value of $-60 \pm 1{\cdot}35$ mV (S.E.M., $N = 25$). In radial fibres, membrane potential varied between −49 and −79 mV with a mean value of $-66{\cdot}6 \pm 1{\cdot}37$ mV (S.E.M., $N = 32$). A Student's t-test showed that the two mean values differ significantly (for $\alpha = 0{\cdot}05$).

The ionic basis of the membrane potential was investigated by varying the external potassium concentration $[K^+]_o$. When $[K^+]_o$ was lowered from 10 to 1 mmol l^{-1}, the membrane potential in both longitudinal and radial fibres hyperpolarized by 20 mV instead of the 58 mV predicted from the Nernst equation. In the range 10–100 mmol l^{-1} K^+, the potential showed a linear relationship with $\log[K^+]_o$, in close agreement with the Nernst equation, with a slope per decade of 51·2 mV in radial fibres and 54 mV in longitudinal fibres (Fig. 3). These results were obtained on freshly isolated fibres, but following a prolonged incubation (24–36 h) at 8–12°C in the recovery medium (2 mmol l^{-1} Ca^{2+}, 0·2% BSA) the shift of membrane potential for a 10-fold change in extracellular K^+ concentration (10–100 mmol l^{-1}) did not exceed 40 mV.

Membrane responses to current pulse injections

The input resistance of isolated muscle fibres at the end of 100–400 ms hyperpolarizing pulses of 10–15 mV amplitude ranged from 12·5 to 25 MΩ. Both longitudinal and radial muscle fibres produced spikes when depolarized to approximately −35 mV by outward current pulses of 10–80 ms (Fig. 4). Usually, a single action potential was triggered in response to a single injected current pulse. Action potentials were elicited either during current pulses or subsequent to them, depending upon the intensity of the stimulation current. In the case of threshold current pulses, the membrane potential did not recover to its resting level as soon as

the current pulse was switched off, but could remain depolarized for 200–300 ms before the action potential was produced.

There were marked differences in the time course of action potentials recorded from radial and longitudinal fibres. Action potentials from longitudinal fibres were prolonged by a short plateau potential (Fig. 4A) followed by a fast repolarization. The plateau duration was highly variable and ranged from 4·5 to 17·5 ms. This extreme variability was even observed in signals produced by one fibre when

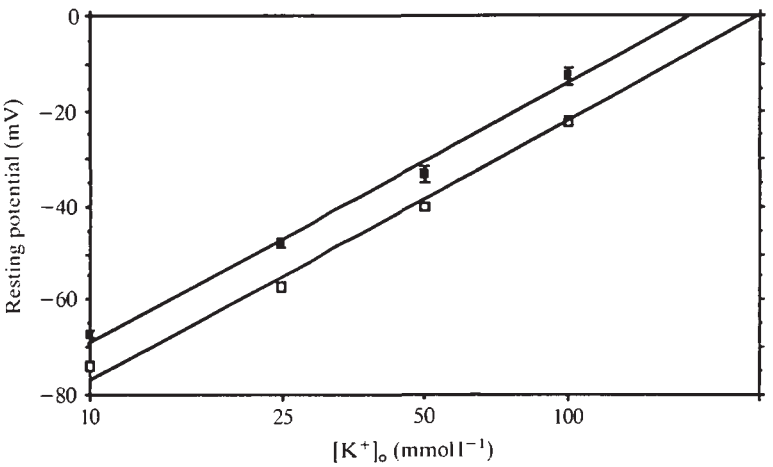

Fig. 3. The relationship between membrane potential and the external potassium concentration in longitudinal (■) and radial (□) fibres, for $10 < [K^+]_o < 100$ mmol l^{-1}. Each point represents the mean of five measurements for longitudinal fibres and eight measurements for radial fibres. $[K^+]_o$ was changed by pumping ASW of increasing $[K^+]$ into the experimental dish. Membrane potential was measured after it had stabilized (generally 5 min after solution change). The fibres used in this experiment originated from the same animal and were taken from the same batch of isolated cells. In this sample the initial values of resting potentials appear higher than the average values given for each fibre type in the Results section, but fit in the range of values obtained in this study. The standard error bars are not shown if they are smaller than the symbols used (<1).

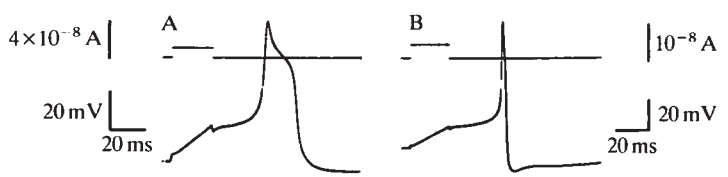

Fig. 4. (A) Action potential (lower trace) evoked by a current pulse (upper trace) intracellularly injected into a longitudinal smooth muscle fibre. The depolarizing phase has a plateau that is of variable duration in different fibres; it is followed by an abrupt repolarization of the cell membrane. This recording shows the longest action potential that has been observed in standard ASW. (B) Action potential (lower trace) elicited in a radial smooth muscle fibre in response to an intracellularly injected current pulse (upper trace). There is no evidence for a plateau phase. Current trace indicates zero potential.

successive spikes were obtained by iterative stimulation. A plateau of this kind was never observed in action potentials from radial muscle fibres (Fig. 4B). The repolarizing phase of action potentials from both kinds of fibres was followed by a variable undershoot.

The overall amplitudes of the action potentials recorded from longitudinal and radial fibres were $79 \pm 1{\cdot}5$ mV (S.E.M., $N = 18$) and $84{\cdot}9 \pm 2$ mV (S.E.M., $N = 21$), respectively. A Student's t-test showed that the two mean values differ significantly (for $\alpha = 0{\cdot}05$). The overshoot was $20{\cdot}4 \pm 1{\cdot}3$ mV (S.E.M., $N = 18$) in action potentials from longitudinal fibres and $20{\cdot}0 \pm 1{\cdot}1$ mV (S.E.M., $N = 21$) in action potentials from radial fibres. The two mean values do not differ significantly as demonstrated by a Student's t-test. The duration of the repolarizing phase of all action potentials measured at half-amplitude of peak potential ranged between 1 and 2 ms. The duration of the repolarizing phase of action potentials from radial fibres measured at 20% of peak potential amplitude was about 2 ms, whereas in action potentials from longitudinal fibres it varied between 4 and 18 ms. In normal ASW, action potentials were never observed to occur spontaneously in either fibre type. Long-lasting repetitive membrane firing could follow the microelectrode penetration into radial muscle fibres. Such behaviour was not observed subsequent to micropipette penetration into longitudinal muscle fibres.

Contractile activities as examined with a stereomicroscope appeared to be different for the two types of fibre. A single action potential of a longitudinal muscle fibre induced a twitch-like contraction characterized by a fast-contracting phase immediately followed by relaxation. In radial fibres a single spike had no visible effect, but a twitch could be elicited by a train of 2–3 spikes. Under repetitive long-lasting stimulation (2 Hz) both fibre types produced a progressive and sustained shortening. When electrical stimulation was turned off, the fibres relaxed slowly and partially.

No contraction could be elicited in longitudinal or radial fibres if the $[Ca^{2+}]_o$ was less than 1 mmol l^{-1}.

Ionic dependence of action potentials of longitudinal muscle fibres

Action potentials could be recorded from longitudinal muscle fibres in nominally Ca^{2+}-free ASW (Fig. 8Aii) although the amplitude of the overshoot was decreased, falling in most experiments to 50–60% of its initial value. In some fibres the reduction was as great as 90%. The reduction in amplitude was associated with the disappearance of the plateau potential. In most cases the durations of the rising and falling phases were not appreciably modified, although in some experiments they were lengthened.

In the presence of EGTA (1–2 mmol l^{-1}) added to nominally Ca^{2+}-free ASW, the membrane potential slowly depolarized by up to 30 mV. Action potentials were maintained but they were broadened and their overshoot was reduced in amplitude. Repetitive firing of the cell membrane could occur during stimulation but long-lasting plateau potentials were never observed. If the fibres were exposed for more than 30–40 min to ASW containing EGTA, action potentials disappeared irreversibly.

Action potentials were unaffected by the presence of TTX (10^{-5} mol l^{-1}) added to either ASW or Ca^{2+}-free ASW.

Action potentials were also elicited in Na^+-deficient ASW in which the external Na^+ concentration had been lowered to 25 mmol l^{-1} (Fig. 5A). They were similar to those produced in normal ASW except that the overshoot was decreased by 5–6 mV. In every experiment, the muscles fibre contracted after about 30 s of exposure to the Na^+-deficient ASW. Isolated fibres progressively shortened until they became spherical. This slow process could cease before complete contraction of fibres and was not associated with action potentials. However, during the shortening induced by Na^+-deficient ASW, the membrane potential first hyperpolarized slowly (10–20 mV), then regained its initial value when isolated fibres were fully shortened. In this state of contraction, fibres often failed to produce action potentials in response to intracellularly injected current pulses. Action potentials observed in 25 mmol l^{-1} Na^+ ASW originated mainly from incompletely contracted fibres.

Muscle fibres failed to spike if they were bathed with Na^+-deficient ASW in which the external Ca^{2+} concentration had been lowered to 1 mmol l^{-1}. No contraction could be observed, as in any Ca^{2+}-free medium.

These observations show that the ionic dependence of the depolarizing phase of action potentials recorded from longitudinal muscle fibres may involve both Na^+ and Ca^{2+}. This assumption was tested by using divalent cations known to block membrane Ca^{2+} conductance (namely Mn^{2+}, Co^{2+} and Cd^{2+}) or by using substitutes for Ca^{2+} (namely Sr^{2+} and Ba^{2+}) during Ca^{2+}-dependent membrane electrogenesis.

Action potentials of longitudinal fibres were unaffected by the presence of Mn^{2+} (20 mmol l^{-1}) added to ASW.

In ASW containing 20 mmol l^{-1} Co^{2+}, the resting membrane potential remained stable although, in some experiments, the presence of Co^{2+} could depolarize the cell membrane by about 10 mV. The overshooting amplitude of the spikes diminished reversibly by 20–25 % and the plateau potential was also reversibly suppressed. Identical results were obtained when the extracellular Co^{2+} concentration was increased by up to 50 mmol l^{-1}. Full recovery was obtained following the return to

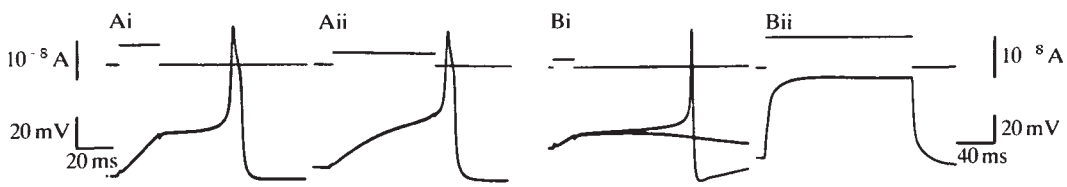

Fig. 5. Effect of Na^+-deficient ASW on the action potential (lower trace) recorded from longitudinal (A) and radial (B) muscle fibres. (Ai) Action potential in ASW; (Aii) the overshooting action potential is maintained after 16 min in Na^+-deficient ASW. (Bi) Action potential in ASW; (Bii) after 1 min in low external [Na^+] (25 mmol l^{-1}), the action potential is abolished. In this experiment, the membrane potential has been depolarized to −10 mV by a 200 ms current pulse. Upper trace: current monitor and zero potential.

ASW. Action potentials were completely and reversibly abolished when Co^{2+} (20 mmol l^{-1}) was added to Na^{+}-deficient ASW (Fig. 6A).

Cd^{2+} had a more ambiguous effect than Co^{2+} on action potentials of longitudinal fibres. In the presence of 1 or 2 mmol l^{-1} Cd^{2+} added to ASW, the overshoot amplitude decreased reversibly by about 10 mV and the action potential duration was increased threefold (Fig. 7A). Initially the resting membrane potential became rapidly depolarized by 10–20 mV, reaching −40 mV. It then decreased again slowly until it attained −20 mV and, in this state, injected current pulses failed to elicit action potentials. However, action potentials were produced once again when the cell membrane was hyperpolarized by a constant current passed into the fibre. Action potentials were irreversibly abolished when the extracellular Cd^{2+} concentration was higher than 5 mmol l^{-1}.

Sr^{2+} (10 mmol l^{-1}) and Ba^{2+} (10 mmol l^{-1}) added to Ca^{2+}-free ASW in place of Ca^{2+} fully restored the action potential of longitudinal fibres. In Sr^{2+} ASW, the resting membrane potential was unaffected and the overshoot amplitude was similar to that initially observed in normal ASW. The duration of the action potential was lengthened to twice its initial value by the prolongation of the depolarizing plateau (Fig. 8A).

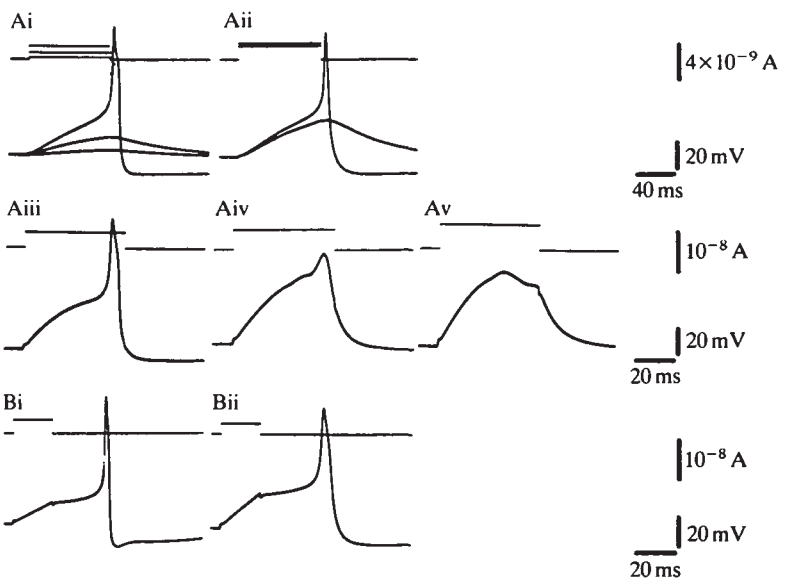

Fig. 6. Effect of Co^{2+} on the action potential (lower trace) recorded from longitudinal (A) and radial (B) muscle fibres. (Ai) Overshooting action potential in ASW; (Aii) 4 min after the addition of Co^{2+} (15 mmol l^{-1}) in ASW, the amplitude of the action potential overshoot is decreased and the plateau phase is suppressed; (Aiii) action potential in Na^{+}-deficient ASW; (Aiv,v) progressive disappearance of the action potential overshoot in the presence of Co^{2+} (20 mmol l^{-1}) (after 7 and 9 min, respectively, in Co^{2+} ASW). (Bi) Overshooting action potential in ASW; (Bii) decrease of the action potential overshoot after 4 min in Co^{2+} (20 mmol l^{-1}) ASW. Upper trace: current monitor and zero potential.

Ba^{2+} had multiple effects on the electrical activity of longitudinal fibres. In the presence of Ba^{2+} added to Ca^{2+}-free ASW, the resting membrane potential depolarized slowly by 5–10 mV; the peak of the action potential was 4–8 mV more positive than that in normal ASW. The depolarizing phase was prolonged by a long-lasting plateau which was steady 10 mV below the maximum amplitude of the spike

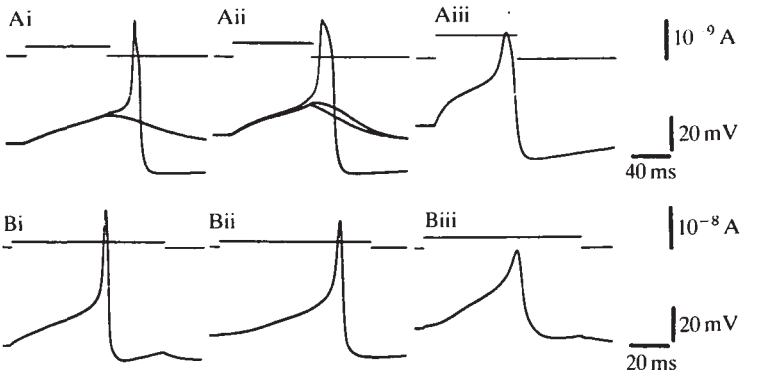

Fig. 7. Effects of Cd^{2+} on the action potentials (lower trace) recorded from longitudinal (A) and radial (B) muscle fibres. (Ai) Action potential in ASW; (Aii,iii) in the presence of Cd^{2+} ($1{\cdot}5\ mmol\ l^{-1}$), the membrane potential slowly depolarizes and the action potential overshoot decreases (3 and 7 min in Cd^{2+} ASW, respectively). (Bi) Action potential in ASW; (Bii,iii) progressive decrease of the membrane potential and action potential overshoot following the addition of $2\ mmol\ l^{-1}$ Cd^{2+} in ASW (6 and 15 min in Cd^{2+} ASW, respectively). Upper trace: current monitor and zero potential.

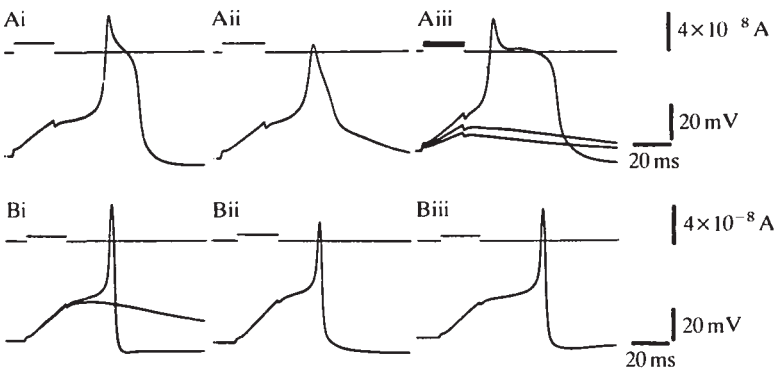

Fig. 8. Effects of Sr^{2+} ($10\ mmol\ l^{-1}$) on the action potential (lower trace) recorded from longitudinal (A) and radial (B) muscle fibres. (Ai) Action potential in ASW; (Aii) after 4 min in Ca^{2+}-free ASW, the action potential overshoot decreases and the slow repolarizing inflection disappears; (Aiii) the longitudinal fibre action potential is restored 90 s after the addition of Sr^{2+} in Ca^{2+}-free ASW. Compared with the action potential evoked in ASW, the duration of the depolarizing plateau has been lengthened by a factor of two. (Bi) Action potential in ASW; (Bii) decrease in spike overshoot after 3 min in Ca^{2+}-free ASW; (Biii) restoration of the radial fibre action potential 2 min after the addition of Sr^{2+} to Ca^{2+}-free ASW. Note the absence of a plateau. Upper trace: current monitor and zero potential.

potential (Fig. 9A). Plateau duration could reach 900 ms and ended with a fast repolarizing phase followed by a strong hyperpolarizing undershoot. Following the removal of Ba^{2+}, the full recovery of both resting and action potentials needed several minutes.

Two voltage-dependent K^+ conductance blockers, TEA^+ and 4AP, were tested on longitudinal muscle fibres isolated from *Beroe*.

When added to ASW, TEA^+ ($10\,mmol\,l^{-1}$) induced a slow depolarization of 10–12 mV of the resting membrane potential, an increase of overshoot amplitude by 8–10 mV and a broadening of 10–12 ms of the repolarizing phase. Action potentials were similarly modified when the extracellular TEA^+ concentration was increased up to $50\,mmol\,l^{-1}$. TEA^+ was equally effective when Ca^{2+} was omitted from ASW. If $10\,mmol\,l^{-1}$ TEA^+ was added to Ca^{2+}-free ASW, the overshoot increased by 8–10 mV and the membrane potential depolarized by the same value. The repolarizing phase was prolonged by a long depolarizing plateau which could last 400 ms. At the end of the plateau potential, the fast membrane repolarization was followed by j hyperpolarizing undershoot of 10–15 mV (Fig. 10A). The effects of TEA^+ were fully reversible.

The effects of 4AP ($1–2\,mmol\,l^{-1}$) on the repolarizing phase of the action potentials were comparable to those of TEA^+. If 4AP was added to ASW, the resting membrane potential and action potential overshoot were unaffected, whereas the duration of the repolarizing phase increased by 5–6 ms. If 4AP was added to Ca^{2+}-free ASW, the overshoot was greater by 5–6 mV than that observed in control Ca^{2+}-free solution. The repolarizing phase began with brief membrane oscillations followed by a plateau potential 400–450 ms long (Fig. 11A). The plateau ended with

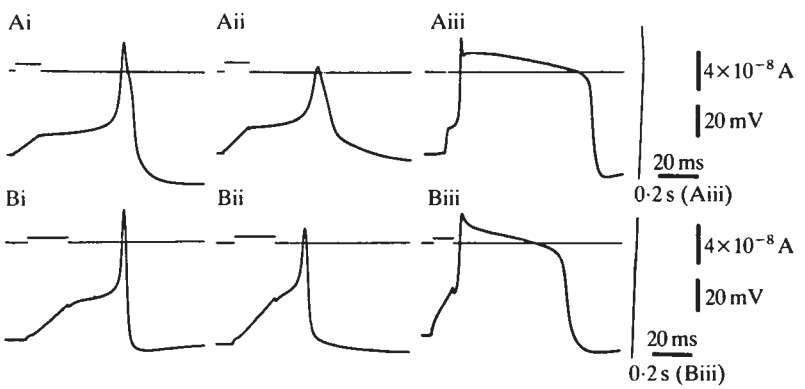

Fig. 9. Effect of Ba^{2+} ($10\,mmol\,l^{-1}$) on the membrane response (lower trace) of longitudinal (A) and radial (B) muscle fibres. (Ai) Action potential in ASW; (Aii) membrane response after 2 min in Ca^{2+}-free ASW; (Aiii) long-lasting depolarizing plateau potential induced in the presence of Ba^{2+} added to Ca^{2+}-free ASW (1 min incubation). (Bi) Action potential in ASW; (Bii) membrane response in Ca^{2+}-free ASW; (Biii) restoration of the radial fibre action potential 4 min after the addition of Ba^{2+} to Ca^{2+}-free ASW. The repolarizing phase is prolonged by a depolarizing plateau potential. Upper trace: current monitor and zero potential.

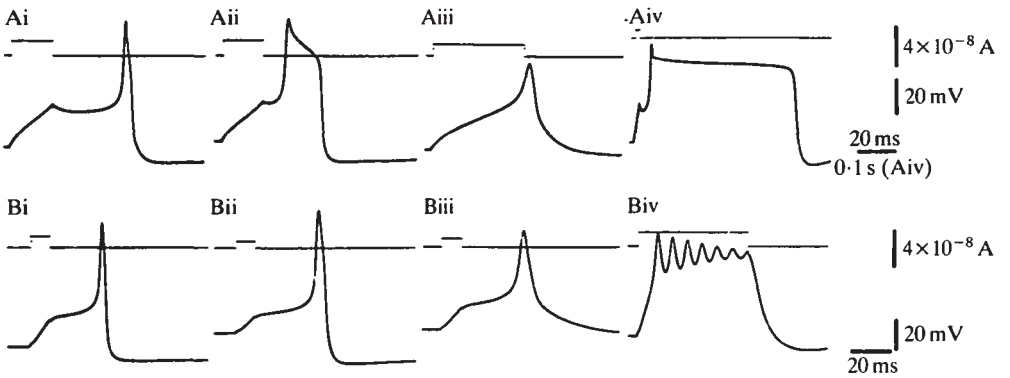

Fig. 10. Effects of tetraethylammonium (TEA^+) ($10\,mmol\,l^{-1}$) on the membrane responses (lower trace) of longitudinal (A) and radial (B) muscle fibre. (Ai) Action potential in ASW; (Aii) prolongation of the action potential 2 min after the addition of TEA^+ to ASW; (Aiii) membrane response in Ca^{2+}-free ASW; (Aiv) long-lasting depolarizing plateau induced in the presence of TEA^+ added to Ca^{2+}-free ASW. (Bi) Action potential in ASW; (Bii) TEA^+ added to ASW slightly prolongs the duration of the radial fibre action potential after 90 s; (Biii) membrane response in Ca^{2+}-free ASW; (Biv) oscillations in the plateau potential induced during current pulse injection after 2 min in Ca^{2+}-free ASW + TEA^+. Note the increase in current duration and amplitude. The membrane potential was held at −60 mV with a constant hyperpolarizing current. Upper trace: current monitor and zero potential (in Aiv, the current trace has been shifted upwards).

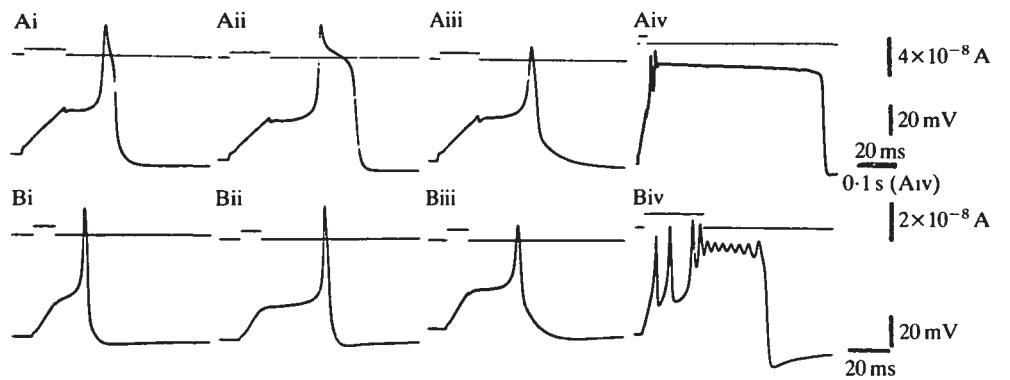

Fig. 11. Effects of 4-aminopyridine (4AP) ($2\,mmol\,l^{-1}$) on the membrane responses (lower trace) of longitudinal (A) and radial (B) muscle fibres. (Ai) Action potential in ASW; (Aii) prolongation of the action potential 30 s following the addition of 4AP; (Aiii) action potential in Ca^{2+}-free ASW; (Aiv) long-lasting depolarizing plateau induced 90 s after the addition of 4AP to Ca^{2+}-free ASW. (Bi) Action potential in ASW; (Bii) after 1 min in 4AP added to ASW, the action potential is not significantly affected; (Biii) single action potential evoked 2 min after 4AP was added to Ca^{2+}-free ASW; (Biv) an increase of the intensity and duration of the depolarizing current pulse induces repetitive firing of the cell membrane that leads to oscillations of the prolonged plateau (same fibre as in Biii 1 min later). Upper trace: current monitor and zero potential (in Aiv and Biv, current trace has been shifted upwards).

an abrupt membrane repolarization followed by a hyperpolarizing undershoot of 4–6 mV.

Action potentials were not modified by the presence of apamine (10^{-6} mol l^{-1}) added to ASW or ASW containing TEA^+ (20 mmol l^{-1}).

Ionic dependence of action potentials of radial muscle fibres

The ionic dependence of action potentials recorded from radial muscle fibres was studied in the same way as described for longitudinal fibres.

Action potentials were recorded in nominally Ca^{2+}-free ASW (Fig. 8B). In the absence of Ca^{2+}, the membrane potential underwent slow fluctuations which were no higher than 6 mV in either the depolarizing or the hyperpolarizing direction. The action potential overshoot was decreased by 85–90 % from its control value while neither the rising nor the falling phase was appreciably modified in duration.

In EGTA-containing Ca^{2+}-free ASW, the action potential overshoot was drastically reduced and no depolarizing plateau was observed.

In 25 mmol l^{-1} Na^+ ASW no action potentials could be triggered by a depolarizing current pulse injected into a fibre irrespective of the potential reached by the membrane during the stimulation (Fig. 5B). In all experiments, isolated radial fibres contracted strongly as soon as they were exposed to 25 mmol l^{-1} Na^+ ASW. As previously described, this contractile activity was associated with a large transient membrane hyperpolarization. Reintroduction of normal ASW restored the action potential. Action potentials were not produced in Na^+- and Ca^{2+}-deficient ASW (25 mmol l^{-1} and 1 mmol l^{-1}, respectively).

Action potentials were unaffected by the presence of TTX (10^{-5} mol l^{-1}) added to ASW or Ca^{2+}-free ASW.

When Co^{2+} (20 mmol l^{-1}) was added to ASW, the membrane potential depolarized slowly by 8–12 mV. The spike overshoot was reversibly decreased by more than 60 % whereas the durations of the rising and falling phases were unaffected (Fig. 6B).

Cd^{2+} (1–2 mmol l^{-1}) induced a more pronounced modification of the membrane potential and the action potentials of isolated radial muscle fibres than did Co^{2+}. In the presence of Cd^{2+}, the membrane potential depolarized by about 20 mV and the spike overshoot was reduced (Fig. 7B). Action potentials could not be obtained if the membrane potential was depolarized by more than 20 mV in the presence of Cd^{2+}. Action potentials reappeared if the membrane was hyperpolarized by a constant inward current injected into the muscle fibre. Upon reintroduction of standard ASW, the membrane potential and the action potential overshoot were slowly and partially restored. The action potentials of radial fibres were irreversibly abolished by 5 mmol l^{-1} Cd^{2+}.

If Sr^{2+} or Ba^{2+} was added to Ca^{2+}-free ASW the action potential was restored. In the presence of Sr^{2+} (10 mmol l^{-1}), action potentials were similar to those elicited in normal ASW (Fig. 8B). The effects of Ba^{2+} (10 mmol l^{-1}) were threefold: (1) a depolarization of the resting membrane potential by 15–25 mV, (2) an increase in the

action potential overshoot of 8–10 mV and (3) an increase in the duration of the repolarizing phase which could last up to 400 ms (Fig. 9B).

TEA^+ (20–50 mmol l^{-1}) added to ASW rapidly depolarized the cell membrane by 8–10 mV. The action potential overshoot was increased by 5–6 mV whereas the repolarizing phase was slightly lengthened.

In Ca^{2+}-free ASW, TEA^+ did not noticeably modify the action potential. However, the presence of TEA^+ in Ca^{2+}-free ASW appeared to induce oscillation of the cell membrane potential in response to an increase in the intensity and/or duration of injected current pulses. Bursts of spikes or an oscillating plateau potential were elicited during, or subsequent to, current injection (Fig. 10B). 4AP (1–2 mmol l^{-1}) added to ASW or Ca^{2+}-free ASW did not affect the action potentials. As observed in the presence of TEA^+, repetitive firing of the cell membrane was observed in the presence of 4AP following an increase of the intensity and/or duration of current pulses. In ASW or Ca^{2+}-free ASW, membrane responses started with rhythmic spikes which, in Ca^{2+}-free ASW, could be prolonged by a post-stimulatory oscillating plateau potential. Membrane responses ended with an abrupt undershooting repolarization (Fig. 11B).

The action potentials were unaffected by apamine (10^{-6} mol l^{-1}) added to ASW or ASW containing 20 mmol l^{-1} TEA^+.

DISCUSSION

The results presented in this study establish that in isolated smooth muscle fibres of *Beroe* the action potentials recorded from longitudinal fibres are different from those recorded from radial fibres. There are not only differences in resting membrane potential but also differences in the amplitude and shape of the action potential which appear to be related to the presence of different ionic conductances. Although significant differences in resting membrane potential were established in an earlier study of *in situ* fibres (Hernandez-Nicaise *et al.* 1980), they were attributed to the difficulties associated with penetrating the longitudinal fibres within the mesogloea and also to the different diameters of the two fibre types. It was not until it became possible to record from isolated fibres that we could establish the differences between the fibre types. Here we shall compare the results from isolated fibres with those previously obtained from muscle fibres of *Beroe in situ*.

In isolated muscle fibres the waveform of the action potential recorded from the longitudinal fibres has a short plateau that precedes the fast terminal repolarization. A similar plateau may be seen in the recordings from longitudinal fibres published for *in situ* fibres (Hernandez-Nicaise *et al.* 1980). This plateau is absent in isolated radial fibres and in all but one record taken from the *in situ* fibres. This last discrepancy may be attributed to the difficulties associated with the identification of fibres in the body wall where the fibre endings were not always visible. The prolonged broadening of the action potential sometimes observed in longitudinal fibres has been attributed by Hernandez-Nicaise *et al.* (1980) to a possible decremental reflection of propagated action potentials from the resealed end of the fibre, because the

action potential duration was found to depend upon the position of both the recording and the current-passing microelectrodes along the fibre. Such reflections would not be expected in fibres with branched ends. Although it is possible that reflections may be responsible for the exceptionally prolonged action potentials, the short plateau normally observed in longitudinal fibres could be recorded at any position of the double-barrelled electrode along the fibre and it is unlikely that it arises simply from the passive membrane properties of the longitudinal muscle fibres.

From these two sets of data, we conclude that (a) action potentials produced by isolated muscle fibres appear identical to those produced by *in situ* fibres of the same type, and (b) the waveform of action potentials arising from longitudinal muscle fibres is different from that of action potentials arising from radial muscle fibres. In *Mnemiopsis*, the giant muscle fibres are grouped into two sagittal bundles and constitute a homogeneous cell population (Anderson, 1984; Hernandez-Nicaise, Nicaise & Malaval, 1984). Action potentials produced by either isolated or *in situ* muscle fibres are alike and their waveforms are similar to that of longitudinal muscle fibres of *Beroe*.

The membrane potential of single muscle fibres of *Beroe* is mainly K^+-dependent when the extracellular potassium concentration ranges from 10 to 100 mmol l^{-1}. However, the straight line obtained by plotting membrane potential against the logarithm of external potassium concentration has a slope 4–7 mV lower than the value expected from the Nernst equation (58 mV for a tenfold change at 20°C). In *in situ* radial muscle fibres of *Beroe* (Hernandez-Nicaise *et al.* 1980) this slope fits more closely the value predicted from the Nernst relationship. It is therefore possible that, in isolated fibres, the resting permeability of the cell membrane may have been altered by the proteolytic enzymes. In isolated muscle fibres of *Mnemiopsis* (Anderson, 1984) the relationship between extracellular $[K^+]$ and resting potential is not linear. However, if impermeant anions are substituted for extracellular Cl^-, the relationship follows the Nernst equation. Anderson (1984) proposed in *Mnemiopsis* fibres the existence of a strong resting membrane Cl^- permeability. In isolated muscle fibres of *Beroe*, membrane Cl^- permeability cannot be preponderant, at least in the range of extracellular K^+ concentrations used, since variations of the resting potential fit the Nernst equation rather closely.

From our study of the ionic mechanism of action potentials evoked in isolated smooth muscle fibres from *Beroe* we conclude that in both fibre types the depolarizing phase is Na^+- and Ca^{2+}-dependent. A sodium component may be inferred from the following observations: (a) action potentials could be elicited in nominally Ca^{2+}-free ASW and in Ca^{2+}-free ASW containing EGTA or in the presence of the Ca^{2+} channel blockers Co^{2+} or Cd^{2+}; (b) muscle fibres failed to spike if they were bathed with Na^+-deficient ASW in which the external Ca^{2+} concentration had been lowered to 1 mmol l^{-1}. Other observations indicate the presence of a calcium component in longitudinal fibres: (a) overshooting action potentials could be recorded in Na^+-deficient ASW; (b) Co^{2+} and Cd^{2+} reversibly reduced the action potential overshoot; (c) Co^{2+} added to Na^+-deficient ASW blocked the action

potentials reversibly; (d) Sr^{2+} and Ba^{2+}, known to substitute for Ca^{2+} as charge carriers (Fatt & Ginsborg, 1958), fully restored the action potentials evoked in Ca^{2+}-free ASW.

The presence of a calcium component in radial fibres is more questionable as action potentials could not be elicited in Na^+-free media but, just as in longitudinal fibres, action potentials could be partially blocked by Co^{2+}- or Cd^{2+}-containing ASW and fully restored in the presence of Sr^{2+} or Ba^{2+} added to Ca^{2+}-free ASW. As these divalent cations are known to act chiefly on the Ca^{2+} conductance, we consider that Ca^{2+} may also participate in the depolarizing phase of action potentials recorded from radial muscles fibres. This suggests that the respective contributions of Na^+ and Ca^{2+} to the generation of the two forms of action potential might be different for each type of fibre. The Na^+-dependence of the action potential from the radial fibres may be more significant than in longitudinal fibres, where Ca^{2+} may be more important. However, an indirect effect of the Na^+-free solution on the composition of the cell cytoplasm cannot be excluded in radial muscle fibres. Incidentally, a Na^+/Ca^{2+} exchange may take place in *Beroe* muscles as both types of fibres contract strongly in Na^+-deficient ASW.

The participation of Na^+ and Ca^{2+} in action potentials recorded from *in situ* muscle fibres of *Beroe* (Hernandez-Nicaise *et al.* 1980) and in isolated muscle fibres of *Mnemiopsis* (Anderson, 1984) has also been proposed. In a number of other preparations the membrane Na^+conductance can be distinguished from the membrane Ca^{2+} conductance on the basis of its sensitivity to TTX (Geduldig & Gruener, 1970; Hagiwara & Kidokoro, 1971). As already established for *in situ* muscle fibres of *Beroe* (Hernandez-Nicaise *et al.* 1980) and isolated muscle fibres of *Mnemiopsis* (Anderson, 1984), the action potentials of isolated muscle fibres of *Beroe* were unaffected by TTX added to ASW or Ca^{2+}-free ASW. So it is not possible to use TTX as a means of showing whether the Na^+ and Ca^{2+} pathways are the same or separate.

Further differences between the action potentials of longitudinal and radial fibres were deduced from the action of Sr^{2+}. When Sr^{2+} was added to Ca^{2+}-free ASW, the amplitude of the action potential returned to normal in both types of fibre and, in longitudinal fibres, the duration of the plateau potential was lengthened. This broadening of the action potential of longitudinal fibres may be attributed to several different mechanisms. It is well known that an increase in internal Ca^{2+} concentration arising from an inward Ca^{2+} current can modify membrane conductances either by activating a Ca^{2+}-dependent K^+ conductance (Meech, 1978) or by inactivating Ca^{2+} currents as reported in smooth muscle cells (Jmari, Mironneau & Mironneau, 1986) or other preparations (Brehm & Eckert, 1978; Plant & Standen, 1981). In guinea pig taenia coli smooth muscle cells (Inomata & Kao, 1979) and in insect muscles (Ashcroft & Stanfield, 1982), the inactivation rate of the inward current is slowed if Sr^{2+} is used to replace Ca^{2+} in the bathing solution. In invertebrate nerve cells, Gorman & Hermann (1979) have reported that Sr^{2+} is less effective than Ca^{2+} in activating membrane K^+ conductances. If we assume that in longitudinal muscle fibres of *Beroe* the Ca^{2+} component of the action potential

inactivates the inward Ca^{2+} current (or at least part of it) and/or activates the outward K^+ conductance, the substitution of Sr^{2+} for Ca^{2+} should lead to a lengthening of the action potential. In vascular smooth muscle cells of vertebrates Hotta & Yamamoto (1983) have reported that Sr^{2+} induces a Na^+-dependent plateau potential. They suggested that this resulted from an unmasking of the Na^+ conductance usually blocked by Ca^{2+} bound to membrane sites.

Ba^{2+} added to Ca^{2+}-free ASW induced the same kind of membrane responses in both longitudinal and radial muscle fibres, although the effects were more pronounced in longitudinal muscles: (a) the resting potential became less negative; (b) the spike overshoot was increased and (c) the spike was prolonged by a long-lasting plateau potential. This prolonged plateau may be attributed to the blockade of K^+ conductances by Ba^{2+} (Werman & Grundfest, 1961; Sperelakis, Schneider & Harris, 1967; Armstrong, Swenson & Taylor, 1982) and/or to the fact that Ba^{2+} carries more current than Ca^{2+} (Hagiwara, Fukuda & Eaton, 1974).

The membrane responses of longitudinal fibres obtained in the presence of TEA^+ added to ASW or Ca^{2+}-free ASW suggest that a voltage-activated K^+ conductance and a Ca^{2+}-activated K^+ conductance may be involved in the repolarizing phase of the action potential. In ASW containing TEA^+, the resting membrane potential was affected in the same way as in other smooth muscle cells (Kirkpatrick, 1975; Harder & Sperelakis, 1979): the action potential overshoot increased and it was five times longer than in ASW. Both effects may be attributed to the known effect of TEA^+ in decreasing both resting and voltage-activated K^+ conductances. Following the change to Ca^{2+}-free ASW with TEA^+, the action potential duration was 150 times longer than that seen in the presence of Ca^{2+} in TEA^+-containing ASW. This suggests that the repolarization of the action potential in longitudinal muscle fibres depends in part on a Ca^{2+}-activated K^+ conductance such as has been characterized in a number of smooth muscle cells of vertebrates (Vassort, 1975; Mironneau & Savineau, 1980; Walsh & Singer, 1983). Similar observations were made when 4AP replaced TEA^+ in Ca^{2+}-free ASW.

Radial muscle fibres were much less sensitive than longitudinal muscle fibres to TEA^+ and to 4AP when added to ASW. The only noticeable effect of 4AP was to promote the oscillatory membrane behaviour of radial fibres in response to an increase of intensity and/or duration of the injected electrical pulse. Such behaviour was also observed in the presence of TEA^+ or 4AP added to Ca^{2+}-free ASW. TEA^+ did increase the spike duration slightly but neither it nor 4AP was able to induce a long-lasting smooth depolarizing plateau potential like that usually characterizing the effect on longitudinal fibres. Consequently, the repolarizing phase of the action potential of radial fibres is likely to depend on several membrane K^+ conductances, only one of them being TEA^+-sensitive.

Our results may be compared with the situation in vertebrate smooth muscle cells. In vertebrates the ionic dependence of the action potential depends on the type of organ involved. Although Ca^{2+} appears to be the major component of the rising phase of the action potential in most smooth muscle cells (Brading, Bülbring & Tomita, 1969; Mironneau, 1973; Walsh & Singer, 1980; Isenberg & Klöckner,

1985), the contribution of Na^+ to membrane electrogenesis has been reported in a number of preparations (Anderson, Ramon & Snyder, 1971; Kao & McCullough, 1975; Keatinge, 1968). In such cases, the action potential is unaffected in the presence of TTX. In several smooth muscle cells, e.g. visceral (Prosser, Kreulen, Weigel & Yau, 1977) or uterine muscle (Mironneau, Eugene & Mironneau, 1982) long-lasting Na^+-dependent plateau potentials were observed when the extracellular Ca^{2+} concentration was lowered by the addition of EGTA. These plateau potentials were TTX-resistant but were blocked by Mn^{2+} or D600 (Prosser *et al.* 1977; Mironneau *et al.* 1982). In fact, various excitable cells display a long-lasting Na^+-dependent plateau potential when the external $[Ca^{2+}]$ is lowered (e.g. cardiac myocytes, Garnier, Rougier, Gargouïl & Caraboeuf, 1969; invertebrate neurones, Yang & Lent, 1983). It is thought that under these experimental conditions the Na^+ current flows through the same channels as the Ca^{2+} current. In smooth muscle fibres isolated from *Beroe*, the membrane responses evoked in Ca^{2+}-free ASW were unaffected by the presence of TTX, which suggests that Na^+ might be able to enter the cell through Ca^{2+} channels. If this is correct, the action potentials should be abolished in the presence of blockers of membrane Ca^{2+} conductance. In fact, as reported in this paper, the action potential overshoot was decreased in the presence of the divalents cations Co^{2+} or Cd^{2+} but the action potential was not fully abolished. Such effects were observed for extracellular Co^{2+} concentrations ranging from 20 to 40 mmol l^{-1} and it seems likely that under our experimental conditions Co^{2+} and Cd^{2+} selectively blocked the Ca^{2+} component of the action potential. However, plateau potentials were not observed when single smooth muscle fibres of *Beroe* were exposed to EGTA-containing Ca^{2+}-free ASW and it is possible that they may have Ca^{2+} channels distinct from TTX-resistant Na^+ channels.

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