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ORIGINAL PAPER

Conduction velocities in amphibian skeletal muscle fibres exposed to hyperosmotic extracellular solutions

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Abstract Early quantitative analyses of conduction velocities in unmyelinated nerve studied in a *constantly* iso-osmotic volume conductor were extended to an analysis of the effects of *varying* extracellular osmolarities on conduction velocities of surface membrane action potentials in *Rana esculenta* skeletal muscle fibres. Previous papers had reported that skeletal muscle fibres exposed to a wide range of extracellular sucrose concentrations resemble perfect osmometers with increased extracellular osmolarity proportionally decreasing fibre volume and therefore diminishing fibre radius, a. However, classical electrolyte theory (Robinson and Stokes 1959, Electrolyte solutions 2nd edn. Butterworth & Co. pp 41–42) would then predict that the consequent increases in intracellular ionic strength would correspondingly decrease sarcoplasmic resistivity, R_i. An extension of the original cable analysis then demonstrated that the latter would precisely offset its expected effect of alterations in a on the fibre axial resistance, r_i, and leave action potential conduction velocity *constant*. In contrast, other reports (Hodgkin and Nakajima J Physiol 221:105–120, 1972) had suggested that R_i *increased* with extracellular osmolarity, owing to alterations in cytosolic viscosity. This led to a prediction of a *decreased* conduction velocity. These opposing hypotheses were then tested in muscle fibres subject to just-suprathreshold stimulation at a Vaseline seal at one end and measuring action potentials and their first order derivatives, dV/dt, using 5–20 MΩ, 3 M KCl glass microelectrodes at defined distances away from the stimulus sites. Exposures to hyperosmotic, sucrose-containing, Ringer solutions then reversibly *reduced* both conduction velocity and maximum values of dV/dt. This was compatible with an *increase* in R_i in the event that conduction depended upon a discharge of membrane capacitance by propagating local circuit currents through initially passive electrical elements. Conduction velocity then showed graded decreases with increasing extracellular osmolarity from 250–750 mOsm. Action potential waveforms through these osmolarity changes remained similar, including both early surface and the late after-depolarisation events reflecting transverse tubular activation. Quantitative comparisons of reduced-χ^2 values derived from a comparison of these results and the differing predictions from the two hypotheses strongly favoured the hypothesis in which R_i *increased* rather than *decreased* with hyperosmolarity.

Keywords Conduction velocity · Osmolarity · Skeletal muscle

Introduction

A classic paper by Hodgkin (1954) (see also: Adrian 1975; Noble 1979) performed a quantitative cable analysis of the local circuit currents thought responsible for action potential propagation in unmyelinated nerve fibres studied in large volumes of constantly iso-osmotic extracellular fluid and demonstrated that their conduction velocities should vary as the square root of fibre radius, a. Action

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potential conduction velocity is also physiologically important in striated muscle: it ensures a rapid, sarcomeric activation leading to near-synchronous muscle contraction. The present paper accordingly extends these early analyses to effects of *varying* extracellular osmolarity on conduction velocities of surface membrane action potentials in *Rana esculenta* skeletal muscle. Skeletal muscle membrane differs from unmyelinated nerve in including an excitable transverse (T-) system whose activation initiates muscle contraction (Adrian et al. 1969, 1970; Huang 1993; Nielsen et al. 2004; Stephenson 2006). Nevertheless, it is likely that the initial sarcolemmal membrane activation *precedes* the subsequent inward tubular excitation and that the latter may take place partially independently of the propagation of the surface excitation wave. Sheikh et al. (2001) suggested a partially separable T-tubule excitation initiated by Na^+ channels selectively clustered around the mouths of the T-tubular lumina: detubulation produced by osmotic shock left surface membrane conduction velocities unchanged. Furthermore, increases in extracellular osmolarity did not alter the tubular diameters at the necks of the T-tubular system important in electrical connectivity between T-tubule network and remaining extracellular space (Launikonis and Stephenson 2002, 2004).

However, variations in surface conduction velocities in skeletal muscle studied under *varying* osmolarities would be expected to differ from those of nerve fibres studied under *constant*, iso-osmotic conditions owing to the consequent changes in their cell volumes and fibre diameters. Nevertheless, skeletal muscle volume exhibits close to ideal osmotic behaviour, varying inversely with changes in extracellular osmolarity that would in turn predictably increase solute concentration. The Results section of this paper accordingly first develops the original treatment (Hodgkin 1954; Adrian 1975; Noble 1979) for situations in which fibre diameter is varied by alterations in extracellular osmolarity that would in turn alter intracellular ionic strength. This provided quantitative expectations for any resulting change in conduction velocity that corresponded to two contrasting situations. First, classical electrolyte theory (Robinson and Stokes 1959; Atkins 1998) would predict that increased intracellular ionic strength should proportionally *decrease* sarcoplasmic resistivity R_i. Our analysis then indicated that this would precisely offset any expected changes in fibre axial resistance, r_i, produced by the osmotically induced alterations in fibre diameter and thereby leave conduction velocity *unchanged*. Secondly, Hodgkin and Nakajima (1972) reported that R_i increased with extracellular osmolarity, possibly due to increases in myoplasmic viscosity, although we certainly do not exclude possible factors arising from the more complex membrane structures found in muscle as opposed to nerve (see e.g. Sheikh et al. 2001). This led to predictions that conduction velocity would *decrease* with increasing extracellular osmolarity.

These predictions were then investigated by experimental determinations of conduction velocity obtained from action potential records derived from microelectrode measurements made at known distances from defined stimulation sites in surface muscle fibres of *Rana esculenta* studied at a fixed ($\sim$7°C) temperature at varied extracellular osmolarities. These findings demonstrated reversible changes in the conduction velocities of action potentials the nature of whose waveforms, including early surface and later tubular components, were otherwise unchanged, and distinguished between hypotheses through the observed dependence of conduction velocity on extracellular osmolarity.

Materials and methods

Cold-adapted frogs, *Rana esculenta*, were killed by concussion followed by pithing (Schedule I: Animal Procedures Act, Home Office, UK). The skin was removed and the tendon of insertion of the sartorius into the patella ligated, cut distally and dissected along its borders up to and including its origin at the pelvic girdle and acetabulum. This was performed at room temperature (21.7 ± 0.51°C; mean ± standard error of the mean; n = 15) in standard Ringer solution consisting of (mM): 115 NaCl, 2.5 KCl, 1.8 $CaCl_2$, 3 Hepes, titrated to pH 7, osmolarity 250 mOsm. The muscle was then stretched to 1.4 times its in situ length and secured in a transilluminated methacrylate polymer (Perspex) chamber by pinning the cleaned acetabulum and the ligature to minimize contractile artefact, which became less evident in solutions of higher osmolarities, and entirely absent with the higher (>350–400 mM) sucrose concentrations. The isolated sartorius muscles had an in situ length of 30.6 ± 0.94 mm and a length of 41.6 ± 1.15 mm (n = 15) when stretched. The muscle was laid over a supporting ramp with its dorsal surface uppermost.

A watertight Perspex partition, coated with a layer of Vaseline, with the muscle running through a notch at its lower surface electrically isolated the recording chamber into two compartments. The only path conducting the brief voltage stimuli applied from two platinum electrodes at opposite ends of the chamber was therefore through the muscle across the partition. The platinum electrode on the side of the chamber containing the acetabulum was the cathode. The temperature of the bath solution was lowered by circulating distilled water cooled in a magnetically stirred ice bath. The water was circulated through a glass coil placed in the chamber in close proximity to the muscle, using a Minipuls 3 peristaltic pump (Gilson, France). A digital thermometer incorporating a remote thermistor (P. Frost, Department of Physiology, Development and Neuroscience, Cambridge), previously

linearised and calibrated against a platinum film resistor was placed in the bathing solution near the muscle to allow the temperature to be continuously monitored and adjusted to remain constant throughout the recordings. The temperature was kept constant at 7.0 ± 0.03°C (n = 15) (Sheikh et al. 2001). Action potentials are temperature-sensitive so this allowed conditions to be optimised. Cooling also prolonged fibre viability especially in the hyperosmotic conditions.

The muscle was studied in the range of extracellular osmolarities from 250 to 750 mOsm. Solutions of different osmolarities were made by adding to the standard Ringer solution the membrane-impermeant solute sucrose at varying (150, 250, 350, 400, 450, 500 mM respectively) concentrations to yield solutions whose increased osmolarities (mOsm) were calculated from their total solute concentrations, and checked against measurements using a standard vapour pressure osmometer. Hepes was obtained from Sigma-Aldrich (Gillingham, Dorset, UK) and all other reagents from BDH Limited (UK). Solutions were changed, avoiding contact with the muscle, between tests using a vacuum pump (Edwards, UK) after which approximately 5–10 min elapsed before recording to permit time for both temperature (7°C) and osmotic equilibration; previous reports (Ferenczi et al. 2004) have indicated that 200 s sufficed to permit complete volume changes in response to hyperosmotic solutions.

3 M KCl-filled glass capillary microelectrodes (resistances 5–20 MΩ; tip potentials <5 mV: Adrian 1956) drawn from 1.2 mm (inner diameter) glass tubing were used to obtain membrane potentials. These were mounted via Ag/AgCl junctions to a high impedance voltage follower balanced by matching Ag/AgCl junctions earthing the bath. Only surface fibres showing clear-cut penetrations and stable resting membrane potentials were studied. Rates of rise and fall, dV/dt, in the voltage traces, were obtained by electrical differentiation of the output, with voltage and dV/dt channels filtered between cutoffs of 0 Hz/5 kHz and 0 Hz/20 kHz respectively. Action potentials were elicited by direct just-suprathreshold stimulation which varied between 1.5 and 5 V, to minimise electrotonic spread of the stimulus voltage and the number of fibres in which electrical activity was initiated, across the Perspex partition, through two platinum stimulating electrodes.

Conduction velocity was calculated by dividing the distance from the site of action potential initiation to the position of the recording microelectrode tip, ascertained by a Vernier scale, which gave measurements in cm to two decimal places, by the latency given by the time from a clear stimulus artefact generated to the peak of the dV/dt input, which will be referred to as the maximum dV/dt. The corresponding resting membrane potential, action potential overshoot and the value of maximum dV/dt were also noted. These relatively early events in the timecourse of the regenerative response often could be measured even in records showing small contractile artefact, which often only became evident at later times in the recorded traces.

Results

The experiments utilized an extension of early quantitative analyses (Hodgkin 1954; Adrian 1975) of conduction velocity in unmyelinated nerve studied in a *constantly* iso-osmotic volume conductor to changes that would result from *varying* the extracellular osmolarities in *Rana esculenta* skeletal muscle fibres. The initial analysis used the cable equation for the current density through any patch of flat membrane, expressed as current per unit membrane area,

$$I_{\mathrm{m}} = \frac{1}{s r_{\mathrm{i}}} \frac{\partial^2 V_{\mathrm{m}}}{\partial x^2}. \tag{1}$$

This describes conduction in a cable whose own finite internal volume is small compared to that of the extracellular conducting fluid in which it is studied to give large ratios between longitudinal intracellular, r_i, and extracellular, r_o, resistances to current flow per unit length, i.e., $r_o << r_i$, permitting extracellular terms relating to r_o to be dropped.

Equation (1) is combined with the expression for conduction velocity, $\theta = \frac{\partial x}{\partial t} \quad \Leftrightarrow \partial x = \theta . \partial t$, using the chain rule of differentiation, to give:

$$I_{\mathrm{m}} = \frac{1}{s r_{\mathrm{i}} \theta^2} \frac{\partial^2 V_{\mathrm{m}}}{\partial t^2}, \tag{2}$$

where V_{m} is the internal potential across the membrane at distance x along the fibre. Conduction at constant velocity through local circuit spread of excitation then requires I_m to be a single valued function of V_m. The term containing the intrinsic membrane parameters consisting of the area of membrane per unit length of fibre s, and the axial cytoplasmic resistance per unit length r_i, is then constant, whence $\frac{1}{s r_{\mathrm{i}} \theta^2} = \frac{1}{k}$, and where the constant k only depends on passive local membrane electrical properties. Thus:

$$\theta = \sqrt{\frac{k}{s r_{\mathrm{i}}}}. \tag{3}$$

For unmyelinated cylindrical fibres for which the axial cytoplasmic resistance per unit length, r_i, is related to fibre radius a and sarcoplasmic resistivity R_i by $R_i = r_i \pi\, a^2$, the total fibre volume, vol, $= \pi\, a^2 L$ where L is the length of the fibre and for which the total surface area of the fibre, $A = sL$:

$$\theta = \sqrt{\frac{k.vol}{R_{\mathrm{i}} A}}. \tag{4}$$

In the case of skeletal muscle fibres exposed to solutions of different osmolarities, osm, the volume, vol, behaves as a perfect osmometer following the relationship:

$$vol \propto \frac{1}{osm} \tag{5}$$

and reductions in fibre volume due to increased extracellular osmolarity would correspondingly increase solute concentration. Classical electrolyte theory (Robinson and Stokes 1959) then predicts that each participating ion contributes a specific conductance K_{sp} determined by its solute concentration η_C and a constant Λ defined for any given ion in any specified solute and referred to the conductivity of the ion at a 1 M concentration:

$$K_{sp} = \Lambda\eta_C. \tag{6}$$

The overall conductivity of such an ideal solution is then the algebraic sum of the conductivities of individual component ions. Decreases in fibre volume resulting from increased extracellular osmolarities then would increase intracellular ionic strength and in turn increase K_{sp} and proportionally decrease R_i:

$$R_i \propto vol. \tag{7}$$

Equation (7) then gives:

$$R_i \propto \frac{1}{osm}. \tag{8}$$

Equations (5) and (8) then give,

$$\theta = \sqrt{\frac{k'/osm}{A/osm}} = \sqrt{\frac{k'}{A}}, \tag{9}$$

thereby predicting a *constant* conduction velocity in view of the fact that the terms k', resulting from the additional proportionalities above, and A are both constants.

In contrast, Hodgkin and Nakajima (1972) suggested that sarcoplasmic resistivity, R_i, increased with increasing extracellular osmolarity, possibly owing to an increased myoplasmic viscosity, with reductions in fibre volume in hyperosmotic solutions, reporting a linear increase in R_i with increasing extracellular osmolarity:

$$R_i = D(osm) + E, \tag{10}$$

where D and E are constants.

From (5) and (10):

$$\theta = \sqrt{\frac{k'/osm}{(D(osm) + E)A}}. \tag{11}$$

Because k' and A are constants, we have:

$$\theta = \sqrt{\frac{1}{D'(osm)^2 + E'(osm)}}, \tag{12}$$

where D' and E' are also constants. This forms a contrasting expectation in which θ is expected to *decrease* with increasing extracellular osmolarity in a relationship modelled by Eq. (12) as a result of an increase in R_i. The alternative hypotheses could then be quantitatively tested by measurements of θ under different conditions of extracellular osmolarity.

Reversible effects of hyperosmotic extracellular solutions on action potential waveforms and latencies

Figure 1 shows typical action potential and dV/dt waveforms obtained at a temperature of ~7°C before, during and after exposures to solutions with increased extracellular osmolarity with column *A* showing the action potential waveforms and column *B* their corresponding dV/dt records. Panel *a* shows typical results from fibres in iso-osmotic Ringer at 7°C. The muscle was then exposed to a hyperosmotic (600 mOsm) Ringer solution and recordings resumed 5–10 min following this solution change (Panel *b*). Finally, the muscle was then returned to iso-osmotic Ringer and recordings again resumed 5–10 min after the solution was restored (Panel *c*). Latencies were measured from the clear stimulus artefact to the maximum point of the dV/dt

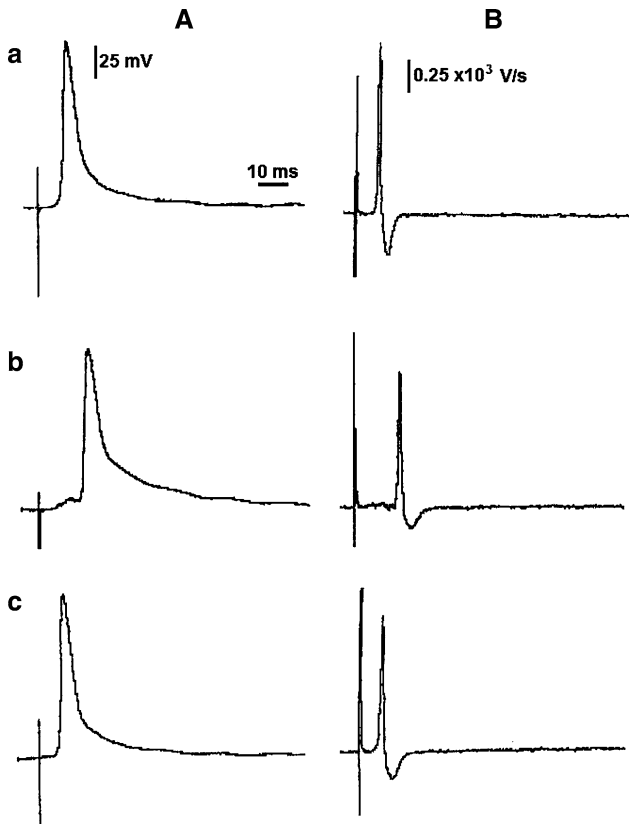

Fig. 1 Typical action potential (**A**) and dV/dt traces (**B**) obtained from muscle fibres exposed successively to (**a**) iso-osmotic Ringer (**b**) hyperosmotic (600 mOsm) Ringer and (**c**) returned to iso-osmotic Ringer, in the same sartorius muscle

trace as seen in column *B*: this approach provided more consistent measurements of action potential latency than using arbitrarily chosen points on the action potential traces (see also Sheikh et al. 2001).

The action potential traces showed prolonged positive after-depolarisation phases lasting well beyond 20 ms following the surface action potential deflections (Fig.1Aa), consistent with persistent excitation of an intact transverse (T-) tubular system (Adrian and Peachey 1973). These persisted both in the hyperosmotic solution (Fig. 1Ab) and the iso-osmotic solution to which the fibres were finally returned (Fig. 1Ac), confirming a persistence in transverse tubular excitation and its excitation following initiation of the surface component of the action potential through these manipulations.

Use of 600 mOsm as opposed to standard Ringer increased the action potential latencies and consequently the calculated values of conduction velocity. Thus, conduction velocities in the initial iso-osmotic Ringer solution, then at 600 mOsm-Ringer solution and finally, the returned iso-osmotic Ringer were 0.98 ± 0.092 m s^{-1} (n = 11 fibres), 0.58 ± 0.051 m s^{-1} (n = 10) and 0.72 ± 0.101 m s^{-1} (n = 6) respectively. The effect of hyperosmolarity on conduction velocity was thus at least partly reversible. However, this decrease in conduction velocity with increased osmolarity (from 250 to 600 mOsm) was not accompanied by qualitative changes in action potential or dV/dt waveform. Likewise, the waveforms showed no qualitative changes following return to the iso-osmotic Ringer solution after hyperosmotic exposure.

Grading of conduction velocity changes with extracellular osmolarity

Figure 2 summarises typical traces of action potentials (column *A*) and dV/dt (column *B*) for experiments that systematically investigated the effects of graded changes in extracellular osmolarity reflecting different extracellular sucrose concentrations on conduction velocity, action potential waveform, and dV/dt obtained at 7°C. Action potential waveforms again included both early rapid surface action potential deflections following the stimulus artefact and prolonged after-depolarisation phases that lasted well beyond 20 ms, reflecting T-tubular activation, at all the extracellular osmolarities studied (cf. Adrian and Peachey 1973). Measurements of latencies between the stimulus artefacts to the maximum value of the dV/dt traces then suggested a noticeable overall *decrease* in the calculated conduction velocities with increasing osmolarity. All these changes occurred in the absence of any excessive depolarization in resting membrane potential over the range of explored osmolarities. Thus resting potential at an osmolarity of 250 mOsm was –85.27 ± 1.05 mV (n = 87); that at an osmolarity of 700 mOsm was –75.94 ± 2.20 mV (n = 33).

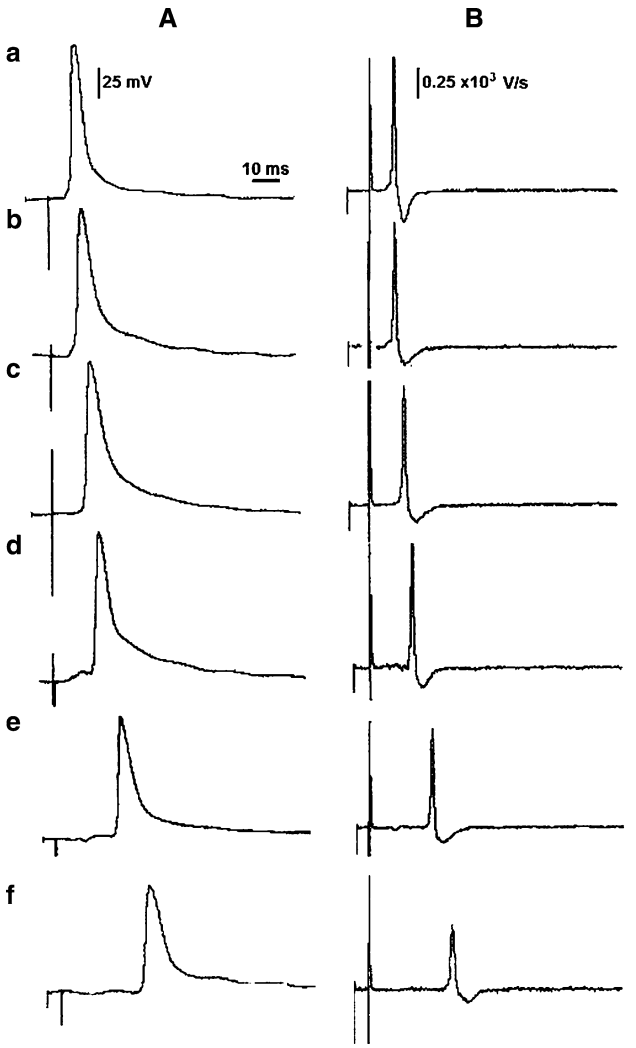

Fig. 2 Typical action potential (**A**) and dV/dt (**B**) traces recorded in the following extracellular osmolarities: (**a**) 250, (**b**) 400, (**c**) 500, (**d**) 600, (**e**) 650 and (**f**) 750 (all values given in mOsm). There is a notable overall increase in the latencies measured from (**B**)

However, resting potential at an osmolarity of 750 mOsm was –62.71 ± 2.82 mV (n = 17) (cf. Fraser et al. 2006). At the latter extracellular osmolarity a small proportion of action potentials appeared to display markedly reduced amplitudes resulting in peak deflections that failed to show overshoots, and a firing of two consecutive action potentials after a single stimulation in the case of one fibre. Accordingly, the investigations were not performed at higher sucrose concentrations.

Plots of conduction velocity and maximum dV/dt with increasing extracellular osmolarity

Figure 3 summarizes the results of systematic studies of the dependence of conduction velocity on extracellular osmolarity in a statistically larger number of muscle fibres. Action potentials and their corresponding dV/dt were measured

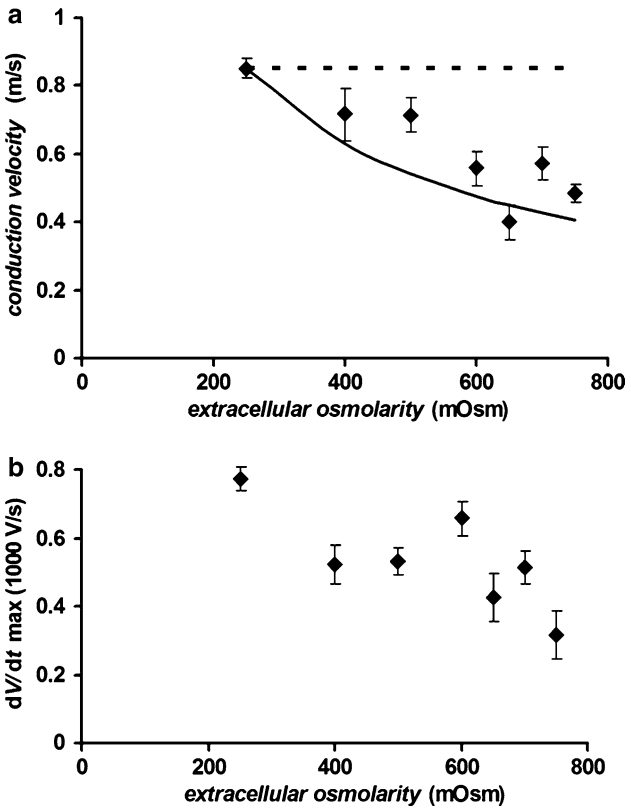

Fig. 3 Experimentally measured conduction velocities (**a**) and maximum d*V*/d*t* (mean ± standard error of the mean) plotted against extracellular osmolarity (◆). Dashed lines in (**a**): velocities predicted for a situation in which R_i decreases proportionally as a result of increased intracellular ionic strength produced by changes in cell volume brought about by the osmolarity changes. Continuous line: predictions when R_i increases with increasing extracellular osmolarity suggested by Hodgkin and Nakajima (1972). The changes in (**a**) were accompanied by decreases in maximum d*V*/d*t* (**b**) with increasing osmolarity, as expected for propagation brought about by local circuit currents

systematically in n = 87, 18, 27, 11, 10, 33 and 13 fibres at sucrose concentrations of 0, 150, 250, 350, 400, 450 and 500 mM corresponding in turn to osmolarities of 250, 400, 500, 600, 650, 700 and 750 mOsm respectively. Figure 3 demonstrates that both conduction velocity (a) and maximum d*V*/d*t* (means ± standard errors of the mean) (b) monotonically decreased with extracellular osmolarity. Figure 3a then compares these experimental values of conduction velocity against predictions from the two hypotheses for the dependence of R_i on osmolarity. In both cases, θ was calculated from R_i and the extracellular osmolarity, *osm*, using the relationship derived from Eqs. (4) and (5) above that:

$$\theta \propto \sqrt{\frac{1}{R_{\mathrm{i}}(osm)}}.$$

The dotted line denotes expectations from the first situation outlined above in which R_i decreases proportionally with increasing extracellular osmolarity: this predicts that conduction velocity does not alter with osmolarity from its control value in iso-osmotic solutions corresponding to a constant value of 0.85 m s^{-1} obtained in iso-osmotic solutions. The continuous line represents the second hypothesis in which R_i depends both on temperature and extracellular osmolarity as reported by Hodgkin and Nakajima (1972). The function displayed assumes the sarcoplasmic conductivity $G_i(= 1/R_i)$ to have a Q_{10} = 1.37, and a value of R_i at 2°C, of 298.51 Ω cm in a muscle fibre within an extracellular solution of 250 mOsm osmolarity and of 390.63 Ω cm in a muscle fibre within an extracellular solution of 600 mOsm osmolarity, as suggested by Hodgkin and Nakajima (1970, 1972). Then, let θ_1, R_{i1} and osm_1 correspond to conditions in iso-osmotic Ringer and θ_2, R_{i2} and osm_2 correspond to conditions in any hyperosmotic solution. Thus:

$$\frac{\theta_1}{\theta_2} = \sqrt{\frac{R_{\mathrm{i2}}(osm_2)}{R_{\mathrm{i1}}(osm_1)}},$$

Since θ_1 = 0.85 ± 0.029 m s^{-1} in iso-osmotic solution,

$$\theta_2 = 214.78\sqrt{\frac{1}{R_{\mathrm{i2}}(osm_2)}},$$

where

$$R_{\mathrm{i2}} = \frac{1000}{5.91\times1.37^{(temperature/10-2)}\times\frac{250}{\left[250+\left(\frac{osm_2-250}{370}\right)\times86\right]}}.$$

The values generated by the above equations predicted a decline in conduction velocity with increasing extracellular osmolarity.

To test the statistical significance of the goodness-of-fit of the two contrasting predictions to the experimental data obtained, an F_x test that takes into account the difference of the two reduced-χ^2 values in proportion to the first χ^2 term was performed (Bevington 1969). This derived for each hypothesis a value of χ^2 depicting the summed squared deviation of the original data, y_i, to the predicted findings, $y(x_i)$, obtained at any extracellular osmolarity, x_i, such that:

$$\chi^2 = \sum\left(\{y_{\mathrm{i}} - y(x_{\mathrm{i}})\}^2\right).$$

Values for χ^2 obtained in the two cases, χ_1^2 where the predicted conduction velocity is a constant value and χ_2^2 where there is an increase in R_i with extracellular osmolarity were 21.360 and 15.141 respectively. These values were then used to compute a F-statistic, given by:

$$F_1 = \frac{\left|\chi_1^2 - \chi_2^2\right|}{\chi_1^2}(n-1),$$

where n, the sample size = 200 and n–1 is the number of degrees of freedom.

The reduced-χ^2 tests for goodness-of-fit yielded a F-statistic of 81.73 consistent with a significantly better fit ($P << 0.001$) to predictions from a situation in which R_i increased as described by Hodgkin and Nakajima (1972) rather than a decrease in R_i with increasing extracellular osmolarity.

Discussion

This paper begins from classic analyses (Hodgkin 1954; Adrian 1975) of the dependence of action potential conduction velocities upon the diameter of nerve fibres studied in large volume conductors under iso-osmotic extracellular conditions. This had employed cable analysis that attributed these propagation processes to local circuit currents driven by Na^+ currents, I_{Na}, generating the rising phase of the action potential that in turn discharged initially passive circuit components (Valdiosera et al. 1974) equivalent to a membrane capacitance per unit area, C_m and resistance R_m of unit membrane area in series with an axial cytoplasmic resistance per unit length, r_i. The latter is related to fibre radius a and sarcoplasmic resistivity R_i (kΩ cm) by $R_i = r_i \pi a^2$ giving the original result that conduction velocity would vary as the square root of a.

The present paper extends this analysis to the effects of varying extracellular osmolarity on conduction velocities of surface membrane action potentials in *Rana esculenta* skeletal muscle, a situation that differed in a number of respects. First, skeletal muscle contains excitable transverse (T-) tubular membrane system responsible for initiating excitation-contraction coupling (Adrian et al. 1969, 1970; Huang 1993; Nielsen et al. 2004; Stephenson 2006). Nevertheless, the rapid initial sarcolemmal membrane activation that ensures rapid action potential propagation producing a synchronous sarcomeric activation likely largely precedes full tubular excitation (Adrian and Peachey 1973). Recent detubulation experiments left surface membrane conduction velocities unchanged suggesting a separation of surface and T-tubule excitation, the latter possibly initiated separately by Na^+ channels localized around the T-tubular luminal mouths (Sheikh et al. 2001). Increasing extracellular osmolarity did not increase the diameter of the necks of the T-tubules important in electrical connectivity between T-tubule network and the fibre membrane (Launikonis and Stephenson 2002, 2004). These findings would permit muscle fibre conduction velocities to be analysed in terms of surface cylinders. Second, skeletal muscle volume alters inversely with extracellular osmolarity (Blinks 1965; Ferenczi et al. 2004) in turn correspondingly increasing solute concentration, in contrast to the situation represented by comparisons of nerve fibres of different diameters in similarly iso-osmotic extracellular solutions.

This paper then derived quantitative consequences from two possible hypotheses emerging from the above conditions. On the one hand, classical electrolyte theory (Robinson and Stokes 1959; Atkins 1998) predicts a specific conductance K_{sp} attributable to each intracellular ion species increasing proportionally with solute concentration following decreases in cell volume in hyperosmotic solution. This led to a prediction of a decrease in R_i precisely correcting out effects of any osmotically induced diameter change together leaving conduction velocity constant. On the other hand, empirical observations suggesting increases in R_i with extracellular osmolarity (Hodgkin and Nakajima 1972) permitted construction of a quantitative formula for the resulting variations in R_i with extracellular osmolarity as well as temperature. This led to predictions of a conduction velocity that *decreased* with increasing extracellular osmolarity.

The experiments described in this paper then sought to distinguish between the two hypotheses. It investigated the effects of extracellular osmolarity on conduction velocities of surface membrane action potentials in surface fibres, that would be maximally exposed to these solution changes, in frog skeletal muscle, following stimulation at a Vaseline seal at defined distances from the microelectrode recording site. Simultaneous records were made of the rate of change of membrane potential dV/dt that would be provided a consistent time point at which there would be a maximal action potential slope as well as rate of discharge of the membrane capacitance, C_m, by local currents driven by the Na^+ current I_{Na}.

These studies showed that conduction velocity declined with increasing extracellular osmolarity along with maximum dV/dt as expected for a process dependent upon a local circuit current flow, despite relatively constant resting membrane potentials. These changes in conduction velocity in hyperosmotic solution were at least partially reversible. Furthermore, there were no changes in the nature of the action potential waveforms including early surface deflections and late after-depolarisation phases observed, consistent with a minimal change in the capacity for T-tubular excitation, through a still intact tubular system, that nevertheless followed generation of the initial surface component of the action potential wave.

Further systematic study in larger fibre numbers through a range of osmolarities (250–750 mOsm) demonstrated corresponding declines in conduction velocity and maximum dV/dt. At the highest osmolarity (750 mOsm), a small proportion of fibres variously showed low action potential overshoots which however did not correlate with the situations where there was a reduced conduction velocity, as well as multiple firing in a single muscle fibre. Nevertheless, further studies were not made at these and higher osmolarities; in any case, muscle fibres are thought only to

act close to perfect osmometers at osmolarities up to around *four* times that of the iso-osmotic solution (Blinks 1965).

These findings are thus consistent with the second hypothesis in which sarcoplasmic resistivity, R_i, increases with the fibre shrinkage observed in hyperosmotic solutions, as suggested by Hodgkin and Nakajima (1972), as opposed to the first possibility outlined above in which conduction velocity should be constant. This was borne out by objective statistical analysis of the goodness-of-fit of the predictions derived from the two contrasting hypotheses as expressed in their resulting reduced-χ^2 values, with the experimental values of conduction velocity generally assuming slightly higher values than predicted. The latter might reflect minor departures from a purely continuous conduction as postulated for *unmyelinated* cylindrical fibres, either due to contributions from peripheral regions of tubular membrane less isolated than the remaining tubular system (Hodgkin and Nakajima 1972), or the clustering of sodium channels around the tubular necks as reported by Sheikh et al. (2001) that may contribute to a more saltatory-like conduction in the muscle that would be expected to generally speed up propagation of electrical activity.

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