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TEMPERATURE DEPENDENCE OF THE ACTION POTENTIAL IN *NITELLA FLEXILIS*

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SUMMARY

1. The action potential and resting potential of internodal cells of *Nitella flexilis* have been measured between 3 °C and 28 °C. The amplitude of the spike diminishes with decreasing temperature from about 125 mV at 28 °C to about 65 mV at 3 °C. Over the same temperature interval the resting potential decreases from about 170 mV to about 100 mV.

2. The rise and decay times of the action potential increase by about two orders of magnitude between 28 °C and 3 °C, although the shape of the action potential remains unaltered.

3. Relatively sharp discontinuities in slope of the Arrhenius plots of the rise and decay times are found near 13.5 °C.

4. In each of the two temperature regions the activation energies for rise and decay times appear to be nearly identical, about 24 kcal/mole for $T > 13.5$ °C and 68 kcal/mole for $T < 13.5$ °C.

5. It is suggested that the rate limiting process for transport of Cl^- and K^+ across the excited membrane is probably associated with the activation of transport proteins in the membrane, and that the discontinuity near 13.5 °C may be related to a "phase change" of the membrane.

6. Threshold depolarization current and refractory period are also influenced by temperature, the latter more strongly than the former.

INTRODUCTION

Internodal cells of the fresh-water algae, the *Characeae*, have been the object of extensive investigations. Much of the interest in these cells stems from their electrical properties which parallels in many respects those of nerve axons and other electrically excitable cells [1–3]. Work aimed at elucidating these properties has involved the study of the permeabilities of plasmalemma and tonoplast membranes

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to Na^+, K^+ and Cl^- as functions of membrane potential using voltage-clamp techniques [4–6]; the determination of ionic fluxes under resting and active conditions [7–10]; the dependence of the resting and action potentials on ionic concentrations of the bathing medium [11–15], on metabolic inhibitors and other pharmaceuticals [15–18], on illumination [19–25], and on magnetic field [26].

As a result of these investigations the following appear to be fairly well established. The resting potential between the vacuole and the exterior is the sum of the potentials across the tonoplast and plasmalemma. In *Nitella flexilis* these potentials are about + 15 mV, and − 170 mV respectively, at room temperature [10]. From the ionic concentrations of the external medium, the cytoplasmic fluid, and the vacuolar sap, it appears that in *N. flexilis* K^+ is near the equilibrium concentration, but that Cl^- is actively transported into and Na^+ actively extruded from the cell. For several *Characeae*, among them *N. flexilis*, there are indications of Na^+/K^+ and Cl^- pumps at both plasmalemma and tonoplast membranes [10]. In addition, Kitasato [27] has demonstrated the existence of an active H^+ extrusion mechanism in *Nitella*; his work and that of others [15, 28, 29] on the role of H^+ transport suggest that the resting potential derives, at least in part, from an electrogenic H^+ pump. Measurements of the membrane resistance have yielded widely divergent results [30–32], but generally agree in ascribing to the plasmalemma a resistance an order of magnitude greater than that of the tonoplast, and suggest that the tonoplast is relatively permeable to these ions. Radioactive tracer measurements of ion fluxes in *Chara* [7] and *Nitella* [8] support the view that the action potentials in these cells arise from a depolarizing efflux of Cl^- with repolarization due to a delayed efflux of K^+. These effluxes are accompanied by a pronounced reduction in effective membrane resistance. Measurements on *Chara australis* [32] and *Nitellopsis obtusa* [32] indicate that the spikes are predominantly set up across the plasmalemma, although the tonoplast does contribute somewhat to the action potential as measured between the vacuole and the exterior. In the present experiments the voltage-electrode was inserted into the vacuole and no attempt was made to distinguish between tonoplast and plasmalemma potentials by locating an electrode in the cytoplasm. It is well known that a glass microelectrode, if left in the cytoplasm for more than about 20 min, will become sealed [13]. Typically, the duration of one experiment on a single cell extended over 48 to 72 h, precluding continuous reading of a cytoplasmic electrode.

Although the use of temperature as a means to study the activation of physiological processes is well known, work on *Nitella* has, for the most part, been carried out at a fixed temperature, generally near 20 °C. The influence of temperature was first investigated by Umrath almost 40 years ago [34]. Most recent investigators [15, 35, 36] have confined their attentions to steady state properties, the resting potential and membrane resistance. Kishimoto has studied the characteristics of the excitable *Chara* membrane, and reported data on the temperature dependence of the resting potential, conductance, and threshold [37]. Thus, since the early work of Umrath, the temperature dependence of the action potential in *Nitella*, its amplitude, rise and decay times, and other critical parameters such as threshold for stimulation, refractory period and propagation velocity have not been subjected to careful, systematic study, although it is known that at least some of these parameters do depend on temperature in squid axons [38, 39]. This paper reports the temperature depen-

dence of the amplitude, rise and decay times of the action potential in *N. flexilis* between 3 °C and 28 °C.

In addition to providing information that may lend support for, or argue against particular models of excitability in these systems, the study of temperature dependent effects is of particular interest in view of recent experiments on artificial as well as naturally occurring membranes which indicate the existence of one or more "phase transitions" [40, 41]. Such a transition manifests itself in various ways, among them a break in the Arrhenius plot of transport properties, i.e. a fairly sharp change in the activation energy for transport of the relevant ion or molecule across the membrane at the critical temperature of the transition [42].

MATERIALS AND METHODS

Specimens used in this study were purchased from a local biological supplier and kept at room temperature in APW (1 mM NaCl, 0.1 mM $CaCl_2$, 0.1 mM KCl, 0.1 mM $MgCl_2$) for a period of 1–4 weeks. Cells of approximately the same length (3.0–3.5 cm) and diameter (400–450 μm) and comparable age (third or fourth internodal cell) were used.

Two glass capillary microelectrodes, with tips between 7 and 10 μm diameter and filled with 150 mM KCl were inserted transversely into the vacuole. One was used to pass stimulating current pulses, the other to sense the potential between vacuole and bathing medium, APW of the above composition. The separation between the two electrodes was always less than 1 mm to reduce time delays due to the relatively slow propagation velocity to a minimum. The indifferent (ground) electrode was a silver wire, placed parallel to and about 1 mm from the internodal cell. The temperature of the bathing fluid was measured with a gold–iron versus chromel thermocouple whose junction, covered by a thin layer of epoxy, was located less than 1 cm from the glass electrodes. The chamber housing the sample, thermocouple, electrodes and micro-manipulators was made of Perspex and so constructed that it could be submerged in a constant temperature bath.

The voltage signal from the potential electrode was fed to the input of a WI Instruments Model 750 amplifier whose output was connected to an oscilloscope and fast strip chart recorder. The response of the recorder was adequate to follow the action potential faithfully below 12 °C. At higher temperatures the spike was recorded photographically from the oscilloscope display.

Stimulation was by means of a depolarizing pulse of 50 ms duration. The current was set at 1.5 times the threshold current for excitation of a spike near 4 °C. In a series of preliminary experiments it was found that the threshold current increases with decreasing temperature, consistent with results of Kishimoto [37]. As it was considered inadvisable to change the strength of the stimulus during the course of an experiment, the current was fixed at this value even though this was bound to result in stimulation well above threshold at more elevated temperatures. The use of a stimulating current very near threshold was also avoided because such currents sometimes gave spurious results for the rise time of the action potential [43].

Not only did the threshold current increase with decreasing temperature, but the refractory period was also substantially increased. Although at 20 °C spikes evoked at 3-min intervals were identical to those produced if stimulated once every

15 min, near 4 °C the refractory period was of the order of 1 h. At the lowest temperatures, therefore, stimulating pulses were applied at 90 min intervals; above 10 °C this was reduced to 30 min, and between 20 °C and 28 °C pulses were applied at 10 min intervals. Following a temperature change the specimen was left at the new temperature for at least 15 min before measurements were commenced; below 10 °C the cell was allowed to equilibrate for half an hour.

Visual observation of cytoplasmic streaming was used as an indication of thermal equilibration since the velocity of cyclosis is a linear function of temperature [34]. Observation of the duration of arrest of cyclosis following the application of a stimulating pulse suggests that this interval may bear a close relation to the refractory period.

RESULTS AND DISCUSSION

4 oscilloscope traces of the action potential in 1 *N. flexilis* cell at temperatures of 20.2 °C, 12.8 °C, 9.3 °C, and 7.4 °C are shown in Fig. 1. The vertical scale is 20 mV/div., the time scale 2 s/div. (This picture was obtained for illustrative purposes only; normally, the time scale was adjusted so that the spike extended over the entire screen.) The diminution of the amplitude of the spike and the increase in its duration are evident, as is the fact that the general shape of the action potential does not change with temperature

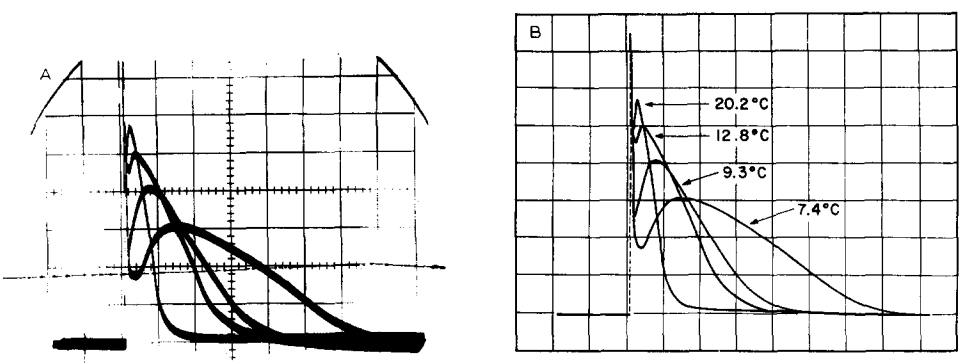

Fig. 1. Oscilloscope traces of the action potential of a *N. flexilis* internode at 20.2 °C, 12.8 °C, 9.3 °C and 7.4 °C. Ordinate scale: −20 mV/div.; time scale: −2 s/div. A, photograph; B, tracing.

Fig. 2 shows the amplitude of the spikes obtained from four different cells as functions of temperature. Also shown is the change in resting potential with temperature. The change in resting potential is about 2.5 mV/°C, in agreement with results on *Nitella translucens* [20, 35]. As found also by others, the action potential is somewhat smaller than the resting potential, i.e. the vacuole remains at all times negative with respect to the external medium, in contrast to the behavior of squid axons.

Membrane depolarization with decreasing temperature has been attributed by Hogg et al. [35] and by Hope and Aschberger [36] to changes in membrane permeability. However, since the initial experiments of Kitasato [27], additional experiments

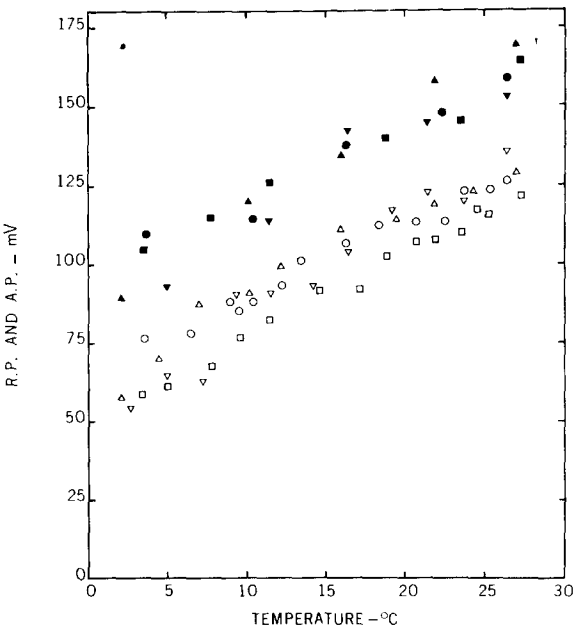

Fig. 2. Magnitude of the resting potential (R.P., solid symbols) and the amplitude of the action potential (A.P., open symbols) of four *N. flexilis* internodes as functions of temperature. Note: The resting potential is negative at all temperatures; the amplitude of the action potential is defined as the difference between the membrane potential at rest and the peak of the action potential response.

have provided further support for the existence of an electrogenic H^+ pump which contributes in some measure to the observed resting potential. The temperature dependence of the resting potential is, therefore, probably associated with the temperature dependence of this electrogenic contribution [20]. It is, therefore, surprising to find close parallelism of the action potential and resting potential since the former depends presumably on the Cl^- and K^+ equilibrium potentials and their activation and inactivation rates.

Fig. 3 shows the rise and decay times of the action potential from the same four samples, plotted on a logarithmic scale against the reciprocal of the absolute temperature. These times are somewhat arbitrarily defined as the intervals between the stimulus and the peak of the action potential, and between the peak of the action potential and its repolarizing mid-value, respectively. Another choice might have been to plot the maximum slopes on the rising and decaying portions of the action potential, but the constancy of the shape of the action potential assures that, except for a multiplicative factor, identical results would have been obtained.

Both times are well represented by two straight line segments on an Arrhenius plot, intersecting at 13.5 ± 2 °C. The slopes of these two straight line segments are nearly equal for both times (as they must be to preserve the shape of the action potential), and correspond to activation energies of 24 ± 4 kcal/mole for $T > 13.5$ °C and 68 ± 8 kcal/mole for $T < 13.5$ °C.

The dependence of rise time with temperature reported here is in qualitative agreement with measurements by Umrath on *Nitella mucronata* [34]. His data also

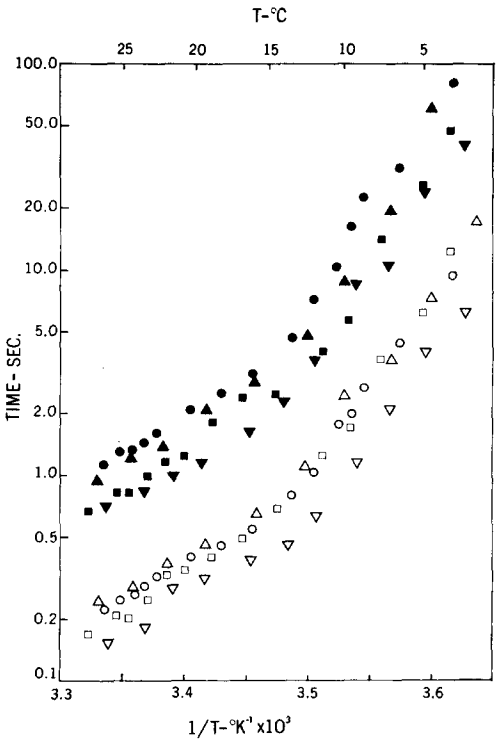

Fig. 3. Rise time (open symbols) and decay time (solid symbols) of the action potential of four *N. flexilis* internodal cells as functions of the reciprocal of the absolute temperature. Rise time is defined as the interval between the stimulating pulse and the peak of the action potential; decay time is defined as the interval between the peak of the action potential and the instant when the potential has decayed to the mid-value of the action potential response.

suggest a fit using two straight line segments on an Arrhenius plot with a break near 15 °C and activation energies, though smaller, of comparable magnitude.

Two features of these results appear particularly noteworthy, namely the fairly well-defined break of the Arrhenius plots near 13.5 °C and the close agreement of the activation energies for depolarization and repolarization.

The latter is quite remarkable especially as different ions, Cl^- and K^+, traversing the membrane presumably through different channels, are responsible for the rising and falling portions of the action potential. As was pointed out previously, this agreement reflects the constancy of the shape of the action potential, a feature also observed in other systems. If the activation energies are associated with diffusion through static pores, such close agreement of the activation energies would be most fortuitous. These energies may, therefore, relate to a rate limiting process other than simple ionic diffusion, for example, to the activation of Cl^- and K^+ "gates" or transport proteins. The fact that these energies are nearly identical then suggests that the two activation processes may be closely coupled, perhaps mediated by the same ion or molecular complex. Moreover, if the transport proteins are conformationally coupled to the membrane, the activation energies will reflect the dynamical proper-

ties of the membrane and exhibit drastic changes as a result of a "phase transition".

As regards the break in the Arrhenius plots, similar changes in activation enthalpies for the permeation of K^+ and Na^+ in *Chara corallina*, at nearly the same temperature, were interpreted as indicative of either a change in membrane structure, i.e. a "phase transition", with cooling, or of different permeation processes dominating in different temperature ranges [36]. Either model could account for our observations, although the absence of a similar discontinuity in the resting potential and action potential amplitude might argue against the "phase transition" model, since such a transition could be expected to influence the steady-state permeabilities and hence the membrane potential. The change in these potentials is, however, only about 50% between 28 °C and 3 °C, contrasted to two orders of magnitude for the rise and decay times, and the scatter of the data is large enough to obscure a possible slope discontinuity. The critical temperature of such phase transitions is known to depend on the degree of saturation of the lipid hydrocarbon chains [42], and it would, of course, be desirable to compare the temperature of the Arrhenius plot break with estimates based on lipid composition of the plasmalemma membrane. Unfortunately, this information does not appear to be at hand, precluding direct comparison. It is, however, interesting that in the two algal systems in which such apparent transitions have been found, they occur at nearly the same temperature.

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