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TEMPERATURE DEPENDENCE OF THE IONIC CURRENT KINETICS OF *MYXICOLA* GIANT AXONS

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SUMMARY

1. The temperature dependence of the sodium and potassium conductances of *Myxicola* giant axons was studied in the range of 1–18° C.

2. Analysis of voltage clamp records for step depolarizations of 20–150 mV yielded Q_{10}'s for $\bar{g}_{Na}$, $\bar{g}_K$, and g_l of 1·3–1·5. The steady-state parameters $m_\infty(v)$, $h_\infty(v)$, $n_\infty(v)$ were independent of temperature. The time constants had Q_{10}'s of: $2{\cdot}64 \pm 0{\cdot}2$ for τ_m; $2{\cdot}56 \pm 0{\cdot}2$ for τ_h; $3{\cdot}02 \pm 0{\cdot}3$ for τ_n.

3. τ_h determined from the effects of prepulse duration rather than the decline in sodium current during a step depolarization had a Q_{10} of $2{\cdot}47 \pm 0{\cdot}2$.

INTRODUCTION

Results concerning the effect of temperature on the rate constants governing conductance changes in excitable membranes are limited. For the squid axon a Q_{10} of 3 was found to be adequate, allowing rate constants from different axons to be represented as single functions of potential (Hodgkin, Huxley & Katz, 1952; Hodgkin & Huxley, 1952), and yielding calculated responses in good agreement with experiment (Huxley, 1959). The maximum conductances, $\bar{g}_{Na}$ and $\bar{g}_K$, on the other hand, had Q_{10}'s more in line with those expected for simple electrolyte solutions (Hodgkin *et al.* 1952; Moore, 1958; Wang, Narahashi & Scuka, 1972).

Such data were next obtained in *Xenopus* myelinated nerve (Frankenhauser & Moore, 1963), where the Q_{10}'s for sodium activation (m) were found to be somewhat less than 2, substantially lower than those for sodium inactivation (h) and potassium activation (n), which agreed with the squid values. However, in *Rana* fibres, Moore (1971) recently found no such difference, τ_m having a Q_{10} of nearly 3, similar to that for τ_h and τ_n.

This difference is of some importance, since in the case of the m para-

meters of *Xenopus* fibres application of reaction rate theory (Tsien & Noble, 1969; Noble, 1972) yields a negative entropy of activation, whereas for all other processes a positive entropy is calculated.

The rate constants themselves are strongly voltage-dependent, and thus the measured Q_{10}'s might perhaps be expected to show similar behaviour. However, except for Moore (1971), who reported data from three different potentials in which no systematic variation of the Q_{10} was apparent, most studies of the temperature dependence of rate constants have been confined to a single potential. From a theoretical point of view, it would be extremely useful to have accurate data on temperature dependence over a wider range of potential.

For these reasons, and to characterize further the properties of *Myxicola* axons, a complete voltage clamp analysis of the sodium and potassium conductances was performed over a temperature range of 1–18° C and a full range of potentials.

METHODS

Myxicola giant axons were voltage clamped by methods previously described (Binstock & Goldman, 1969). Compensated feed-back was used throughout. Temperature was continuously monitored by a thermistor located in the perfusion solution just before its entry into the chamber. The actual temperature at the axon proper for various flow rates and temperatures was determined in a series of calibration procedures by placing a YSI Type 524 thermistor in the location to be occupied by the axon during an experiment. All temperatures given here refer to such temperatures, which were never more than 2° C higher than the measured temperature at the lowest flow rates used.

The pH of the artificial sea water (430 mM-NaCl; 10 mM-KCl; 10 mM-$CaCl_2$; 50 mM-$MgCl_2$) was controlled with 5 mM-Tris, to which sufficient 6 M-HCl was added before an experiment to maintain the pH at $8{\cdot}0 \pm 0{\cdot}05$ at all temperatures studied.

In the first series of experiments, membrane currents for step depolarizations of 20–150 mV were recorded in artificial sea water (ASW) and ASW containing 10^{-6} M tetrodotoxin. The separated sodium and potassium currents were then analysed by the procedures described in detail by Goldman & Schauf (1973) to yield the Hodgkin–Huxley parameters $\bar{g}_{Na}$, $\bar{g}_K$, $\tau_m(v)$, $\tau_h(v)$, $\tau_n(v)$, $m_\infty(v)$, $h_\infty(v)$, and $n_\infty(v)$ as a function of temperature.

In a second procedure, two pulse experiments were performed at various temperatures. Here, $h_\infty(v)$ and $\tau_h(v)$ were obtained from the effect of prepulse duration on the peak sodium current during a subsequent fixed test pulse using the methods described by Goldman & Schauf (1972). Test pulses were always chosen to be on the positive limb of the sodium current–voltage relation, and all data was leak corrected by repetition of the protocols in 10^{-6} M tetrodotoxin.

RESULTS

Experiments using step depolarizations

In three axons a limited voltage clamp analysis was performed at two temperatures (about 10° C apart), with runs at the colder temperature bracketing that at the higher. A linear correction for changes in the

maximum conductances was applied and the averaged Q_{10}'s found to be: $1{\cdot}37 \pm 0{\cdot}06$ for $\bar{g}_{Na}$; $1{\cdot}50 \pm 0{\cdot}10$ for $\bar{g}_K$; $1{\cdot}34 \pm 0{\cdot}09$ for g_l. The somewhat higher Q_{10} for $\bar{g}_K$ is not significant. A regression analysis of the pooled data from all axons, the majority only studied at a single temperature, also produced slopes between 1·3 and 1·5, but the variance was considerably higher because of the scatter in absolute conductances.

A total of ten axons was used to define the temperature dependence of the rate constants. First, the quantities $m_\infty(v)$, $h_\infty(v)$, $n_\infty(v)$ were plotted for all axons. As pointed out by Goldman & Schauf (1973), values for $h_\infty(v)$ determined by step depolarizations are near zero for all potentials. The other functions showed no systematic variation with temperature, implying that the forward and reverse rate constants (α_i and β_i) had a similar temperature dependence. Therefore all data were left in terms of the experimentally measured time constants.

Goldman & Schauf (1973) derived an empirical set of equations which were a good description of *Myxicola* rate constants measured using step depolarizations at 5° C (eqns. (4), (5), (9), (10) and (12) of that paper). These are reproduced here for the reader's convenience (note that α_h was assumed to be zero):

$$\alpha_n = \frac{1}{2{\cdot}85\,[\exp(V-21/-22{\cdot}8)+1]},$$

$$\beta_n = 0{\cdot}045\exp(-V/138),$$

$$\alpha_m = \frac{0{\cdot}066\,(V+45)}{1-\exp[(V+45)/5{\cdot}95]},$$

$$\beta_m = 0{\cdot}075\exp(-V/23{\cdot}8),$$

$$\beta_h = \frac{1}{0{\cdot}714\,[\exp(V-34/-23)+1]} + 0{\cdot}4.$$

In order to describe the temperature data, the equations for the appropriate α_i and β_i were scaled by the same factor and τ_i calculated from $\tau_i = (\alpha_i+\beta_i)^{-1}$.

Figs. 1 and 2 show typical results obtained by this procedure. In Fig. 1 the solid symbols represent time constants τ_m, τ_h, τ_n measured at 13·5° C in a single axon, while the continuous lines are computed from the equations of Goldman & Schauf (1973). In Fig. 2 the measured time constants τ_n (filled symbols) for four different axons at four temperatures are shown. Again, the continuous lines are computed by scaling the *Myxicola* equations at 5° C.

In both cases the continuous lines adequately fit the measured time constants over the entire range of potentials investigated. This implies that the measured Q_{10}'s are not significantly voltage-dependent. The time

constants decrease by a factor of 4–5 over the range of potentials for which data are available, and a deviation of as much as 10% should be evident.

The pooled data are shown in Fig. 3, where the factors by which the *Myxicola* equations at 5° C were scaled are plotted as a function of temperature for τ_m, τ_h and τ_n. A regression analysis of the data yields Q_{10}'s (± S.E. of the slope) of: 2·64 ± 0·2 for τ_m; 2·56 ± 0·2 for τ_h; 3·02 ± 0·3 for τ_n. An analysis of variance shows that the value for τ_n is not significantly different from the others. Most axons of Fig. 3 were studied at only a single temperature, but in two cases the same axon was examined at two temperatures with no deviation of the measured Q_{10}'s.

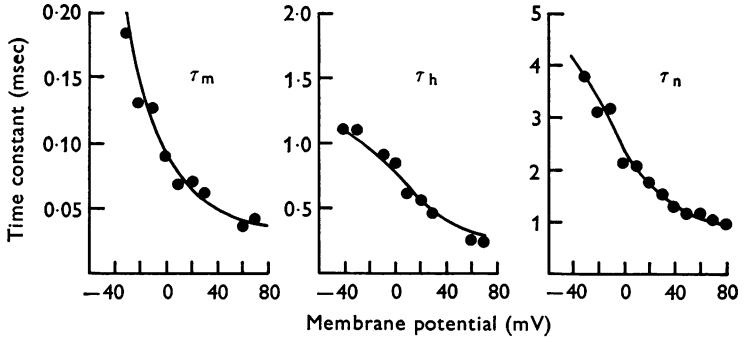

Fig. 1. Measured time constants (filled symbols) for the m, n, and h processes in a single axon at 13·5° C. The continuous lines are calculated from the equations describing *Myxicola* kinetics at 5° C by scaling eqns. (4), (5), (9), (10), (12) of Goldman & Schauf (1973). Scaling factors required for this particular experiment were 3·6 for τ_m, 2·0 for τ_h, 2·9 for τ_n.

Two-pulse experiments

Goldman & Schauf (1973) demonstrated that the values of τ_h obtained by fitting a time constant to the decline in sodium currents during a step depolarization are significantly lower than those obtained from the effects of prepulse duration on the peak sodium current during a subsequent fixed test pulse, when comparison is made at the same membrane potential. Thus, in general the temperature dependence of τ_h need not be the same in both cases.

A total of five axons were examined using prepulses. No temperature dependence of $h_\infty(v)$ was observed. Fig. 4 shows the values of τ_h (filled symbols) measured experimentally in axons at two different temperatures. The continuous curves in this case have been computed using eqns (6) and (7) of Goldman & Schauf (1972), which are a good description of the inactivation rate constants for *Myxicola* at 5° C. Both α_h and β_h were scaled by the same factor and the time constant computed as

$\tau_h = (\alpha_h + \beta_h)^{-1}$. Again the continuous curves seem to adequately describe the data without any systematic deviation with prepulse potential.

Fig. 5 shows the scaling factors by which α_h and β_h were multiplied to give a best fit to the measured time constants as a function of temperature. A regression analysis of this data yields a Q_{10} of $2{\cdot}46 \pm 0{\cdot}2$, which is no

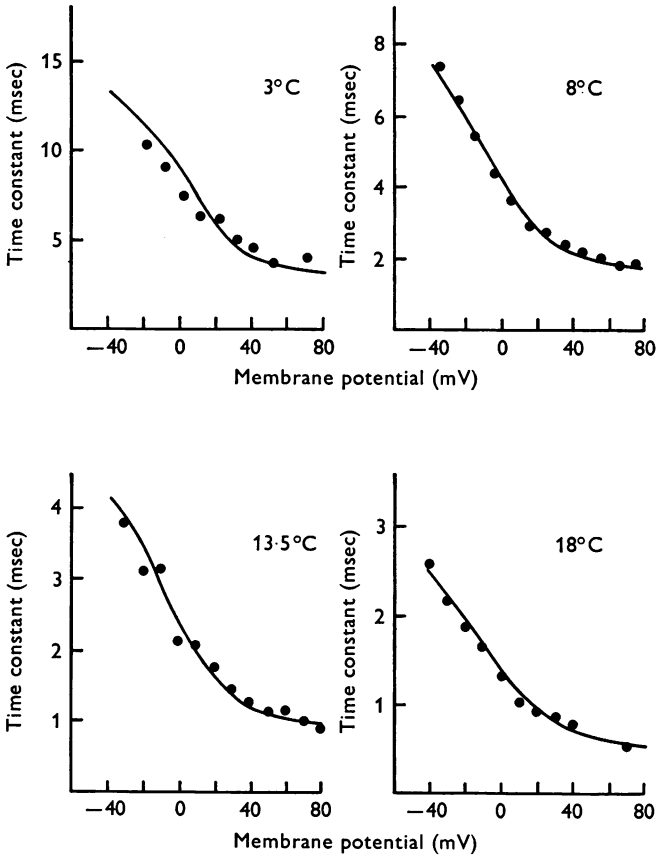

Fig. 2. Measured time constants (filled symbols) for potassium activation (τ_n) for four different axons at four different temperatures. The continouus lines are calculated from the equations describing *Myxicola* kinetics at 5° C by scaling. Scaling factors required for best fit were 0·95 (3° C), 1·65 (8° C), 2·9 (13·5° C), 5·0 (18° C).

different from that obtained earlier. Most axons were examined at a single temperature, but in one case two temperatures (1·5 and 12° C) were studied with the same result.

A useful check on the results is the computation of membrane action potentials as a function of temperature using the measured temperature

dependence of the rate constants. A computational procedure identical to that of Goldman & Schauf (1973) was followed except for scaling all rate constants by the appropriate factor. The duration of the computed action potentials, measured at a level of 50 mV depolarized from rest, had a Q_{10} of 2·58. Experimentally, the action potential duration in *Myxicola* axons varied with temperature with a Q_{10} obtained by regression analysis of $2{\cdot}47 \pm 0{\cdot}2$, which agrees quite closely with the computed value.

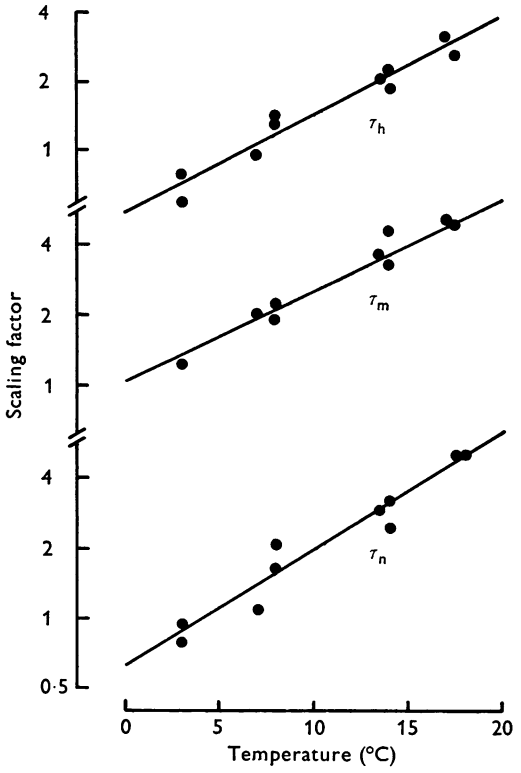

Fig. 3. Factors by which the equations describing *Myxicola* rate constants at 5° C were scaled for best fit as a function of temperature (filled symbols). The continuous lines (of the form $\log_{10} y = aT + b$) were calculated using a linear regression analysis. The Q_{10} is calculated from the slope of this regression line. The sample standard error of the slope permits the calculation of upper and lower bounds on the Q_{10} which are slightly asymmetrical because of the logarithmic transformation. Using the largest deviations we obtain Q_{10}'s ($\pm$ maximum S.E.) of $2{\cdot}64 \pm 0{\cdot}2$ for τ_m, $2{\cdot}56 \pm 0{\cdot}2$ for τ_h, and $3{\cdot}02 \pm 0{\cdot}3$ for τ_n.

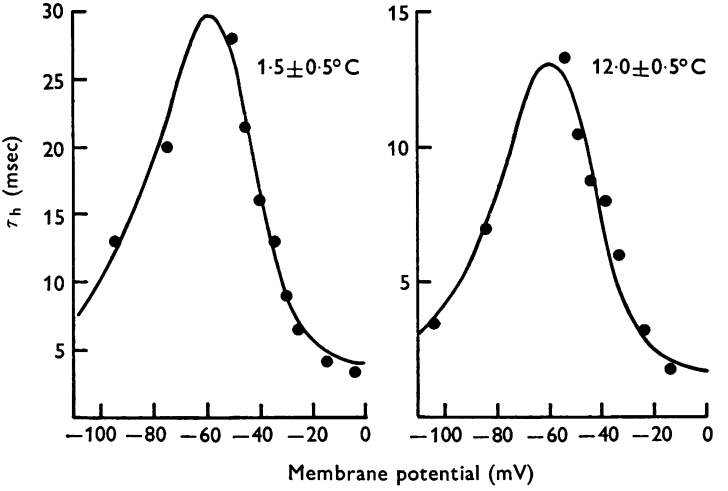

Fig. 4. Measured inactivation time constants from two pulse experiments (filled symbols) at 1·5° C and 12° C. The continuous lines were calculated from the equations describing *Myxicola* kinetics at 5° C (eqns. (6) and (7) of Goldman & Schauf, 1972) by scaling. The scaling factors required in these particular experiments were 0·80 for 1·5° C and 1·85 for 12·0° C.

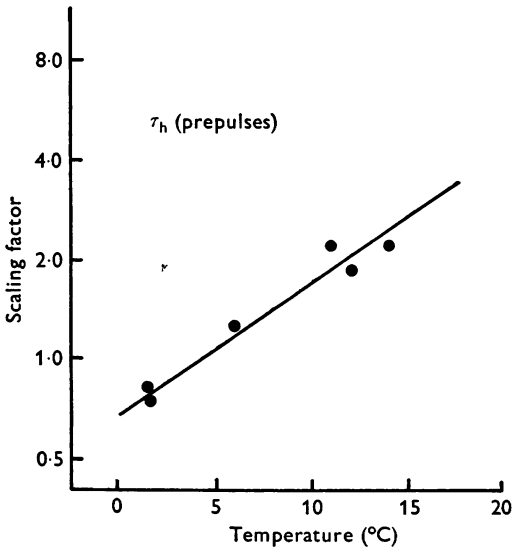

Fig. 5. Pooled data on the inactivation time constant measured by prepulses. The factors by which the equations describing *Myxicola* rate constants at 5° C were scaled for best fit are plotted as a function of temperature. The continuous line was calculated from a linear regression and yields a Q_{10} of 2·47 ± 0·2.

DISCUSSION

The Q_{10}'s for $\bar{g}_{Na}$, $\bar{g}_K$, and g_l for *Myxicola* axons agree with previous determinations (Hodgkin *et al.* 1952; Moore, 1958; Frankenhauser & Moore, 1963) in being in the range observed for conduction in electrolyte solutions. The conclusion that $m_\infty(v)$, $h_\infty(v)$, and $n_\infty(v)$ are temperature independent was also implicit in the use by Hodgkin *et al.* (1952) of a Q_{10} of 3 for all rate constants. Differences of 4–12 % in the forward and reverse rate constants were tabulated by Frankenhauser & Moore (1963) but are probably not statistically significant. Wang *et al.* (1972) did, however, observe a small but significant shift in $h_\infty(v)$ with temperature for squid axons.

The temperature dependence of *Myxicola* kinetics resembles that observed in squid (Hodgkin *et al.* 1952) and in myelinated fibres of *R. pipiens* (Moore, 1971) in having Q_{10}'s for nearly 3 for all rate constants. Thus, nodes of *X. laevis* remain the only completely analysed system in which significant differences are observed. Noble & Tsein (1968) found a Q_{10} of 6 for the S process in cardiac Purkinje fibres, but data is not yet available concerning the temperature dependence of the other kinetic processes.

There is no measurable effect of potential on the temperature dependence of *Myxicola* kinetics over a range in which the absolute rates vary by a factor of 4–5. This conclusion agrees with the more limited data of Moore (1971) on *Rana* fibres. For example, his activation energies for τ_n at a membrane potential of -30 mV were 14·7 and 16·6 kcal/° mole in two experiments, while at $+10$ mV (where τ_n is about half as large), values of 16·2 and 20·8 kcal/° mole are obtained. No decrease in the temperature dependence for the faster process is apparent.

For a simple single barrier model in which the variations in absolute reaction rate (free energy of activation) is solely attributable to variations in the activation enthalpy, the Q_{10} should decrease by about 10 % for a 20-fold increase in rate (Tsien & Noble, 1969). For the range of variation seen in Figs. 1, 2 and 4, this would correspond to at most a few per cent. Such a deviation would not be apparent, and this data seems therefore to be consistent with such a hypothesis. Certainly there is no evidence from this study that entropy changes are markedly different for the different permeability reactions, or that there is any significant systematic variation in the activation entropy with potential for a particular process. The calculated entropies are all in the range of 5–9 entropy units and resemble those calculated for the h and n process of *Xenopus* fibres (Tsien & Noble, 1969).

The temperature dependence of the inactivation rate constants is the

same even though the absolute magnitudes measured by prepulse experiments are significantly slower than those obtained from the response to a step depolarization to the same potential (Goldman & Schauf, 1973). This serves to further define the requirements which a detailed model of the sodium conductance must satisfy.

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