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EFFECT OF TEMPERATURE ON THE ANOMALOUS RECTIFICATION OF THE MEMBRANE OF THE EGG OF THE STARFISH, *MEDIASTER AEQUALIS*

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SUMMARY

1. The effect of temperature upon the anomalous or inward rectification of the K conductance in the immature egg membrane of the starfish, *Mediaster aequalis*, was studied by using voltage clamp technique.

2. The K conductance decreases with a relatively small Q_{10} (1·62) down to about 10 °C; below 10 °C, the Q_{10} is much greater (5·8 at $[K^+]_o$ = 25 mM). The smaller Q_{10} is independent of $[K^+]_o$, whereas the larger one depends on $[K^+]_o$.

3. The activation of the rectification depends on $V - V_K$, rather than V alone, at all temperatures at constant internal K concentration.

4. The K conductance at a given $V - V_K$ is approximately proportional to the square root of $[K^+]_o$ at a fixed $[K]_i$ above 10 °C while the conductance depends substantially less on $[K^+]_o$ below this temperature.

5. The logarithm of the activation time constant of the inward rectification depends linearly on the membrane potential at all temperatures.

6. The slope of the relation is strongly temperature dependent above about 10 °C whilst the dependence is much less below 10 °C: i.e. the Q_{10} of the activation time constant is membrane potential-dependent above 10 °C.

7. The results suggest that the mechanism of ion permeation during anomalous rectification changes at about 10 °C.

INTRODUCTION

The resting membrane of the starfish egg is predominantly permeable to K^+, and this K permeability shows the anomalous or inward rectification (Hagiwara & Takahashi, 1974; Miyazaki, Ohmori & Sasaki, 1975; Hagiwara, Miyazaki & Rosenthal, 1976; Ciani, Krasne, Miyazaki & Hagiwara, 1978; Hagiwara & Yoshii, 1979). The inward rectification includes instantaneous and time-dependent components. The time-dependent component shows first order kinetics. When the external K concentration is varied at a fixed internal K concentration, the activation of the kinetics depends on $V - V_K$ rather than V alone, where V and V_K are the membrane potential and the K equilibrium potential. At a given $V - V_K$, the K conductance of

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the membrane is roughly proportional to the square root of the external K concentration. All of these results were obtained at room temperature (20 ~ 22 °C).

In the present work the inward rectification of the immature egg of the starfish, *Mediaster aequalis*, was studied by using voltage clamp technique under varying temperatures. We have examined the effect of temperature on (1) the amplitude of the K conductance for the inward rectification, (2) the kinetics of the time-dependent component, (3) the dependence of activation on $V-V_K$, and (4) the dependence of the membrane conductance on the external K concentration.

METHODS

Materials: Immature eggs of the starfish, *Mediaster aequalis*, were used. These eggs were about 1 mm in diameter. The collection of the animals and the methods of preparing the eggs were similar to those described previously (Hagiwara, Ozawa & Sand, 1975).

Temperature control: An egg was immobilized at the centre of a chamber in the manner described by Hagiwara *et al.* (1975). The chamber was perfused at 2 ml./min throughout the experiment by a micropump (Minipulse 2, Gilson Medical Electronics, Middle Town, Wisc. U.S.A.). The bath temperature was controlled by a thermoelectric device (Cambion, Cambridge Thermionics Corp., Cambridge, Mass. U.S.A.) through which the perfusion solution flowed. The desired temperature was obtained by selecting an appropriate voltage applied to the device. A thermistor with a 2·4 mm head (Type 402, Yellow Springs Instr. Co., Yellow Springs, Ohio, U.S.A.) was placed adjacent to the egg usually downstream from it. The chamber was narrow (3 mm wide) and long (1·7 cm), so that the flow was usually even. The measured temperature was likely the true temperature of the egg, because shifting the thermistor upstream or downstream from the egg did not change the reading significantly.

Electrical recordings: All recordings were made with the two-micropipette voltage clamp technique described previously (Hagiwara *et al.* 1975). The membrane potential was recorded differentially between an intracellular and an extracellular electrode (both 2–5 MΩ). The membrane current was recorded as a voltage drop across a 100 kΩ resistor inserted between the output of the feed-back amplifier and the intracellular current electrode. This method was selected in preference to measuring the bath current, since there was the possibility of a leakage current between the bathing system and ground due to waterdrops formed during cooling. Recording systems other than those mentioned above were the same as described before (Hagiwara, Miyazaki & Rosenthal, 1976).

Solutions: The compositions of the major solutions used are listed in Table 1. The test solutions of various K^+ concentrations were prepared by mixing A_1 and A_2 or B_1 and B_2. K ions were replaced with Na ions (solution A) or with Tris (solution B). The pH of the solutions was adjusted to 7·6–7·7 by HCl. At this pH, approximately one quarter of Tris-OH was undissociated. Essentially no difference was found between results obtained with solutions A and B in the present experiments.

TABLE 1. Composition of master solutions (mM)

	KCl	NaCl	$CaCl_2$	$MgCl_2$	TrisOH	HCl*
Solution A_1	—	470	10	50	10	7·5
A_2	200	270	10	50	10	7·5
Solution B_1	—	—	10	50	537	403
B_2	200	—	10	50	308·5	231·5

* Used for titration to pH 7·6–7·7.

RESULTS

Effect on the steady-state conductance

A step change of the membrane potential in the negative direction resulted in an instantaneous inward current (Fig. 1; the preceding capacitative currents are not seen in the records). Thereafter, the magnitude of the inward current increased with an approximately exponential time course to a final steady-state value, I_s. The four

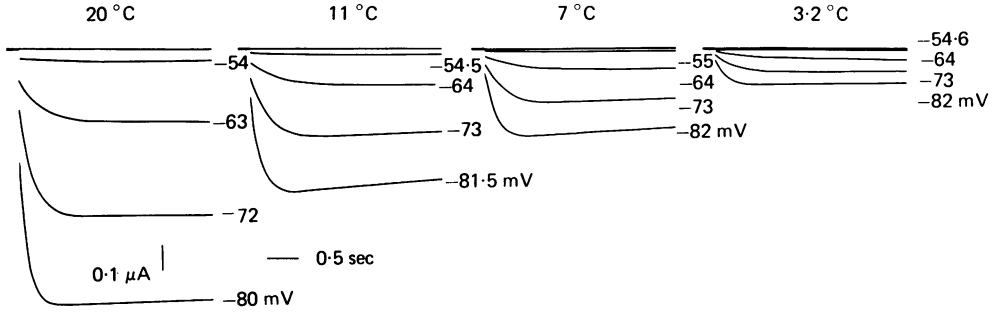

Fig. 1. Membrane currents during voltage clamp of the same immature egg cell membrane of the starfish, *Mediaster aequalis*, obtained at different temperatures. The number listed by each trace indicates the membrane potential during the voltage pulse. External solution, 25 mM-K (solution A). Holding potential: −51 mV at 20 °C, −45 mV at 11 °C, −25 mV at 7 °C and −5 mV at 3·2 °C. Diameter of the cell, 900 μm.

sets of membrane current recordings in Fig. 1 were obtained during voltage clamp to various membrane potentials in 25 mM-K medium at four temperatures (20, 11, 7 and 3·2 °C). The relationship between I_s and the membrane potential V at different temperatures is shown in Fig. 2. For large negative potentials (e.g. −80 mV), the magnitude of the current tended to decline slowly after reaching the maximum value. In such cases the maximum value was taken for the value of I_s. This did not seem to introduce any serious error since the present work was limited to the range of membrane potentials where the slow decline was small.

The effects of the holding potential on I_s are also shown in Fig. 2. The I_s–V relationship was examined after holding at each potential for 30 sec–1 min. At room temperature (20 °C), the holding potential was initially the zero current potential, V_0, which was essentially V_K (Hagiwara & Yoshii, 1979). In this cell V_0 at 20 °C was −51 mV. The I_s–V relationship was unaltered by shifting the holding potential from −51 to −25 mV at 20 °C. The result was similar at 11 °C. However, at 7 and 3·2 °C, the amplitude of I_s increased when the holding potential was shifted from −45 to −25 mV. The increase of I_s reached saturation at 7 °C when the holding potential became more positive than −25 mV; at 3·2 °C the increase saturated at a holding potential more positive than −5 mV. The results suggest the existence of an inactivation-like phenomenon. The phenomenon was found in Na as well as in Na-free Tris media. The time constant of the inactivation, however, may be very large since the amplitude of the current obtained at the holding potential of −25 mV did not decline to the value obtained at the holding potential of −45 mV, at least within 2–3 sec at either 7 or 3·2 °C. To avoid an underestimation of I_s at low temperatures, data obtained at holding potentials giving full removal of inactivation were always used.

The steady-state K chord conductance, G_K, was obtained as $I_s/(V-V_o)$, since the membrane is predominantly permeable to K^+, i.e. V_o was practically identical to V_K. When the temperature was lowered from 20 °C, V_K should change according to the Nernst equation

$$V_K = \frac{RT}{F} \ln \frac{[K^+]_o}{[K^+]_i}. \quad (1)$$

Eqn. (1) predicts that $V_K = -51$ mV at 20 °C should become $V_K = -48$ mV at 3·8 °C if one assumes $[K^+]_o/[K^+]_i$ is unaltered. However, in actuality, V_o showed a substantially more positive value (e.g. -20 mV) at that temperature. Tentatively, this effect was attributed to a small leakage introduced by the insertion of micropipettes which became significant at low temperatures because of the decrease in the K permeability at V_K. Therefore, V_K at a lower temperature was calculated from eqn. (1) by assuming $V_o = V_K$ at room temperature (20–22 °C). Then G_K was calculated by

$$G_K = (I_s - I_s^*)/(V - V_K), \quad (2)$$

where I_s^* is the I_s at the calculated V_K and its observed amplitude was negligibly small compared with that of I_s for $(V-V_K) < -10$ mV.

The amplitude of the K chord conductance G_K at a given $(V-V_K)$ decreased with decreasing temperature. The calculated G_K values at $V-V_K = -30$ mV are plotted against the temperature on a logarithmic scale in Fig. 3. The best fit of the data seems to be two straight lines crossing at about 10 °C. The figures listed by each point indicate the order of measurement; there was no evidence of hysteresis.

The slope conductance of the $I_s - V$ relationship (Fig. 2) increased as the membrane potential became more negative and finally approached a saturation value. This saturation value was called 'limiting slope conductance, G_{lim}, (Hagiwara & Takahashi, 1974) and can be used as a measure of the maximum conductance during inward rectification. Note that the calculation of G_{lim} does not require knowledge of the value of V_K. The temperature dependence of G_{lim} is plotted in Fig. 3, based on the curves used to obtain G_K of the same Figure. The results indicate the Q_{10} calculated for G_K was identical to that calculated for G_{lim}. The temperature dependence of G_{lim} was measured in five other cells and similar results were obtained. The Q_{10} estimated from the slope of the linear relationship above 10 °C was $1·62 \pm 0·13$ (S.D., $n = 6$), whereas that obtained below 10 °C was $5·8 \pm 2·1$ (S.D., $n = 6$). The result suggests that a change occurs in the permeation mechanism at about 10 °C.

Effect of the external K concentration

The membrane currents were observed in the same cell at $[K^+]_o = 25$, 50, and 100 mM at 20 and 3·8 °C. The plot of the calculated values of G_K against V shows clearly that the activation of the K channel during inward rectification depended on $V-V_K$ at 20 °C as well as at 3·8 °C (Fig. 4). There was, however, a marked difference in the relationship between the amplitude of G_K and $[K^+]_o$ at these two temperatures. A log-log plot of the normalized values of G_K against $[K^+]_o$ at $V-V_K = -20$, -30 and -40 mV is given in Fig. 5*A*. At 20 °C, the slope was one half, and so the amplitude of G_K at a given $V-V_K$ was roughly proportional to the square root of $[K^+]_o$ at 20 °C. This result agrees with previous findings obtained in a similar temperature range (Miyazaki, Takahashi, Tsuda & Yoshii, 1974; Hagiwara & Takahashi, 1974; Miyazaki *et al.* 1975; Ciani *et al.* 1978). At 3·8 °C, however, the slope was less than one third, and so G_K at a given $V-V_K$ became substantially less dependent on

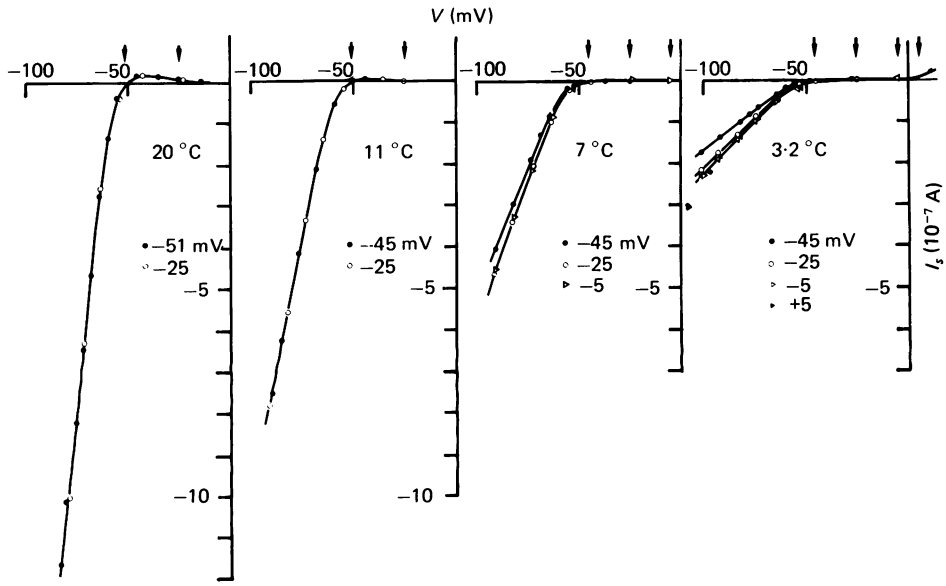

Fig. 2. Relationship between the steady-state current, I_s, and the membrane potential V of the same cell obtained at different temperatures. External solution, 25 mM-K (solution A). Different symbols refer to different holding potentials. Diameter of the cell, 900 μm.

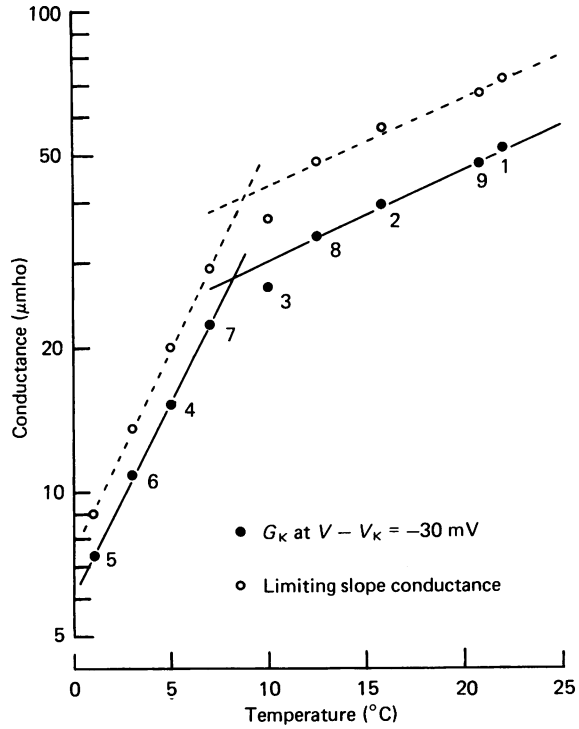

Fig. 3. The temperature dependence of the K conductance during the inward rectification of the starfish egg membrane. Filled circles, K chord conductance at $V - V_K = -30$ mV; open circles, limiting slope conductance. Figures to each point indicate the order of the experiment, showing no evidence of hysteresis. The external solution was 25 mM-K (solution A). Diameter of the cell, 900 μm.

$[K^+]_o$. The reduced dependence was not due to the leakage conductance. Judging from the amplitude of I_s* (see eqn. (2)), the leakage conductance was smaller than 1 μmho, and this was negligible compared with G_K at any value of $V-V_K$. The limiting slope conductance was measured in the same cell for each condition. A plot of the K dependence of G_{lim} normalized to its value at 20 °C and at $[K^+]_o$ = 25 mM

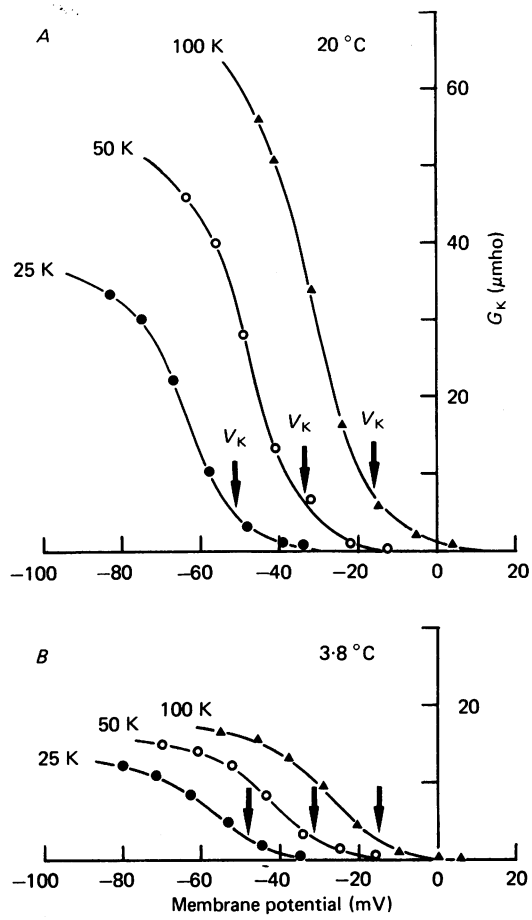

Fig. 4. Relationship between the steady-state K conductance and the membrane potential of the same cell obtained at $[K^+]_o$ = 25, 50 and 100 mM at 20 and 3·8 °C. V_K with an arrow indicates the K equilibrium potential. Those at 3·8 °C were calculated from those at 20 °C and eqn. (1). Solution A and diameter, 900 μm.

of the same cell is shown in Fig. 5*A*. The results indicate that the effects of temperature on G_{lim} and G_K are similar. The dependence of G_{lim} on $[K^+]_o$ as a function of the temperature examined in several other cells is summarised in Fig. 5*B*. The conductance was roughly proportional to the square root of $[K^+]_o$ at both 20 and 10 °C. At 3–4 °C, however, the conductance became substantially less dependent on $[K^+]_o$. In the present experiments no detailed examinations were performed for the temperature range between 4 and 10 °C, and the concentration range was limited between 25 and 100 mM. The results, nevertheless, suggest that the transition of the tempera-

ture dependence for G_K at about 10 °C may have some relation to the change in the dependence of G_K upon the external K concentration. The above results imply that Q_{10} of the conductance is independent of $[K^+]_o$ above about 10 °C whereas Q_{10} is likely to be a function of $[K^+]_o$ below 10 °C, i.e. it tends to increase as $[K^+]_o$ increases.

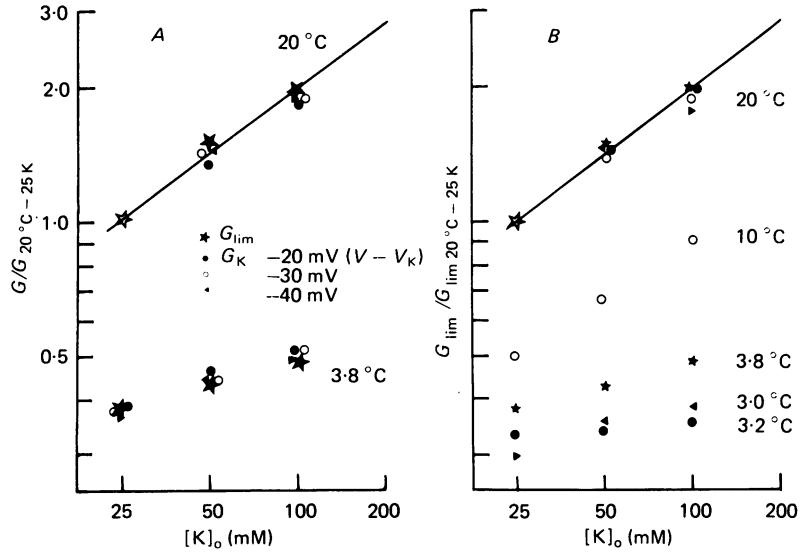

Fig. 5. *A*, steady-state K conductances at $V - V_K = -20$, -30 and -40 mV, and the limiting slope conductance of the steady-state current–voltage relation are plotted against $[K^+]_o$. Data were normalised by dividing by the conductance value at 20 °C and $[K^+]_o$ = 25 mM. All data were obtained from the same cell. Solution A. *B*, limiting slope conductances are plotted against $[K^+]_o$. The same symbols refer to the same cell. The data obtained from each cell were normalized by dividing by the conductance at 20 °C and $[K^+]_o$ = 25 mM. External solutions: solution A, filled stars and triangles; solution B, open and filled circles.

Effect on the kinetics of the time-dependent component

The time-dependent current has been found to follow an exponential time course and the time constant, τ, is a function of $V - V_K$ when the internal K concentration is constant (Hagiwara *et al.* 1976). It has also been shown that log τ is linearly related to V. At room temperature (20 ~ 22 °C), a ten-fold decrease in τ is found for a hyperpolarisation of 45 ~ 55 mV. This is confirmed in the present work (55 mV at 22 °C in Fig. 6*B*). The effects of temperature on the voltage dependence of the time constant are shown in Fig. 6*A*. The linear relationship between the membrane potential and log τ was maintained when the temperature was lowered to 1 °C. However, the slope increased as the temperature was reduced. The amplitude of hyperpolarization necessary to produce a tenfold decrease (it is expressed as $dV/d \log \tau$ and so is the reciprocal of the slope) was calculated and plotted against the temperature in Fig. 6*B*. The results obtained from two different cells show a good agreement. The parameter decreased rapidly with decreasing temperature down to about 10 °C; for lower temperatures, the slope was about independent of the temperature. If one assumes that the time constant, τ for the range of the membrane potential, V more

negative than V_K, follows the equation

$$\tau = A \exp(\alpha FV/RT), \tag{3}$$

where A and α are constants and F, R and T have their usual meaning, logarithm of τ is a linear function of V as in the present experiment. Since eqn. (3) includes T, the slope should change with temperature. However, the expected temperature

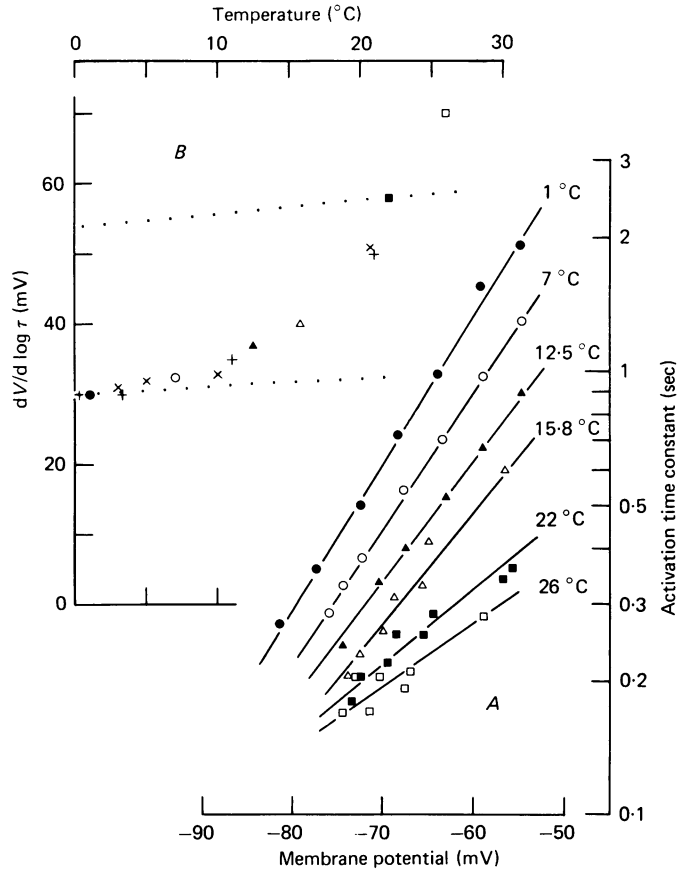

Fig. 6. *A*, relationship between the activation time constant, τ, and the membrane potential obtained from the same cell at different temperatures. *B*, the dependence of τ upon the membrane potential is expressed by the membrane potential necessary to alter τ tenfold (this is expressed as $dV/d \log \tau$), and this parameter is plotted against the temperature. Dotted lines show the slope expected from eqn. (3). The upper and lower lines are calculated so that the line passes the data point at 22 and 0 °C, respectively. All points except those illustrated by + were obtained from the same cell as *A*. Data obtained with two different cells show a good agreement. External solution, 25 mM-K (solution A).

effect is relatively small. The two dotted lines in Fig. 6*B* were drawn according to eqn. (3) by assuming that α is independent of temperature. In fact, α appears to be temperature independent for temperatures below about 10 °C. However, α was clearly temperature-dependent above about 10 °C. This result also suggests that the mechanism of ion permeation changes at about 10 °C.

DISCUSSION

The K conductance during the anomalous or inward rectification of the starfish egg as measured either by the K conductance at a given $V-V_K$ or by the limiting slope conductance shows a relatively small Q_{10} (1·6) above about 10 °C, and this Q_{10} is independent of the external K concentration. Similar values of Q_{10} have been found for the K conductance of the anomalous rectification in other preparations. Almers (1971) obtained a Q_{10} of 1·65 in frog skeletal muscle fibre between 3·5 and 17·5 °C. Similar values of Q_{10} have been found for the Na conductance and K conductance for the delayed rectification (Hodgkin, Huxley & Katz, 1952; Moore, 1958; Frankenhaeuser & Moore, 1963; Wang, Narahashi & Scuka, 1972; Schauf, 1973). In the tunicate egg, Ohmori (1978) obtained a Q_{10} of 1·44 between 5 and 20 °C. He found an almost identical Q_{10} for the conductance of the single channel as estimated by the current noise analysis. This implies that the number of open channels at a given $V-V_K$ is relatively independent of the temperature. This may also apply to the K conductance of starfish eggs above 10 °C, as well as to that of frog skeletal muscle fibre. A Q_{10} of 1·4 $\sim$ 1·6 lies in the range of Q_{10} values found for conduction in simple electrolyte solutions.

In the starfish egg, the Q_{10} for the K conductance during the anomalous rectification below about 10 °C is substantially larger than at higher temperatures. At $[K^+]_o$ = 25 mM it is 5·8 ± 2·1 (S.D., n = 6), which is much greater than that of the conductance of electrolyte solutions. A similar discrete change of the temperature dependence of membrane properties has been a widespread phenomenon in biological and artificial membranes. Examples are the single channel conductance associated with the glutamate receptor at locust nerve muscle junction (Anderson, Cull-Candy & Miledi, 1978), with the acetylcholine receptor of cultured chick myoballs (Fischbach & Lass, 1978), the rate constants of the asymmetrical charge movement in squid axons (Kimura & Meves, 1979), the kinetic parameters and conductance of the Na channel in the Ranvier node and muscle fibre (Chiu, Mrose & Ritchie, 1979; Schwarz, 1979) and ion carrier or channel-induced conductances in lipid bilayers (Krasne, Eisenman & Szabo, 1971). In the present case, the transition temperature is about 10 °C. Q_{10} changes from 1·6 to 5·8 at $[K^+]_o$ = 25 mM. The change roughly corresponds to a shift of the activation energy from about 6 kcal/mole to 26 kcal/mole. Since the present experiment deals with the macroscopic conductance, the large temperature dependence could be an effect either on the number of open channels or on the single channel conductance. As mentioned already, at 10–22 °C the K conductance of the anomalous rectification is roughly proportional to the square root of the external K concentration when the internal K concentration is constant and this is the property of the single channel (Ohmori, 1978). The fact that the K conductance becomes less dependent on $[K^+]_o$ for the range of temperature where a large Q_{10} is found suggests that the properties of the single channel may change. The K channel of the anomalous rectification is likely to have multiple sites with multiple ion occupancy (Hille & Schwarz, 1978). The conductance-concentration relation in such a model may show multiple maxima and even self-blockage. The large Q_{10} below 10 °C may be explained by considering that the ion-site interaction changes in such a way that the conductance becomes less and less dependent on $[K^+]_o$ as the tempera-

ture is reduced below 10 °C. The single channel conductance decreases not only due to the simple effect of reduced temperature (this may be common for temperatures above 10 °C) but also due to the reduced dependence of the conductance on $[K^+]_o$. Thus the value of Q_{10} tends to become greater as $[K^+]_o$ is increased. In order to test this idea, the temperature dependence of the K conductance should be examined at very low K concentration, such as 1 mM. With the present method this was not possible because of the significant leakage conductance.

The normal environmental temperature as well as the temperature of the sea water storage tank for *Mediaster aequalis* was about 15 °C. At this temperature the Q_{10} is still small. In eggs of the tropical Australian starfish, *Nordora punctiformis*, the Q_{10} of the K conductance for the temperature range below 20 °C was found to be 5–6 (Hagiwara & Takahashi, unpublished observation). The environmental temperature was 27 °C where Q_{10} was small. The above comparison suggests (*a*) that the change of the permeation mechanism may relate to the lipid composition of the membrane, and (*b*) that the K conductance of the anomalous rectification decreases drastically when the external temperature becomes 5–10 °C lower than the environmental temperature. Thus, for a further temperature decrease, the ion permeability of the egg cell membrane will rapidly decrease. It is not easy, however, to point out its biological significance.

The gating of the anomalous rectification depends on the membrane potential and the external K concentration regardless of the external temperature. The development of the time-dependent current is expressed by first order kinetics. The time constant, τ, of activation increases as the temperature decreases. At any given temperature, log τ decreases linearly as $V - V_K$ becomes more negative (Hagiwara *et al.* 1976). The most remarkable feature is that the slope of log $\tau - (V - V_K)$ relation increases as the temperature decreases. The change is significantly greater than that expected from Boltzmann relationship, at least for the range of temperature where Q_{10} of G_K is small. In this range, the Q_{10} of the activation time constant is membrane potential-dependent. This feature is different from the temperature effect on the activation kinetics of the Na conductance in *Myxicola* axon (Schauf, 1973) as well as in tunicate egg (Okamoto, Takahashi & Yoshii, 1976). In the latter, the Q_{10} of the activation time constant is practically independent of the membrane potential. This suggests that the mechanism of activation of the inward rectifier channel may differ from that of the Na channel even at room temperature.

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