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Involvement of Photosynthesis in the Action of Temperature on Plasmalemma Transport in *Nitella*

Susanne Stein and Ulf-Peter Hansen
Institute of Applied Physics, New University, D-2300 Kiel, Federal Republic of Germany

Summary. At membrane potentials different from E_K, the temperature effect on membrane potential of *Nitella* consists of two components. One of them changes its sign at E_K, the other one does not. This leads to the assignment of these components to changes in the K^+ channel and in the H^+ pump, respectively. It is shown that the fast time constant (3 to 30 sec) of the temperature effect on the H^+ pump measured as a change in membrane potential and that of the temperature effect on the K^+ channel measured as a change in resistance (having about twice the value of that of the pump) are sensitive to light intensity. Both time constants measured in *Nitella* become smaller if light intensity increases from 0 to 15 Wm^{-2}. This supports the suggestion of Fisahn and Hansen (*J. Exp. Bot.* **37**:440–460, 1986) that temperature acts on plasmalemma transport via photosynthesis via the same mechanism as light does.

Key Words H^+ pump · K^+ channel · light · *Nitella* · temperature

Introduction

Different authors investigated the effect of temperature on the membrane potential of plant cells, and found different values of the temperature coefficient. 0.5 mV/°C was obtained by Nelles and Laske (1982) in corn coleoptiles, whereas other authors reported higher values in *Nitella:* Hogg, Williams and Johnston (1968): 1.6 mV/°C; Blatt (1974): 2.5 mV/°C; Spanswick (1972): 2.5 mV/°C. The value of 0.5 mV/°C is obtained from the factor RT/F (Fisahn & Hansen, 1986) which is found in all theories of membrane transport. The occurrence of this value indicates that the effect of temperature is related to a simple mechanism. Higher values of the temperature coefficient suggest that more sophisticated mechanisms are involved, which are supposed to be of biochemical origin. If biochemical reactions are involved, slow time constants reflecting the rate constants of these reactions are expected to occur resulting in different responses to slow and rapid changes in temperature as observed by Hope and Aschberger (1970) and Nelles and Laske (1982).

Thus, Fisahn and Hansen (1986) have studied the kinetic behavior of the temperature-induced changes in membrane potential of *Nitella*. They came to the following conclusions: temperature influences the electrogenic H^+ pump and the K^+ channel. In cells in the K^+ state (Beilby, 1985), in which membrane potential is close to E_K (Nernst potential of K^+), the temperature effect on the K^+ channel influences mainly membrane resistance R, and the effect on the H^+ pump influences mainly membrane potential V. Thus, a separation of these two effects can be achieved easily in these cells.

The kinetic studies of Fisahn and Hansen (1986) revealed that the temperature effect on membrane potential (H^+ pump) shows the same time constants as the effect of light on membrane potential (Hansen, 1978, 1980, 1985) does. The temperature effect on membrane resistance displayed simple kinetics with one dominating time constant (about 40 sec) different from those of the effect on potential. The mechanism of the temperature effect on the K^+ channel was studied by a current-voltage curve analysis (Hansen & Fisahn, 1987).

In the meantime the reaction related to the time constant of the so-called depolarizing pathway (which depolarizes membrane potential upon illumination) of the light action on membrane potential could be identified. Hansen, Kolbowski and Dau (1987) found the following sequence of events: light increases the uptake of protons into the inner thylakoid space. This leads to a depletion of protons in the outer space which is replenished by protons from the cytoplasm. The resulting alkalinization observed by Felle and Bertl (1986) in *Riccia* and by Steigner et al. (1987) in *Eremosphaera* is supposed to slow down the electrogenic H^+ pump via a substrate effect (Sanders, Hansen & Slayman, 1981; Sanders, Ballarin-Denti & Slayman, 1984), thus es-

tablishing the depolarizing pathway of light action (Hansen, 1985). On the other hand, Köhler et al. (1986) suggested that the acidification induced by darkening opens the K^+ channel in *Eremosphaera*.

Because of the above results, the time constant of the light action of the depolarizing pathway is of special interest. Its investigation is concerned with the following questions:

1. Does temperature act via the same pathway as light does in the case of the fast depolarizing pathway?

2. Does this pathway act on the H^+ electrogenic pump or on the K^+ channel?

These two questions seemed to be answered by the analysis of Fisahn and Hansen (1986) mentioned above. However, the statements were based on the numerical coincidence (question 1) and noncoincidence (question 2) of the time constants obtained from curve fitting of the kinetic data. The numerical determination of critical parameters by curve-fitting is more or less reliable. Thus, support from other approaches is welcome.

In the case of question 1, the findings of Fisahn and Hansen (1986) predict that the time constants of the temperature action should be light-sensitive like those of the light effect according to the assumed involvement of photosynthesis (Hansen & Wittmaack, 1980; Kolbowski, Keunecke & Hansen, 1984).

In the case of question 2, it has to be shown that the time constants of the temperature effect on potential and on resistance are not identical. Fisahn and Hansen (1986) claim to have shown that they are different. Here, the dependence of the kinetic behavior on membrane potential is used as an additional means of showing the involvement of both components.

Materials and Methods

The experimental setup is described by Fisahn and Hansen (1986). Briefly, the most important points are given here:

Nitella flexilis was purchased from R. Kiel in Frankfurt and kept in APW (artificial pond water containing 0.1 mol m^{-3} KCl, 1.0 mol m^{-3} NaCl, 0.5 mol m^{-3} $CaCl_2$, no buffer) In the refrigerator at 10°C at a light intensity of 5 Wm^{-2} (16 hr/day). pH was adjusted to pH 7 by small amounts of HCl, when the pH-meter showed an increase of 0.5 pH units. In the experiments, APW was used if not otherwise mentioned.

The cells in our laboratory showed the peculiarity of low resting potentials of about −120 mV (so-called null-state, Beilby 1985). As mentioned in the previous paper (Fisahn & Hansen, 1986) these low potentials were the key for the success of our kinetic analysis, because at this potential opening and closing of the channel induced by temperature does not interfere with the effect of the electrogenic pump, as the changes of the activity of the channel influences mainly the slope resistance and those of the pump, mainly the potential. Consequent upon this, the complexity of the signals was markedly reduced.

The experimental setup for the modulation of the temperature and for the recording of the electrical parameters of *Nitella* is shown in Fig. 1 of Fisahn and Hansen (1986). The APW flowed first from a 10-l storage vessel to a heat exchanger fed by a Colora cooler, thus obtaining a temperature of 2°C, then through a quartz tube wrapped by a Konstantan-wire acting as an electric heater. The temperature was raised to about 15°C. The electric current through the heater was taken from a control loop which set the temperature at the outlet of the tube to the desired value sensed by a thermistor. This sensor had a time constant of 6 sec. The resulting attenuation of the feedback sensor led to an increase of the temperature modulation at high frequencies. This increase was compensated in the case of the resistance measurements by the time constant of the phase-locked rectifier. In the case of the potential measurements this effect of the slow sensor had to be corrected. The corrections could be tested by the data from a second thermistor measuring temperature in the bathing medium of the cell. However, also in this case, the time constant of the thermistor (2 sec) had to be taken into account. Its signal was used as a reference in the kinetic studies in order to correct for the delay between the heater and the cell. The induced changes in temperature could be described by the following equation:

$$T(t) = T_o + T_v \sin(2\pi ft) \quad (1)$$

with T being temperature, t = time, T_o = mean value of temperature, T_v = amplitude of temperature modulation, f = frequency of the sinusoidal modulation ranging from 0.2 Hz to 1 cycle/1 hr.

Membrane potential was recorded by an inserted 1 k mol m^{-3} KCl microelectrode. The signal from the microelectrode and from the thermistor was fed into a data acquisition system taking 720 samples during one period of a sine wave. Resistance measurements were done by the injection of a sinusoidal current of 1 Hz into short cells (length 1.0 cm, diameter 0.2 to 0.25 mm) via a second microelectrode in series with a 50-MΩ or 5-MΩ resistor (depending on whether APW or 3 mol m^{-3} KCl was used as bathing medium) connected to a high-voltage amplifier (Burr Brown 3584JM) which could provide +/− 100 V. This setup acted as a current source as could be seen from the fact that the voltage at the output of the amplifier was more than 100 times greater than membrane potential. The 1-Hz signal recorded by the voltage electrode was detected by a phase-sensitive rectifier with a low-pass filter (6 sec). This signal could also be fed into the data acquisition system.

The procedure of measuring frequency responses is described by Fisahn and Hansen (1986). The sinusoidal changes in temperature (ranging from 0.2 Hz to 1 cycle/1 hr as described by Eq. 1) induced sinusoidal changes in membrane potential and in resistance if the amplitude is restricted to 1°C (linear range). Two quantities were recorded for each frequency: amplitude of the output signal (V, R) and phase-shift φ (temporal difference between input sine wave and output sinewave). The data had to be corrected, because the temperature signal increased at high frequencies because of the slow time constant of the sensor of the control loop as mentioned above.

Amplitude and phase were plotted versus the related frequency in a so-called Bode plot, as shown in Fig. 2 and in Fig. 4. The data in the Bode plots were subject to curve-fitting.

The data acquisition system consisted of a TMS-9900 microcomputer which generated the sine wave, controlled the data acquisition, eliminated the drift by the subtraction of a least-

squares-fitted parabola and calculated the Fourier coefficients of the responses to the sine waves. The TMS 9900 handed the Fourier coefficients over to a Z80-CPM system, which calculated phase and amplitude and displayed the Bode plots. For curve-fitting, the data were transferred to the PDP-10 of the Kieler Rechenzentrum.

The cells were illuminated by halogen bulbs (Osram Xenophot HLX, 150 W) resulting in the intensities specified in the text.

Results

Purpose of Kinetic Studies

In an intact cell there is the general problem of identifying individual components included in a usually complex response of the cell to a stimulus. Many workers use so-called specific inhibitors in order to simplify the system by selective inhibition of individual components, e.g. the electrogenic H^+ pump. One problem of this method is to find selective inhibitors which attack only one process, and the other problem is that, especially in long-term experiments, the cell itself may activate compensatory mechanisms which take over the function of the selectively inhibited process. Such a process is the replacement of cyanide-sensitive oxydation in wild-type *Neurospora* by the alternate cyanide-insensitive oxidation (Slayman, 1980), or the induction (new synthesis) of cotransport molecules in the plasma membrane of starved cells (Slayman, Slayman & Hansen, 1977).

In order to circumvent these problems we tried to work on intact cells, and to achieve the identification of components with the aid of mathematical tools. We found that the time constants evaluated by curve-fitting from the responses to a stimulus can be used as labels for the involved reactions. In this study we show that time constants can be used to distinguish the component related to the temperature action on the K^+ channel from that related to the H^+ channel.

Responses to Temperature Changes: Involvement of the K^+ Channel

The first set of experiments showed the involvement of the K^+ channel in the temperature effect on membrane potential. The experiments were performed in a bathing medium of 3 mol m^{-3} KCl. At this concentration the estimation of E_K is more reliable, because a screening effect of the cell wall (Dainty, Hope & Denby, 1960) can be ignored in contrast to experiments at 0.1 mol m^{-3} K^+ (Köhler et al., 1985; Fisahn, Mikschl & Hansen, 1986).

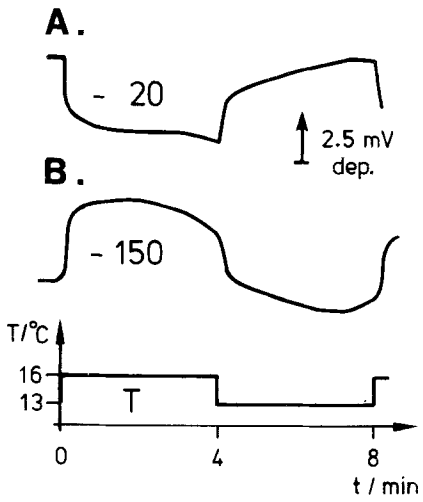

Fig. 1. Responses of membrane potential as induced by changes in temperature between 13 and 16°C measured at different membrane potentials, which are in (*A*) −20 mV, and in (*B*) −150 mV. The experiments were performed in 3 mol m^{-3} KCl resulting in a resting potential of −90 mV. The membrane potential was set to the given values by the injection of a dc current (9 and 7 μA, respectively) via a second microelectrode. Light intensity: 5 Wm^{-2}

Figure 1 shows the response of membrane potential to stepwise changes in temperature between 13 and 16°C. The record of Fig. 1(*A*) was taken at a membrane potential of −20 mV, that of Fig. 1(*B*) at a membrane potential of −150 mV. As the resting potential (being close to E_K) was −90 mV in this cell, the membrane potential was set to the values given in Fig. 1 by means of an adequate dc current injected via the microelectrode which was also used for the injection of the 1 Hz current for the resistance measurements described below.

The responses in Fig. 1 indicate the involvement of the K^+ channel. Opening of the channel with an increase of temperature (Hansen & Fisahn, 1987) pulls the membrane potential closer to E_K. This results in a hyperpolarization at −20 mV (Fig. 1*A*) and in a depolarization at −150 mV (Fig. 1*B*). This Figure corresponds to Fig. 8 in Fisahn and Hansen (1986).

The question is whether besides the K^+ channel the H^+ pump is involved or not. If the pump is involved, at least two components should be detectable in the records of Fig. 1. The time course of the curves in Fig. 1 is complex, but the obvious complexity is related to slow components which are not investigated here. An analysis including the slow components is given by Fisahn and Hansen (1986). These slow components are related to a feedback controller of cytoplasmic pH (Fisahn et al., 1986; Fisahn & Hansen, 1986), and to a light-induced

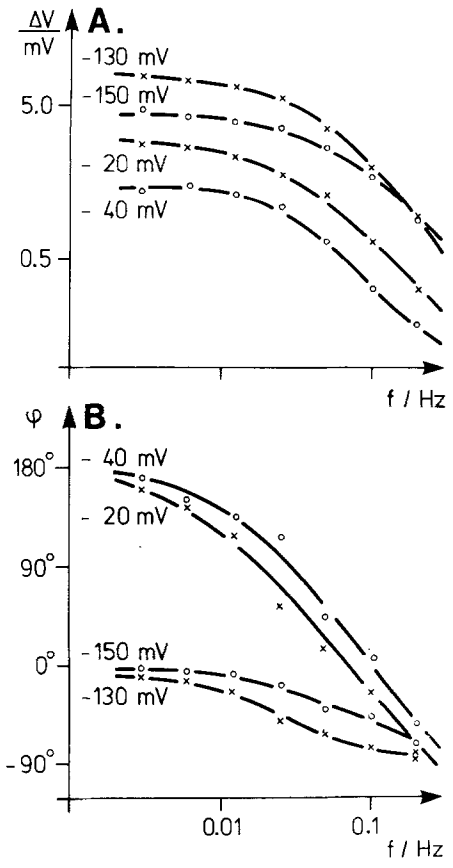

Fig. 2. Frequency responses of the temperature effect on membrane potential (*A*, amplitude, *B*, phase shift) measured at different imposed membrane potentials. The values of the membrane potentials (MP imposed by the currents *I* given below) are attached to the curves. The smooth lines present the fits calculated on the basis of Eq. (7). The fitted parameters are

MP/mV	I/μA	$V_\#$/mV	n/sec	τ_4/sec	τ_r/sec	c_4/mV	c_r/mV
−20	5.0	2.6	−2.5	4	5	4.0	−5.3
−40	4.3	1.6	−1.5	3	5	4.0	−6.4
−130	−4.5	5.8	(14.8)	6	(14)	3.6	0.3
−150	−6.5	5.2	2.0	1	4	3.5	3.7

Different cells. Temperature modulated sinusoidally between 14.5 and 15.5°C, light intensity: 5 Wm^{-2}, solution: 3 mol m^{-3} KCl

stimulation of the H^+ pump which caused the hyperpolarization observed upon illumination (Blatt, 1974; Beilby & Coster, 1976; Kami-ike et al., 1986).

For answering the above question for the two fast components, the kinetic analysis has to be based on a method which provides a better resolution: the measurements of frequency responses.

Detection of the Two Time Constants τ_4 and τ_r in the Temperature-Induced Changes of Membrane Potential

Figure 2 shows frequency responses measured at different membrane potentials by the procedure described in Materials and Methods. The experimental conditions are similar to those in the experiments of Fig. 1. E_K was reduced to *ca.* 90 mV by using a bathing medium of 3 mol m^{-3} KCl. The membrane potential was set to the desired value by the injection of a dc current.

The most striking feature in Fig. 2 is the difference in the frequency responses of the phase (Fig. 2*B*) obtained at hyperpolarizing (−130, −150 mV) and at depolarizing (−20, −40 mV) potentials. The difference of 180° at low frequencies corresponds to the inversion of the temperature-induced changes shown in Fig. 1. The phase $\varphi = 0$ is defined to be related to depolarization upon an increase of temperature; $\varphi = 180°$ means hyperpolarization upon an increase in temperature.

Figure 2(*B*) demonstrates the improved resolution of the frequency response analysis over that in Fig. 1: The high-frequency part of Fig. 2 corresponds to the early step response in Fig. 1, the low-frequency part to the late step response. Figure 2(*B*) shows equal phase shifts at high frequencies (0.2 Hz) for all four membrane potentials. This implies that also the step responses (Fig. 1) of the depolarized cells should start with a depolarization upon an increase in temperature. However, the related amplitude (at 0.1 Hz in Fig. 2*A*) is small. Thus, this very small depolarization is partly overridden by the subsequent dominant hyperpolarization related to the positive phases in Fig. 2(*B*), and partly swallowed by the chart recorder. The only indication of this first depolarization is the edge at the beginning of the responses in Fig. 1(*A*) which is sharper than that in the responses of Fig. 1(*B*).

The problem of identifying this edge demonstrates the higher resolution of the analysis in the frequency domain: Theoretically, the same information is provided by the step responses in Fig. 1 and in the frequency responses of Fig. 2. However, nobody would dare to conclude the existence of two components from the edge in Fig. 1(*A*), whereas this conclusion is obvious in Fig. 2(*B*).

This and the following conclusions are based on general laws of network theory. We make use of the statement that the frequency response of a single reaction displays a slope decaying with $1/f$, and that the change of the (negative) phase shift with increasing frequency cannot exceed 90° (Hansen, 1985). Thus, if the temperature effect would comprise only one time constant, the frequency-dependent change in the phase in Fig. 2(*B*) with temperature must not exceed 90°. The overall change of 270° measured with increasing frequency in the depolarized cells indicates that there are at least two components involved. In the case of the records from the hyperpolarized cells the indication of two components is weaker. According to the statement men-

tioned above we can get this information from the flater-than-$1/f$ high-frequency slope of the amplitude of the -150-mV response (Fig. 2*A*).

More reliable is an evaluation based on curve fitting. The two components of the responses of membrane potential found at membrane potentials different from E_K occur separately in membrane potential and in membrane resistance if membrane potential is equal to E_K. We know from previous investigations (Fisahn & Hansen, 1986) that the following transfer function (ratio of amplitude of the output signal/amplitude of the input signal) has to be used for curve-fitting of the temperature effect on membrane potential (if membrane potential = E_K)

$$V(p) = \frac{V_=(1 + pn_1)(1 + pn_2)(1 + pn_3)(1 + pn_4)}{(1 + p\tau_1(1 + p\tau_2)(1 + p\tau_3)(1 + p\tau_4)} \tag{2a}$$

(with n_i being called the inverse zeros and τ_i being the time constants) and on membrane resistance

$$R(p) = \frac{R_=}{(1 + p\tau_r)} \tag{2b}$$

with p including the frequency f

$$p = 2\pi\sqrt{-1}f. \tag{3}$$

However, in the analysis of Fig. 2 we are interested only in the high-frequency time constant. Thus, a simplified version of Eq. (2a) which is restricted to the high-frequency range is used for fitting potential. This version includes only τ_4 of Eq. (2a).

$$V(p) = \frac{V_=}{(1 + p\tau_4)}. \tag{4}$$

An example of such a fit is given by the smooth lines in Fig. 4. It has to be kept in mind that

$$V(p) = V_o \exp(\sqrt{-1}\varphi) \tag{5}$$

is a complex function providing both amplitude (V, R in Figs. 2*A*, 4*A*,*C*) and phase φ (Figs. 2*B*, 4*B*,*D*). The same holds for $R(p)$. The index "=" indicates the "dc amplification" (steady-state value of this component).

Equations (2b) and (4) hold for the temperature-induced changes measured at a membrane potential which is equal to E_K as mentioned above. In the experiments of Fig. 2, this premise does not hold. The effect of the K^+ channel on membrane potential is clearly indicated by Figs. 1 and 2. Consequent upon this, both time constants τ_4 and τ_r are expected to show up in the temperature action on membrane potential. Thus, the effect of temperature on membrane potential is a superposition of the two transfer functions related to the H^+ pump (Eq. 4) and to the K^+ channel. The parallel action implies that the two transfer functions (Eq. 4 and Eq. 2b) are added.

$$V(p) = \frac{c_4}{(1 + p\tau_4)} + \frac{c_r}{(1 + p\tau_r)}. \tag{6}$$

The employed curve-fitting routine delivers the evaluated transfer function in a different, but equivalent form

$$V(p) = \frac{V_\#(1 + pn)}{(1 + p\tau_4)(1 + p\tau_r)} \tag{7}$$

The mathematical conversion of Eq. (6) to Eq. (7) results in the following identities:

$$n = \frac{c_r\tau_4 + c_4\tau_r}{c_4 + c_r} \tag{8a}$$

$$V_\# = c_4 + c_r. \tag{8b}$$

As curve-fitting is based on Eq. (7), the parameters c_4 and c_r of Eq. (6) have to be calculated from an inversion of Eqs. (8a) and (8b).

c_4 and c_r are the "dc amplifications" of the temperature effects on the pump and on the channel, respectively. They are the steady-state values of the changes illustrated by the arrows in Fig. 6. They provide the parameters which give an adequate measure of the observed effects.

In the legend of Fig. 2 the values obtained from curve-fitting of the four displayed frequency responses are given. The value of $\tau_r = 14$ sec of the experiment at -130 mV deviates strongly from the other values. This is because the K^+ component is very small ($c_r = 0.3$), and thus the accuracy of the determination of this component is low. This is also seen from the value of the zero $n = 14.8$ sec which cancels the time constant $\tau_r = 14$ sec almost completely (Eq. 7). The value of 1 sec of τ_4 at -150 MV is a peculiarity of this individual cell, and of no statistical significance as seen from Fig. 3(*A*).

In Fig. 3 the results of fitting 20 frequency responses measured at five different membrane potentials are shown. The data display considerable scatter. This is no surprise as the long-lasting injection of the dc current for off-setting the membrane potential is a strong burden for the cell. However, the important features are clearly shown by Fig. 3.

The time constants τ_4 and τ_r do not depend on membrane potential as shown in Fig. 3(*A*). This is expected because of the location of the underlying

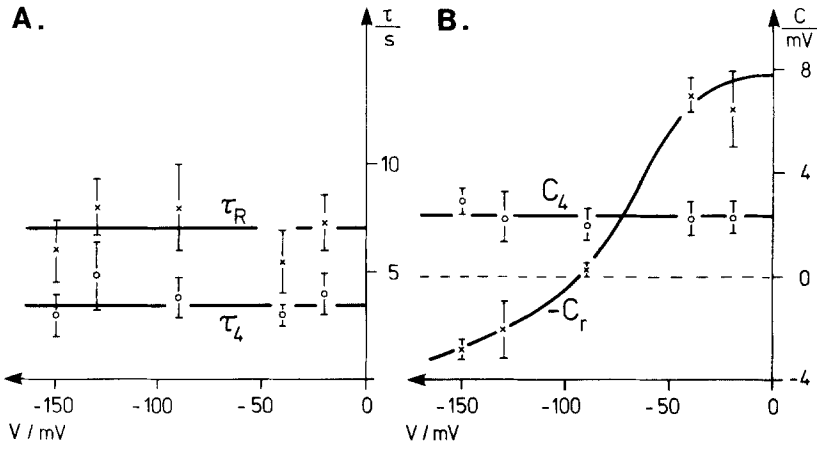

Fig. 3. Dependence of the parameters of Eq. (6) on membrane potential as obtained from curve-fitting of 20 frequency responses like those in Fig. 2. Experimental conditions as in Fig. 2. (*A*) Dependence of the time constants τ_4 and τ_r on membrane potential. (*B*) Dependence of the magnitudes of the temperature effects related to τ_4 (H^+ pump) and to τ_r (K^{K^+} channel) as given by c_4 and c_r (Eq. 6), respectively

processes in the chloroplasts as concluded below from Figs. 4 and 5. The average values are $\tau_4 = 3.5$ sec and $\tau_r = 7$ sec.

In Fig. 3(*B*), the averaged values of c_4 and of $-c_r$ are plotted versus membrane potential. The reason for plotting $-c_r$ is as follows. Firstly, the difference between the c_r curve and the c_4 curve gives the sum $c_4 + c_r$ (Eq. 7) which is a measure of the whole effect on membrane potential. Secondly, the curve of $-c_r$ resembles the I/V curve of the K^+ channel measured by Fisahn, Hansen and Gradmann (1986).

The resulting curves are very interesting: c_r (Eq. 6) changes its sign at E_K, the reversal potential of the potassium channel. This behavior is exactly what is expected for the temperature effect on the potassium channel (Fig. 6). At potentials more negative than E_K, opening of the channel with temperature leads to a depolarization. At potentials more positive than E_K opening induces a hyperpolarization.

c_4 does not change its sign. Also this finding verifies our expectations. At $pH_{cyt} = pH_{medium} = 7$ the reversal potential of the pump is probably −450 mV according to the energy of ATP hydrolysis of −450 meV. Thus, its reversal potential is far out of the range of applied potentials, and the pump acts as a current source in this range.

As mentioned above, the difference between the two curves in Fig. 3(*B*) gives the sum of the temperature effects on membrane potential, because $-c_r$ is plotted instead of c_r. From this difference the behavior shown in Fig. 1 is understood. In a hyperpolarized cell, both effects act in the same direction and a depolarization is induced by increasing temperature. In a depolarized cell a hyperpolarization is observed because the c_r effect has changed its sign and overrides the effect on the H^+ pump. The two effects compensate each other at a potential slightly more negative than E_K. This does not mean that no temperature-induced changes in membrane potential are observed, because the related time constants τ_r and τ_4 are different. Thus, a small wiggle should occur before the zero steady-state value is reached. However, also this steady-state value can be hidden by the subsequent slow processes omitted in the present investigation (Eq. 2 and Fisahn & Hansen, 1986). These slow processes are responsible for the hyperpolarization with increasing temperature observed by Blatt (1974), Beilby and Coster (1976) and Kami-ike et al. (1986).

Fig. 3(*B*) also verifies the premise of the measurements of Fisahn and Hansen (1986): At E_K the temperature effect on potential contains solely the effect on the H^+ pump (c_4), and the effect on the K^+ channel can be seen only in the changes of the resistance.

Dependence on Light Intensity

As mentioned above, the investigations of Fisahn and Hansen (1986) predicted that the time constant of the effect of temperature on the H^+ pump τ_4 should depend on light intensity. According to Fig. 3(*B*), τ_4 can be observed in membrane potential, and τ_r in resistance if the membrane potential is close to E_K. Due to the low pump activity in our cells (which seems to compensate the depolarization by nonpotassium leaks) membrane potential is close to E_K if no dc current is injected.

Frequency responses of the temperature action on membrane potential and on resistance were measured at different light intensities ranging from 0 to 15 Wm^{-2}. Examples are displayed in Figs. 4(*a*) to (*D*). The Figures show that the decay of the amplitude (Fig. 4*A* and *C*) and the shift of the phase (Fig. 4*B* and *D*) start at lower frequencies at an intensity of 2 Wm^{-2} than at 7 Wm^{-2}. This implies that the involved reactions are slower at low intensity than at high intensity, and are less successful in follow-

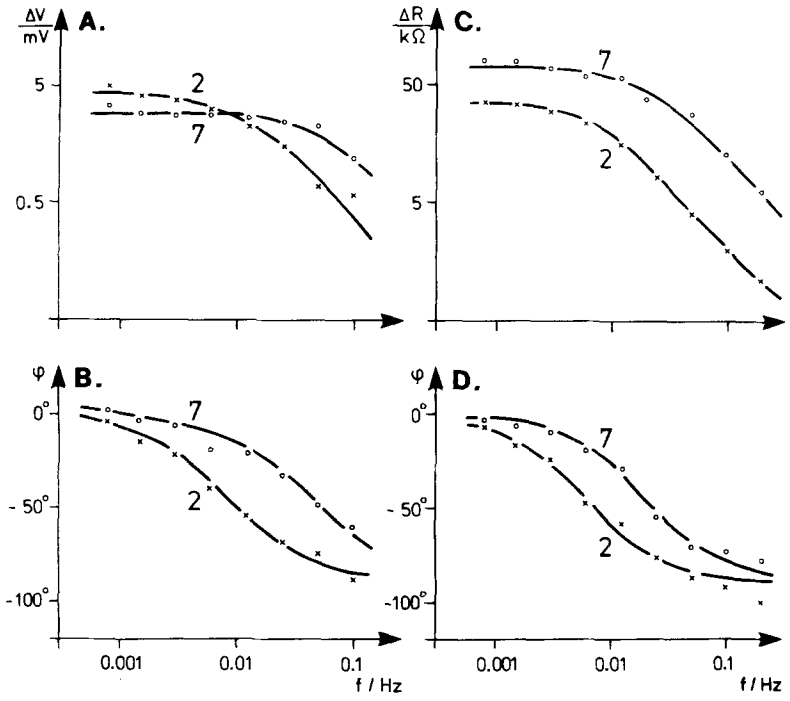

Fig. 4. Frequency responses of the action of temperature on membrane potential (*A*, *B*) and on membrane resistance (*C*, *D*) measured at two different light intensities (2 and 7 Wm^{-2} as given in the Figure). The upper traces show the frequency responses of the amplitude (*A*, *C*), the lower ones those of the phase shift (*B*, *D*). The smooth lines are computer fits on the basis of Eq. (4) (*A*, *B*) and of Eq. (2b) (*C*, *D*). The time constants are: *V*, 2 Wm^{-2}: 18 sec; *V*, 7 Wm^{-2}: 4 sec; *R*, 2 Wm^{-2}: 28 sec; *R*, 8 Wm^{-2}: 8 sec. The measurements were performed at resting potential = −120 mV in APW. Temperature changed sinusoidally between 14.5 and 15.5°C

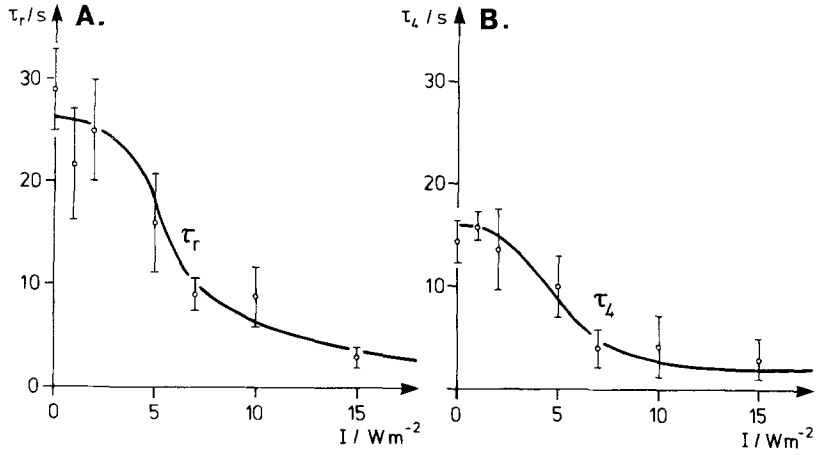

Fig. 5. Dependence of the fast time constants of the temperature effect on light intensity. (*A*) Temperature action on membrane resistance: τ_r. (*B*) Temperature action on membrane potential: τ_4. Experimental conditions as in Fig. 4

ing the imposed changes of the temperature at high frequencies. The exact description of this effect is based on the numerical evaluation of the time constants.

The time constants τ_4 and τ_r of the frequency responses of potential and of resistance, respectively, were determined by curve-fitting (Eqs. 4 and 2b) and plotted versus light intensity I in Figs. 5(*A*) and 5(*B*). These plots show that the time constants of the temperature effect on membrane potential and on resistance depend strongly on light intensity. The time constants get faster at high light intensities. The data also show a small decrease of the values at darkness. However, this decrease is not significant because of the scatter.

The related dc amplifications of Eqs. (4) and (2b), namely c_4 and c_r, are given in the Table. This Table shows that the dc amplifications do not show a significant dependence on light intensity in contrast to the time constants in Fig. 5.

Discussion

Effects on the H^+ Pump and on the K^+ Channel

The experiments in Figs. 2 and 3 show that the fast effects of temperature influences both the H^+ pump and the K^+ channel. The existence of the effect on

Table. Light-dependence of the dc amplifications of Eqs. (4) and (2b) obtained from the experiments of Figs. 4 and 5

Light intensity	0	1	2	5 (Wm^{-2})	7	10	15
c_4/mV	4.0	2.8	2.5	2.7	2.2	2.7	2.3
$scat_4$/mV[a]	1.2	0.5	0.3	0.5	0.4	0.6	0.3
c_r/kΩ	60	69	78	67	94	101	102
$scat_r$/kΩ	14	12	15	12	15	11	3

[a] Scat is the standard error. The average membrane resistance of the whole cell was *ca.* 800 kΩ.

the pump was revealed by the kinetic analysis based on frequency responses. The step responses of Fig. 1 did not provide sufficient resolution for the detection of the effect on the pump besides the obvious effect on the channel.

The results are summarized in the graphs of Fig. 6. This Figure gives an estimation of how the temperature-induced signal influences the H^+ pump and the K^+ channel. In this Figure, putative *I/V* curves are drawn for the H^+ pump and for the K^+ channel. The effects of temperature on the *I/V* curves are introduced according to the results of Hansen and Fisahn (1987).

These fast effects related to τ_4 and to τ_r are followed by the slow effects omitted in this study. According to Fisahn and Hansen (1986) these slow effects include the temperature-stimulated activation of the H^+ pump which leads to the hyperpolarization reported by Blatt (1974), Beilby and Coster (1976) and Kami-ike et al. (1986).

The changes in membrane potential induced by the temperature-induced changes in the currents are

$$c_r = R_M \Delta i_K \tag{9a}$$

$$c_4 = R_M \Delta i_H. \tag{9b}$$

The positive sign of c_4 in Fig. 3(*B*) implies that an increase in temperature results in a depolarization (change to positive potentials). Thus, pump activity decreases with temperature in Fig. 6. This is in line with the suggested mechanism of the light action on membrane potential suggested by Hansen et al. (1987) as discussed below. c_r has a positive sign in hyperpolarized cells, and a negative sign in depolarized cells. Thus, the activity of the K^+ channel in Fig. 6 increases at higher temperature.

In a hyperpolarized cell, both effects (arrows in Fig. 6, index *h*) point into the same direction. In depolarized cells (index *d* in Fig. 6) they are of opposite direction. In accordance with Fig. 3(*B*) also Fig. 6 shows that in cells with a resting potential

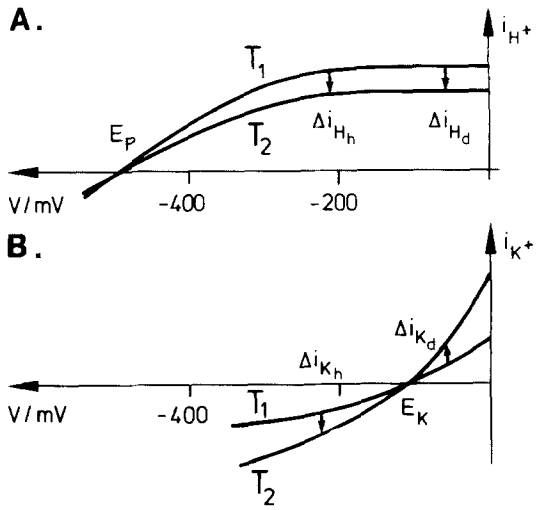

Fig. 6. Putative *I/V* curves of the H^+ pump (*A*) and of the K^+ channel (*B*) used to illustrate the effect of temperature on the individual components of the overall effect on membrane potential. T_2 is higher than T_1

close to E_K (Nernst potential of K^+) the temperature effects on the K^+ channel and on the H^+ pump occur very exclusively in resistance or in potential, respectively. The inherent separation of these effects at E_K led to the simple transfer functions of Eqs. (2b) and (4) and was the premise for curve-fitting the data in Fig. 4.

Light Dependence of the Temperature Effect

The prediction of the light dependence of the time constants of the temperature action on membrane potential (Fisahn & Hansen, 1986) and on membrane resistance is shown to be correct (Fig. 5*A,B*). This implies that the major effect of temperature on membrane transport does not act directly on the transport molecules of the plasma membrane, but primarily on a light-dependent process in the cell. The temperature-induced perturbation of this process leads to an influence on membrane transport obviously via those pathways which also mediate the effect of light on membrane potential. In the case of τ_4, Hansen et al. (1987) showed that this time constant is identical to a time constant observed in oxygen, evolution and in chlorophyll fluorescence. This time constant is assigned to the so-called energy quench related to the uptake of protons into the inner thylakoid space (Krause, Vernotte & Briantais, 1982; Schreiber, Schliwa & Bilger, 1986). From this finding, the chain of events is known as mentioned in the introduction: proton flux into the inner thylakoid space (Junge, 1975);

alkalinization in the outer space, alkalinization in the cytoplasm (Felle & Bertl 1986; Steigner et al., 1987); substrate effect on the H^+ pump (Hansen et al., 1987). The increase of photosynthetic activity leads to a decrease of pump activity in the temporal range given by τ_4. This decrease in activity is also observed if temperature stimulates photosynthetic activity (Fig. 3*B*, Fig. 6).

The mechanism mediating the temperature effect on the K^+ channel is still unknown. According to the dependence of τ_r on light intensity it has to be associated to a strongly light-dependent process. Recently, a higher resolution of the kinetics of chlorophyll fluorescence of leaves of spinach was obtained by using binary noise instead of sine waves for the modulation of the actinic light (Vanselow, Kolbowski & Hansen, 1988). We found a new time constant which had about twice the value of τ_4, which could also be observed in the light-induced changes in membrane potential (this holds for τ_r if membrane potential is different from E_K). The observed value is that which is expected if τ_r is located in the photosynthetic apparatus. Now, we start with simultaneous measurements of chlorophyll fluorescence, membrane potential and of membrane resistance in *Nitella* in order to come to a final statement regarding the association of τ_r to a process in the chloroplasts.

In addition, a messenger has to be found which is different from H^+, because this is already assigned to τ_4, mediating the effect on potential. Miller and Sanders (1987) have found a light-induced depletion of Ca^{2+} in the cytoplasm. This is in line with the light-induced uptake of Ca^{2+} into the chloroplasts as discussed by Heimann et al. (1987). However, there may be more still unknown candidates.

It is an open question whether the light effect on membrane resistance uses the same pathway via τ_r as the effect of temperature does, even though this is suggested by the results of Fig. 5(*B*). Unfortunately, the kinetics of the light effect on resistance were more complicated than those of the temperature effect (Keunecke, 1974). These frequency responses still resist to a meaningful analysis. However, there is no doubt that there are time constants of the light effect which can coincide with τ_r of the temperature effect.

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