Title: Temperature-dependent changes of membrane potentials in cells of thermonastic tepals of Eranthis hyemalis (L.) Salisb

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TEMPERATURE-DEPENDENT CHANGES OF MEMBRANE POTENTIALS IN CELLS OF THERMONASTIC TEPALS OF *ERANTHIS HYEMALIS* (L.) SALISB.

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ABSTRACT

The effect of temperature upon the bioelectric potential across the plasma membrane in cells of tepals of *Eranthis hyemalis* (L.) Salisb. (Winter aconite) is described. Rapid warming of an intact tepal resulted in a transient small increase in the magnitude of transmembrane potential difference followed by a substantial long-lasting depolarization which is considered as an "anomalous" response. Upon rapid cooling the reverse response occurred: a small transient depolarization was followed by a substantial hyperpolarization (also an anomalous response). The anomalous responses were more pronounced in the epidermis on the abaxial side of the tepal than in that on the adaxial side, indicating an electrophysiological dorsiventrality of the tepals. The anomalous responses were much less apparent in cells of isolated tissues than in cells of intact tepals. This difference does not appear to result from wounding or bringing a tissue into direct contact with the external solution because in segments of tepals devoid of the abaxial epidermis only, the PD of the parenchyma behaved in a way similar to that of the intact tepals. It is suggested that the occurrence of the anomalous responses is modulated by the tissue stresses. The functional importance of the responses for thermonastic movements is discussed.

KEY WORDS: *Eranthis hyemalis*, membrane potential, response to temperature change, thermonasty.

INTRODUCTION

The difference in the electric potential across a cell membrane (potential difference, PD) has two additive components: a passive component related to the electrochemical potential and an active one related to electrogenic ion transport. Both components play a role in the dependence of PD on temperature. The passive component allows a proportional effect of temperature (T) on the magnitude of PD, described by the factor RT/zF (where R is the gas constant, F is Faraday's constant, and z is the valency of the ions involved); PD increases with temperature by approximately 0.19 mV per degree centigrade for monovalent ions. This component is also involved when the temperature affects permeability coefficients and/or ion concentrations. The electrogenic component allows a strong temperature effect related to the association of ion transport (ion pumps) with the chemical reactions which have a high Q_{10} . There may also be variable effects of temperature on PD – an increase or a decrease of PD with temperature – related to such processes as light-activated electrogenic pumping (Spanswick 1972), cold-induced enhancement of ion-pump activity (Nelles and Laske 1982) or calcium-mediated temperature sensing (Minorsky and Spanswick 1989). The dependence of PD on temperature may thus be complicated and vary in different cells. Changes in PD well beyond those which would be expected if cells only reacted passively to temperature have already been observed by Umrath (1934). The changes due to rapid cooling have been reviewed extensively by Minorsky (1989). As far as we are aware, the effect of temperature on PD in plant cells has only been studied in characean cells, roots and coleoptiles that are not specialized to employ the temperature stimulation for a particular biological function. Cells participating in thermonastic movements in which a temperature change leads to a functional movement have not been investigated in this respect. However, we would expect that in just such cells the electrical responses to temperature changes may be illustrative of how the temporal changes and/or a spatial gradient of temperature are sensed to initiate or induce the response. A temporal change of temperature is effective in thermonasty (Crombie 1962), while a gradient is effective in thermotropism (Aletsee 1962; Fortin and Poff 1991). Since, as indicated by Fortin and Poff (1991), sensory physiology should be viewed as a network we expect that data pertaining to temperature sensing in thermonasty may also shed light on other movements, especially on the thermotropism of roots in which the faster growth occurs on

the cooler side of the reacting root. In the case of thermonastic flowers a lowering of temperature stimulates the growth rate on the outer side of the floral organs, which is also a peculiar response.

A very clear thermonastic behavior can be observed in *Eranthis hyemalis*. We chose this species because test measurements had indicated an interesting dependence of the PD on temperature in cells of its tepals. Nothing is known about the electrophysiology of *Eranthis* or about the electrophysiology of thermonastic flowers. A short communication on the results of our studies has been published (Hejnowicz et al. 1995).

MATERIALS AND METHODS

Flowering shoots of *Eranthis hyemalis* (L.) Salisb. (approx. 5-cm-long stem ending in the flower with green hypsophylls beneath) growing in the Botanischer Garten, Universität Bonn, were collected in February/March either in the morning or the evening when the temperature outside was below 6°C and the flowers were closed. In order to maintain the shoots at a temperature similar to that outside, they were placed in a container with a layer of ice before being brought into the laboratory. When it was essential that cells should not experience a temperature higher that 10°C between the time of collection of tepals and the first measurement of PD, care was taken to make all preliminary manipulations of the material at a temperature below 10°C.

Experiments were done on cells of the following: intact tepals, tepals or tepal segments lacking the abaxial epidermis, tepal segments lacking both epidermes, labelled "parenchyma", and isolated epidermes (the abaxial and the adaxial epidermis obtained by peeling tepals). To isolate epidermal or parenchymal tissue, an axial segment of a tepal, approximately 4 mm wide, was first obtained by cutting off the lateral parts parallel to the axis. The required epidermis was then peeled from the segment in an apical direction with use of sharp pincers. To obtain parenchyma, the epidermis was peeled from both sides of the tepal segment, beginning with the abaxial epidermis. Both epidermes could be peeled easily without killing their cells; the abaxial epidermis separated completely from the underlying mesophyll but the peeled adaxial epidermis often had patches of hypodermal cells attached. The parenchyma maintained air-filled intercellular spaces when immersed in water. The tepal or tissue was mounted between two horizontally positioned, perforated thin acrilic plates in a flat chamber (5 cm^3) open at the top. Pressing on the material under study was avoided; nevertheless, the isolated, concave tepals were flattened upon mounting. The specimen was immersed in a solution containing 0.1 mM KCl and 1 mM $CaCl_2$ (pH 6.0), stabilized by 10 mM MES/TRIS, and flowing through the chamber. The solution was supplied to the chamber from two reservoirs connected to the chamber by means of a T-valve: one with a cool solution (0-2°C) thermally isolated from the surroundings and another kept at room temperature. The specimen was cooled or warmed by adjusting the flow rates of the cool or warm solutions entering the chamber. The solution entered the chamber at its base and left it at the open top. The temperature in the chamber was monitored by a thermocouple (MP 1300 Tastotherm; Impac Electronic, Frankfurt, Germany) and recorded by a multichannel pen recorder (W+W 520; Kontron, Basel, Switzerland) with a pen offset compensation. The thermocouple was located close to the place where the specimen was impaled with a microelectrode. The temperature of the solution in which the specimens were immersed ranged from 4°C to 25°C (Table 1). The mean temperature at which the experiments started was approx. 19°C when the first change of temperature was cooling, and approx. 8°C when the first change was warming. For convenience, temperatures of 15°C, are denoted as high and those of 10°C as low. Cooling rates ranged from 2 to 15°C min -1 and warming rates from 1 to 11°C min -1. In the case of an intact tepal, either its adaxial or abaxial surface was uppermost to enable measurements on cells of the adaxial or abaxial epidermis, respectively. An isolated epidermis had the cuticle lowermost.

A conventional glass microelectrode with an Ag/AgCl half-cell and a 3 M KCl bridge were used. Borosilicate capillaries with an internal filament (Hilgenberg, Malsfeld, Germany) were pulled to a tip diameter below 1 µm by a vertical electrode puller (model 720; David Kopf Instruments, Tujunga, California, USA), and filled with 3 M KCl solution. The electrode resistance was approx. 4 MΩ. The microelectrode was connected to a custom-made amplifier (10^{14} Ω input resistance) followed by the recorder already mentioned. The insertion of the electrode was approximately perpendicular to the surface of the specimen which was in a thin layer of flowing solution. The insertion was made through a hole in the acrylic plate and was achieved by means of an hydraulic micromanipulator (HMD-2M; Clark Instruments, Reading, England). The impalement of the electrode into a cell or the penetration through individual cells was indicated by voltage recording during insertion. A pipette with an Ag/AgCl half-cell filled with 3 M KCl and closed by means of a short segment of porous, thin ceramic rod was immersed in the bathing solution in the proximity of the microelectrode and served as a reference electrode. The PD was determined as the difference between the potential when the microelectrode was in the cell wall (a few millivolts lower than that in the solution) and that when the microelectrode impaled a cell (indicated by a rapid change of the potential to a more negative level). The tip potential of the microelectrodes (approx. 10 mV) was measured sporadically because we considered only the differences in the potentials recorded by a particular microelectrode (when impaling a cell and when the temperature was changing) which are barely affected by the tip potential. When the microelectrode came into contact with the bathing solution (before impaling the object), the potential was nearly constant, V_o. If the potential after impalement was not stable the electrode was withdrawn. When a microelectrode on withdrawing from the tepal gave a potential which differed by more than 5 mV from V_o it was discarded. Up to four impalements into cells of a tepal were made with a single microelectrode if its potential after withdrawal to the solution differed less than 5 mV from V_o. In the case of a successful impalement the recording was continued until the electrode left the cell (manifested by a strong drop of the potential) or was clogged (manifested by a drift of the potential). Ten to twenty minutes after impalement the temperature of the solution was changed by means of the T-valve connecting the reservoirs with the chamber. Intervals between temperature changes were not shorter than 20 min. For many cells only a single change of temperature was possible because contact with the cell interior was lost at the second change. However, in some cells the temperature could be changed a few times during recording. It was not possible to ascertain whether the measured PD pertained to the cytoplasm or vacuole; however, several symptoms seemed to indicate that the electrode was mostly in the vacuole.

The statistical significance of differences between mean PDs measured at different times of a particular experiment (e.g. PDs before and after the change of temperature, for epidermal cells in intact tepals on cooling) was analysed by means of the multiple t-test (Weber 1986). In the case of independent series (e.g. initial PD in the series for cooling and warming) differences were analysed by means of the Student's t -test.

For microscopy small pieces of tepals were excised in axial and marginal regions at two levels: 1/4 to 1/3 and 2/3 to 3/4 of the tepal length. The pieces were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.0), postfixed in 2% OsO_4 in the same buffer and embedded in epoxy resin (Spurr 1969). Sections, 1 µm thick, were stained with 2% Toluidine Blue O in 1% calcium borate, dried and covered with immersion oil and a coverslip.

RESULTS

General description

The perianth of *Eranthis* consists of 6 tepals in two tripartite circles. Tepals in both circles react thermonastically in a similar way. No apparent differences between the circles were observed with respect to PD.

An intact or detached tepal displays a pattern of curvatures in axial and transverse planes normal to the surface. In closed flowers there is a concavity on the inner side. The curvature (defined as the inverse of the radius of curvature in a particular plane normal to the surface) in the axial plane of a tepal changes during the thermonastic movement of a tepal. Inward curvature (adaxial surface concave) attains a maximum value (0.25 mm^{-1}) for the basal region of the tepal when the flower is closed. In a fully open flower, a tepal shows a concavity on the abaxial side in its basal region. This means that the curvature changes its sign in the basal region of a tepal during the thermonastic opening and closing of the flower. The thermonastic movement is due to the changing curvature mainly in the basal region of a tepal extending for 1/3 of the length of a tepal (motor zone).

Intact, as well as detached tepals grow during flowering, more rapidly when the temperature changes daily so that the flower opens and closes. The irreversible increase of tepal dimension, i.e. its growth, occur mainly during the changes of temperature (data from tensiometric experiments not shown).

The thermonastic movements are caused by differential irreversible extensions on the opposite sides of the tepal. Probably, the differential extensions occurring in response to temperature changes make the main contribution to the growth of tepals during flowering.

The anatomical structure of the tepal is illustrated in Fig. 1. The adaxial epidermis is closely connected to the hypodermal layer, while the abaxial epidermis separates easily from the underlying mesophyll. The thickness of the epidermal cell wall varies both within an individual cell and also at different positions in the tepal. Within a cell, the outer wall is thicker than the others, especially in the movement zone. Within a tepal, the thickness of the outer wall is variable whereas the

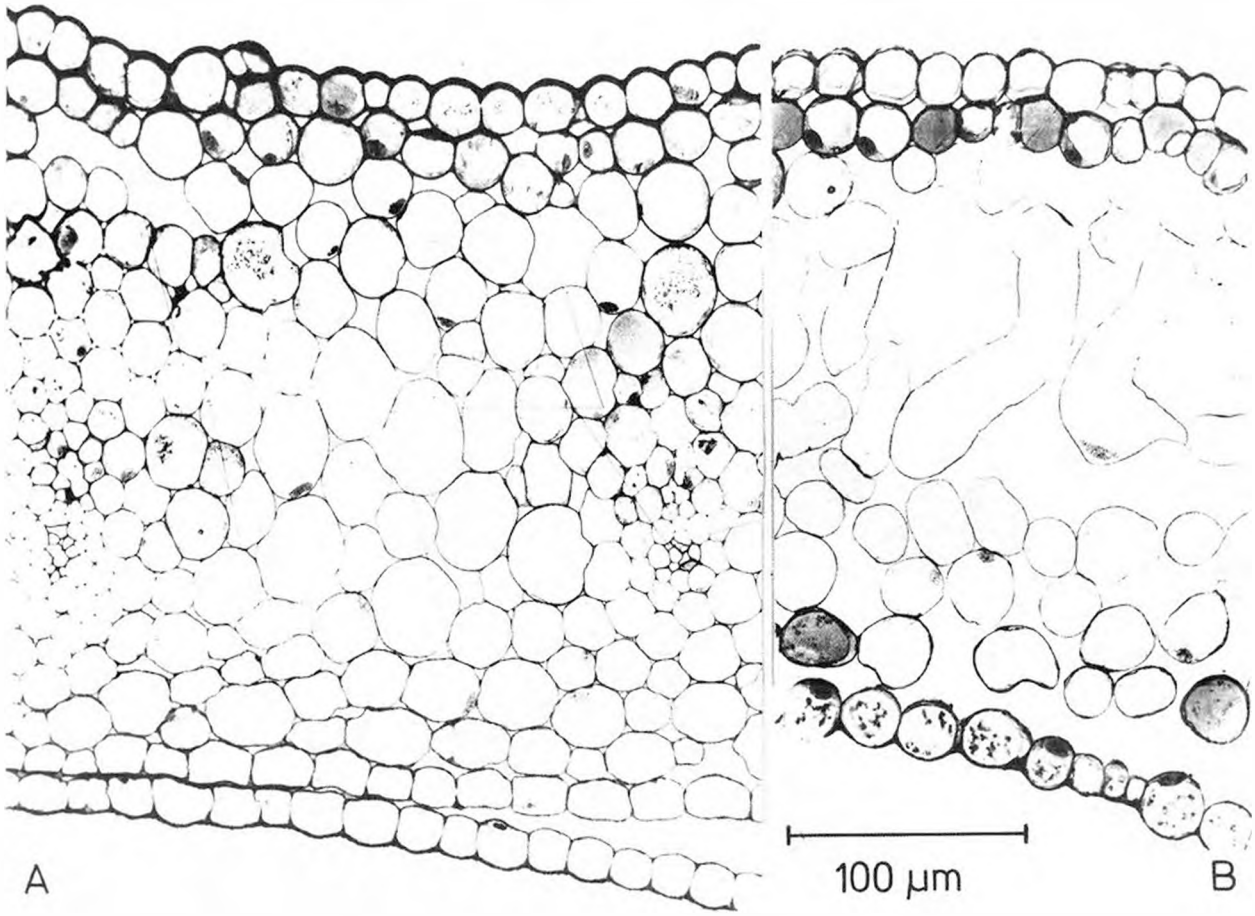

Fig. 1. Transverse cross-sections through the median (A) and lateral (B) regions of an *Eranthis* tepal in the middle part of the motor zone. The adaxial surface of the tepal is at the top of the micrograph.

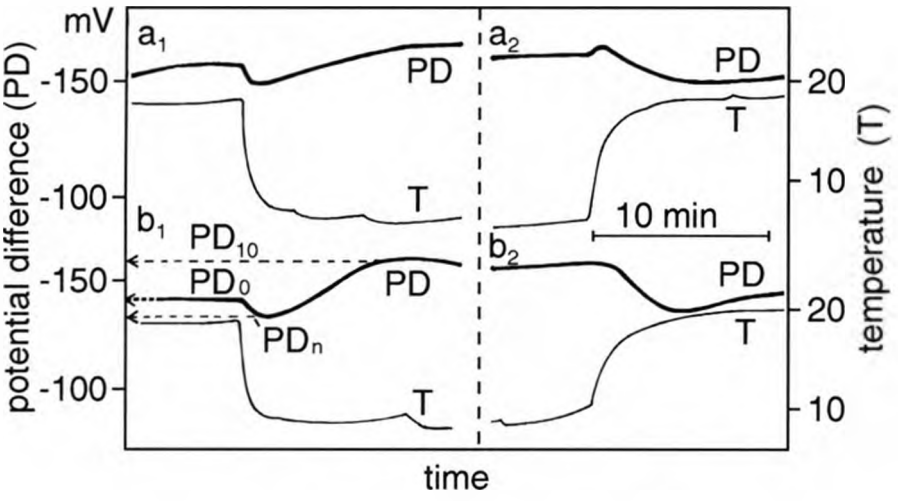

Fig. 2. Representative records of the transmembrane potential difference (PD) and of temperature (T) for a cell of the adaxial (a) or abaxial (b) epidermis of *Eranthis* tepal, respectively. a_1, b_1 – on cooling; a_2, b_2 – on warming. The initial potential difference (PD_o), this at the peak of the normal response (PD_n) and that at 10 min after temperature change (PD_{10}) are indicated in b_1.

other walls are generally consistently thin. The outer wall is thickest in the adaxial epidermis within the motor zone. The ratio of the thicknesses of the outer walls in the adaxial and the abaxial epidermes is approx. 2 : 1 in the motor zone (Fig. 1A), with the maximal thickness approx. 2.5 μm. Close to the margin and in the distal part of a tepal this difference disappears (Fig. 1B).

All cells are elongated mainly in the axial direction of a tepal; in the parenchyma in lateral parts of a tepal, however, the cell axis may deviate considerably from the tepal axis.

The mesophyll is composed of thin-walled cells arranged more densely in the subepidermal layers, especially on the adaxial side and around vascular bundles. The bundles run longitudinally without transverse connections. All cells of a bundle are thin-walled except the tracheary elements which have circular or helical (lignified) thickenings. The vascular bundles can be recognized in an intact tepal as more-translucent stripes. They were omitted from PD measurements as far as possible, but we cannot exclude the possibility that some measurements were done in a parenchymatic cell of a small vascular bundle.

The epidermes of a tepal are under tensile stress, as indicated by shortening of an epidermis on peeling. The parenchyma must then be under compressive stress (though the cell walls are under tensile stress) as is required for static equilibrium. After peeling both epidermes the curvature of the isolated parenchyma tends to decrease indicating that the adaxial layer of the parenchyma was more compressed than the abaxial layer in an intact tepal.

PD in the cells of intact tepals

Potential differences were always considerably above 100 mV (Table 1). There was a tendency for a higher PD in the adaxial epidermis at high temperature; however, the difference was not significant at P = 1%. Warming brought about a substantial, long-lasting depolarization. It was preceded by only a small, transient increase of PD. On cooling, the reverse reaction was observed: a small transient depolarization followed by a substantial hyperpolarization (Fig. 2) (where hyperpolarization or depolarization means an increase or decrease, respectively, of PD above the levels existing at constant temperature before the change). Typically, the PD was further restored to some extent, i.e., the PD in low or high temperature slowly decreased or increased, respectively, but a net effect remained. In consequence, the initial PD, labelled PD_o, in the cooling experiments tended to be different from that of the warming experiments. The mean PD_o was higher at the low temperature in all tissues; however, only in the outer epidermis was the difference significant at P = 0.5%, but not for the remaining tissues.

To facilitate the description of the PD changes, several elements in the PD response to (i) warming or (ii) cooling can be distinguished:

(i) initial hyperpolarization (denoted as "normal" response to facilitate further description), decreasing of PD leading to depolarization (denoted as "anomalous" response) followed by a slow increase of PD;

(ii) initial depolarization ("normal" response), increasing of PD leading to hyperpolarization ("anomalous" response), followed by decrease of PD.

The elements of the PD responses elicited by warming or cooling exhibited a mirror symmetry. However, they often occurred with different magnitudes in a particular cell, or some were even missing. There were considerable variations in the rates at which the response elements developed even in the same tissue, and in the amplitude which they could attain, even at a similar rate of temperature changing.

The transient normal response occurred in the form of a peak only if temperature changed more rapidly than $2^{\circ}C\ min^{-1}$, but sometimes did not occur at all even if the rate was three times as high as this (compare columns N and n in Table 1). The PD at the peak of the normal response was denoted as PD_n. If this response did not occur, i.e., the PD was changing in the "anomalous" direction from the beginning, then we considered PD_n as equal to PD_o in calculation of the mean PD_n. The peak of the initial response occurred, if at all, usually 1 to 2 min after the beginning of the change of temperature.

The anomalous response usually produced a broad peak, with extremum occurring at variable times after temperature changing, usually 8-10 min afterwards. To have a parameter characterizing this response we measured the PD_{10} min after the temperature change (denoted as PD_{10}). In some records the anomalous response did not occur; this happened, however, in less than 20% of the cells (column m in Table 1). The

TABLE 1. Mean values characterizing the magnitude of the transmembrane potential difference (PD) and its alteration following the temperature change from the initial value (T_o) at the rate (T min^{-1}) in the cells of different tissues of intact *Eranthis* tepals and of the isolated tissues. Standard errors are given by ±.

Cell type	N[a]	Initial values		Temperature change			Normal change[d]		Anomalous change[e]	
		T	PD_o	ΔT	ΔT min^{-1}	n[b]	PD_n	time	PD_{10}	m[c]
		°C	mV	°C	°C min^{-1}		mV	min	mV	
in intact tepals on cooling outer epidermis										
outer epidermis	18	19.7 ± 0.6	140 ± 4	11.3 ± 0.6	8.5 ± 0.5	14	132 ± 4	1.6 ± 0.1	154 ± 4	2
parenchyma	17	18.2 ± 0.3	136 ± 4	10.7 ± 0.4	9.0 ± 0.4	12	122 ± 4	1.8 ± 0.3	160 ± 6	1
inner epidermis	10	19.6 ± 0.3	154 ± 7	10.6 ± 0.4	7.8 ± 0.7	9	142 ± 8	2.2 ± 0.4	161 ± 8	1
in intact tepals on warming outer epidermis										
outer epidermis	17	9.6 ± 1.0	160 ± 5	10.2 ± 0.6	5.2 ± 0.5	9	166 ± 5	1.5 ± 0.3	135 ± 3	1
parenchyma	12	7.9 ± 0.5	150 ± 7	10.6 ± 1.0	3.7 ± 0.5	4	152 ± 7	1.6 ± 0.2	129 ± 10	2
inner epidermis	9	8.8 ± 0.8	168 ± 9	9.8 ± 0.7	4.1 ± 0.8	3	169 ± 8	1.5 ± 0.3	151 ± 8	0
in fully isolated tissues on cooling										
outer epidermis	20	18.5 ± 0.5	116 ± 5	10.5 ± 0.9	8.0 ± 0.8	11	113 ± 4	2.3 ± 0.8	116 ± 5	11
parenchyma	7	18.2 ± 0.2	18.2 ± 0.2	116 ± 7	11.3 ± 0.6	6	111 ± 7	1.6 ± 0.2	113 ± 7	6
inner epidermis	7	19.2 ± 1.7	19.2 ± 1.7	107 ± 6	11.9 ± 1.8	3	102 ± 8	1.9 ± 0.4	104 ± 7	4
in fully isolated tissues on warming										
outer epidermis	22	8.5 ± 0.5	122 ± 7	9.4 ± 0.5	5.3 ± 0.5	5	125 ± 7	1.5 ± 0.5	112 ± 6	6
parenchyma	7	8.6 ± 0.3	8.6 ± 0.3	8.5 ± 0.6	5.1 ± 0.7	3	118 ± 8	1.5 ± 0.8	115 ± 7	6
inner epidermis	8	7.2 ± 0.4	7.2 ± 0.4	10.6 ± 0.9	6.8 ± 1.3	3	108 ± 7	1.7 ± 0.2	106 ± 5	5
parenchyma in segments of tepals without the outer epidermis										
on cooling	5	20.7 ± 1.2	138 ± 5	12.3 ± 1.4	9.5 ± 1.1	2	110 ± 8	2.0 ± 0.2	138 ± 8	0
on warming	5	7.8 ± 0.4	163 ± 11	12.6 ± 1.8	7.6 ± 1.6		166 ± 13	2.0 ± 0.5	142 ± 6	3

a – total number of cases investigated;
b – number of cases in which the „normal change" with a peak occurred;
c – number of cases in which the „anomalous change" did not occur;
d – normal change: initial transient decrease or increase of PD on cooling or warming, respectively, PD at the peak of this transient change, or taken as PD_o if the peak did not occur;
e – anomalous change: PD measured 10 min after beginning of the temperature change for the N-m cells (i.e. for the cells in which the anomalous change occurred).

cells which did not show the anomalous response did not contribute to the mean PD_{10} presented in Table 1. In some cells the PD still continued to change in the anomalous direction beyond 10 min after the change of temperature. In the majority of cells, however, the extreme value of the anomalous change had been attained before 10 min and the PD remained either constant or changed slowly in the "normal" direction. The anomalous change, i.e. the difference $PD_o - PD_{10}$, was significant at P = 1% both on cooling and warming in the case of the abaxial epidermis, but was insignificant for the adaxial epidermis. In the case of the parenchyma the difference $PD_o - PD_{10}$ was significant only on cooling.

To visualize better the normal and anomalous changes of PD, fractional values were calculated (Table 2) and expressed as percentages. The mean normal change did not exceed -9% on cooling and +4% on warming, though in individual cells the variation was large. The mean fractional anomalous change was quite high for the parenchyma and the abaxial epidermis. The variance of this change was higher for the parenchyma than for the epidermis.

PD in the cells of isolated tissues

In the cells of the isolated tissues, i.e. in the peeled epidermes, or in the parenchyma remaining after peeling off both epidermes, the magnitude of PD in general was lower than in intact tepals (Table 1). In some cells there was only a normal response to the change of temperature, in others the anomalous response was weak. Often, on cooling, there was only a small compensatory increase of PD magnitude after the initial depolarization, without a final hyperpolarization. The net depolarization on warming occurred in some cases, especially in the abaxial epidermis, but usually there was only a compensatory decrease of PD magnitude after the initial hyperpolarization.

It was interesting to observe that when only the abaxial epidermis was removed, the parenchyma cells showed an anomalous response much better than the cells in the completely isolated parenchyma (Table 1, last part). In these experiments, the microelectrode was inserted from the peeled side into a parenchymatic cell not far from the surface, so the recorded cell was close to the wound surface and also close to the external medium. The initial response on cooling, i.e. the depolarization, was very pronounced, so that, on average, the following increase of the magnitude of PD in more negative direction returned the PD value to PD_o only 10 min after the change of temperature, masking the anomalous response. However, the change of PD continued and eventually a net hyperpolarization occurred. Though the difference between PD_o and PD_{10} was not significant, that between PD_n and PD_{10} was highly significant. On warming, the normal change of PD was slight and the anomalous change was similar to that in intact tepals. It appears that it is not the wounding and/or direct contact of the parenchyma with the external solution which causes the altered PD behavior in the isolated tissues, because both wounding and contact with the solution occurred on removal of the abaxial epidermis.

DISCUSSION

PD reponse to temperature change

The "anomalous" response of PD which occurs in *Eranthis* tepals upon altering the temperature probably resides in the active component of the PD. In general, the active component of the PD increases as the temperature rises, i.e., the PD becomes more negative (Spanswick 1972; Melamed-Harel and Reinhold 1979; Minorsky 1989). In the tepals of *Eranthis*, however, an element of the active component may be inhibited on warming and stimulated on cooling, resulting in the "anomalous" response. Minorsky (1989) did not mention such a case in his review. Blinks (1942), however, observed that the PD in *Valonia macrophysa* was lowest around 25°C, rising towards both 15°C and 35°C (see also Drost-Hansen and Thorhaug 1967). Interestingly, the same species in another study (Thorhaug 1971) did not show the increase of PD with decreasing temperature, but *Valonia ventricosa* did. Nelles and Laske (1982) split up the electrogenic component of the PD of cells in the coleoptile of corn into two parts; one with a positive activation energy, and another that increased linearly with decreasing temperature (coefficient 1.7 mV $°C^{-1}$). They found the second component only on slow cooling, and proposed that this component might be caused either by cold-induced changes of the lipid composition of the membrane enhancing the activity of the electrogenic pump, or by the production of a cold-stress substance which stimulates the activity of the pump. Nelles and Laske (1982) suggested that this component was absent during rapid cooling because the

TABLE 2. Normal and anomalous changes of PD expressed as percentages of the initial PD. Mean values were calculated from the percentages for individual cells in different tissues of intact *Eranthis* tepals.

	on cooling			on warming		
	Normal Change:			$\frac{(PD_n-PD_o)\,.\,100}{PD_o}$		
	N	n	change	N	n	change
outer epidermis	18	14	**-6** ± 2	17	9	**+4** ± 1.5
parenchyma	17	12	**-9** ± 1	12	4	**+1** ± 0.5
inner epidermis	10	9	**-8** ± 3	9	3	**+1** ± 0.5
	Anomalous Change			$\frac{(PD_{10}-PD_o)\,.\,100}{PD_o}$		
	N	n	change	N	n	change
outer epidermis	18	16	**+13** ± 1	17	16	**-16** ± 2
parenchyma	17	16	**+24** ± 7	12	10	**-20** ± 4
inner epidermis	10	9	**+6** ± 1	9	9	**-12** ± 2

N = number of recorded cells;
n = number of cells in which the change occurred;
PD_o = initial PD before the change of temperature started;
PD_o = extremal transiently decreased or increases PD on cooling or warming, respectively. If a cell did not show the normal change then it was given the value PD_o, so the mean was calculated for N cells;
PD_{10} = increased or decreased PD on cooling or warming, respectively measured 10 min after starting the change of temperature. If a cell did not show the anomalous response it did not contribute to the mean, so the mean was calculated for N – n cells.

time period was too short for changes to occur in the lipid composition or for production of a cold-stress substance. In *Eranthis*, rapid cooling hyperpolarizes the plasma membrane, and the hyperpolarization may be considerable. This cannot be explained by changes in lipid composition of the membrane or by production of a cold-stress substance because hyperpolarization starts too early for such changes.

PD and proton secretion

The PD across the plasma membrane is related to the rate of proton secretion which is also related to cell growth, as evidenced by the following examples. The fungal toxin fusicoccin both stimulates proton secretion and hyperpolarizes the transmembrane potential, processes which are accompanied by a stimulation of cell enlargement in many plant organs (reviewed by Marr 1979).

A brassinosteroid, a synthetic analog of brassinolide (a putative plant hormone, isolated from pollen, which promotes growth of the stem of a number of plant species) was found to hyperpolarize the PD and also to stimulate proton secretion and growth in Azuki bean (Cerana et al. 1983) and in maize root segments (Romani et al. 1983).

Auxin also stimulates proton secretion and hyperpolarizes the membrane (see Bates and Goldsmith 1983). The timing coincides with both a hyperpolarization of the PD of parenchyma cells and an increase in growth of the segments (Senn & Goldsmith 1988). In developing root hairs of *Sinapis alba*, application of IAA in very low concentrations (10^{-12} to 10^{-10} M) hyperpolarized the PD, presumably by increasing the activity of an electrogenic H^+ pump (Tretyn et al. 1991). We may close this short review with the conclusion made by Senn and Goldsmith (1988) that the hyperpolarization of the PD is a sensitive indication of enhanced proton secretion.

If it is assumed that *Eranthis* is similar to other systems in which an early hyperpolarization of the PD occurs, it is probable that the anomalous hyperpolarization on cooling is associated with enhanced electrogenic activity of a proton pump, and that the resulting from it decreased pH in the cell walls increases their ability to expand. The depolarization on warming would act in the reverse way. We wish to mention that the epidermal strips isolated from tepals of *Eranthis* and stretched in a tensiometer expand much faster at pH 5.2 than at pH 6.0 (data not shown).

Thermonastic movements of tepals

Thermonastic movements of tepals of such plants as *Tulip* and *Crocus* are due to different growth rates on the two sides of the tepals (reviewed by Crombie 1962). The differential growth is switched on by a change of temperature, so that raising the temperature from an initially low level results in faster growth on the adaxial side side, while a temperature fall from an initially high level results in faster growth on the abaxial side. The ability of the tepals to respond to a change of temperature by differential growth on both sides must reside in their dorsiventrality, which can be anatomical, physiological or both (Böhner 1933; Wood 1953; Crombie 1962). In a separate study we have proved that the thermonastic movement of *E. hyemalis* tepals is also due to differential growth of the inner and outer sides (data not shown). The tepals start to grow faster on the adaxial side than on the outer side when the temperature rises and the flower opens. When the temperature falls the abaxial side shows a more moderate reduction of the growth rate than the opposite side, or even a transient stimulation of growth, and the flower closes.

In different thermonastic flowers there is an anatomical difference between the epidermes on the two sides. Böhner (1933, 1934) drew attention to the fact that in the perianth members of a thermonastic flower the outer walls of epidermal cells are thicker than the others, and that the outer walls on the adaxial side are thicker than those on the abaxial side. A thicker outer wall is a common feature of the epidermal cells of vegetative above-ground organs (leaves, stems) but does not typically occur in perianth leaves; thermonastically active flowers are thus exceptional in this respect. *Eranthis* also conforms to this exceptional pattern; however, a clear difference in the thickness of the external walls of the two epidermes occurs only within the movement zone (at the base of the tepal). It is possible that the difference in wall thickness on the adaxial and abaxial sides of a tepal may be involved in the mechanism of thermonastic movement. Anyway, the tepals of *Eranthis* show an anatomical dorsiventrality.

Böhner (1933) observed that the epidermes in open tulip flowers were under elastic tensile strain. This means that there are tissue stresses in the tepal of an open flower: the epidermes are under tensile stress and the parenchyma under compressive stress though all cell walls are under tensile stress (static equilibrium requires that a tensile force in the epidermis is accompanied by a compressive force in the parenchyma, and the magnitudes of both forces are equal). Böhner (1933) showed that in closed flowers of tulip the adaxial epidermis was under tensile stress while the outer one was free of tissue stress.

Investigations on the physiological dorsiventrality of petals of *Tulipa* and *Crocus* have provided many data (reviewed by Crombie 1962). Perhaps the most important observation is that when a strip of a petal was split tangentially into halves, one consisting of the adaxial epidermis and adaxial mesophyll, the other of the abaxial tissues, the former half responded to a sudden increase in temperature by greater growth whereas the abaxial half only exhibited a slight increase. Following a sudden temperature fall, the abaxial half grew rapidly at first whereas the adaxial half grew slowly (Wood 1953). These observations were interpreted in terms of different temperature optima for cell growth: cells of the abaxial part would have an optimum at about 10^{o}C lower than those of the adaxial part.

Relevance of the anomalous changes of PD to the thermonastic movements

The present study suggests that enhanced proton secretion may be caused by temperature fall, leading to the closing movement in tepals of *Eranthis*. We assume that hyperpolarization on cooling and depolarization on warming means that the electrogenic proton pump is more or less active, respectively. If the hyperpolarization on cooling and the depolarization on warming occurred only in the abaxial epidermis while there was only a "normal" response to a temperature change in the remaining tissues, the mechanism of thermonasty would be simple. On warming, the lowered activity of the proton pump on the abaxial side would mean that the activity on the adaxial side predominates leading to faster growth of this side. On cooling, the proton pump would dominate on the abaxial side allowing the closing type of movement. However, the real situation is not so simple. In *Eranthis*, the anomalous responses occur in both epidermes, and instead of a qualitative difference between them, there is only a quantitative difference. The anomalous responses occur on both

sides of a tepal but the response is more pronounced in the abaxial epidermis.

The difference PD_0-PD_{10}, characterizing the anomalous response, is statistically significant in the abaxial epidermis both on cooling and warming, but is insignificant in the adaxial epidermis. The difference is also significant on cooling in the parenchyma, but not on warming. Electrophysiological dorsiventrality thus exists in *Eranthis*, but not to the extent that the hyperpolarization on cooling occurs only on the abaxial side. However, even if the anomalous response occurred equally on both sides, this would not necessarily mean that the effectiveness of the responses is the same on different sides. There may be additional factors modulating the effectiveness, so that the anomalous response becomes of prime importance in the abaxial epidermis but is only secondary in the adaxial one. We see two possible factors which cooperatively may modulate the effectiveness of the enhanced acidity correlated with the hyperpolarization on both sides: the tissue stresses changing during the thermonastic cycle, and the different thicknesses of the cell walls in the epidermes.

If the tissue stresses in *Eranthis* are such as those observed by Böhner (1933) in tulip, then the adaxial epidermis, having a higher tissue tension in the closed flower, is better prepared to start the expansion on warming than the unstretched abaxial epidermis. The more pronounced depolarization on warming in the abaxial epidermis may contribute to the difference in growth on the two sides – faster on the adaxial side. When the flower is open, both epidermes are under tensile stress. If the temperature then falls, the growth rate decreases generally, but this decrease would be better expressed in the adaxial epidermis which is characterized by the thicker outer cell wall. In addition, the anomalous hyperpolarization on cooling is expressed to a greater extent in the abaxial epidermis. If this means a more pronounced secretion of protons in the abaxial epidermis, the flower must close.

This study indicates that the effect of the change of temperature on the PD depends on whether the recorded cell is in an intact tepal or in an isolated tissue. The state of a cell in an intact organ must surely differ in many aspects from that in a tissue isolated from the organ. Some differences may be trivial, e.g. isolation changes the immediate surroundings of a cell, it also affects the composition of the solution within cell walls. However, isolation also causes a dramatic change in the properties which characterize the organ as a whole: (i) continuity of the symplasm (which is important for signal transmission through plasmodesmata), (ii) continuity of the apoplasm (which is important for electric current between different parts of the organ), and (iii) the tissue stresses (in an organ, a tissue may be under a tensile stress only when another tissue is under compressive stress). We observed a strong reduction of the anomalous response of PD to temperature in isolated tissues. Perhaps this was due to the wounding caused by isolation; however, the anomalous responses in the parenchymatic cells of tepal segments deprived of one epidermis only mitigates such a possibility. If not the wounding, what could be the main difference in the state of the parenchyma in intact tepals, or those peeled on one side and the isolated parenchyma? The difference may pertain to tissue stresses; they still exist in the one-side-peeled tepals but not in the isolated parenchyma. The relationship between stress in the tissues isolated from the tepals and the response of PD to the change of temperature deserves a direct experimental study. By definition, thermonastic movements occur at the organ level. On the same level tissue stresses occur for which the integrity of the organ is obligatory. It is thus possible that temperature-dependent processes specific for this level are involved in the movements. The anomalous changes in PD may be due to temperature-induced changes in the tissue stresses and the changes may be perceived by stretch-activated channels. Such an approach may be important when considering tropic or nastic movements in general, and especially for thermotropism in a root. If there is a gradient of temperature across a root the root grows in the direction of the higher temperature, i.e., the growth rate is faster on the cooler side (Fortin and Poff 1991). Since the growth rate of an isolated tissue or a cell increases with temperature, an explanation of thermotropism is possible only at the organ level. For instance, the distribution of tissue stresses may be changed by the temperature gradient in such a way that the cooler side may grow faster.

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ZALEŻNE OD TEMPERATURY ZMIANY POTENCJAŁÓW BŁONOWYCH W KOMÓRKACH TERMONASTYCZNYCH PŁATKÓW *ERANTHIS HYEMALIS* (L.)

STRESZCZENIE

Opisano wpływ temperatury na mierzony wpoprzek błony potencjał bioelektryczny plazmalemmy (PD) komórek płatków rannika (*Eranthis hyemalis*). Nagłe ogrzanie nie uszkodzonych płatków powodowało niewielki, przejściowy wzrost PD, po którym następowała znaczna i długotrwała depolaryzacja. Efekt ten nazwano reakcją anomalną. Przy nagłym oziębieniu sytuacja była odwrotna: po niewielkiej, przejściowej depolaryzacji następowała znaczna hiperpolaryzacja. To również było traktowane jako reakcja anomalna. Efekty takie były silniej zaznaczone w skórce odosiowej płatka niż w skórce doosiowej. Wskazuje to na elektrofizjologiczną grzbietobrzuszność płatków. Reakcje anomalne były o wiele słabsze w komórkach izolowanych tkanek niż w komórkach nietkniętych płatków. Różnica ta nie wynika jednak ze zranienia lub bezpośredniego kontaktu tkanki z roztworem zewnętrznym, ponieważ w segmentach płatków pozbawionych jedynie odosiowej skórki, PD zmieniało się tak samo jak w nietkniętych płatkach. Zasugerowano, że pojawianie się reakcji anomalnych jest modulowane naprężeniami tkankowymi. Przedyskutowano też znaczenie reakcji anomalnych dla ruchów termonastycznych.

SŁOWA KLUCZOWE: potencjał błonowy, *Eranthis hyemalis*, termonastia, reakcja na zmiany temperatury.