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Electrical properties of *Lupinus angustifolius* L. stem
II. Accommodation and anode break excitation

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Abstract

Under electrical stimulation of *Lupinus* stem phenomena of accommodation and anode break excitation appear. Their characteristic is the same as in the axon or nerve. Only their duration is about 10^3-10^4 longer in plants.

The constant characterizing the rate of accommodation was calculated. A limiting threshold value was found beyond which excitation occurs, irrespective of the rate of stimulus rise (voltage gradient). The accommodation rate is approximately constant, whereas the range of accommodation varies and is dependent on the difference between the rheobase value and the limiting threshold value. Hence plants with a low rheobase are characterized by a wider range of accommodation.

It is suggested that the changes in potential (including AP) recorded on the stem surface are connected with changes of the potential on cell membranes (Sibaoka, 1962).

INTRODUCTION

When a depolarizing current is passed through an axon membrane, the threshold membrane potential slowly rises, and in the case of hyperpolarizing current it slowly falls. When the stimulus is switched off the threshold slowly returns to the resting value. This delayed dependence of the threshold on the membrane potential is known as accommodation (Fig. 1A). If the hyperpolarization reaches a certain value, the threshold may fall below the resting potential. After switching off the stimulus the membrane potential remains temporarily above the threshold (Fig. 1Ad), this elicits an action potential (AP). Such a phenomenon is known as anode break excitation.

When a linearly rising cathodal stimulus is applied, the membrane potential rises almost linearly and so does slowly the threshold. It results

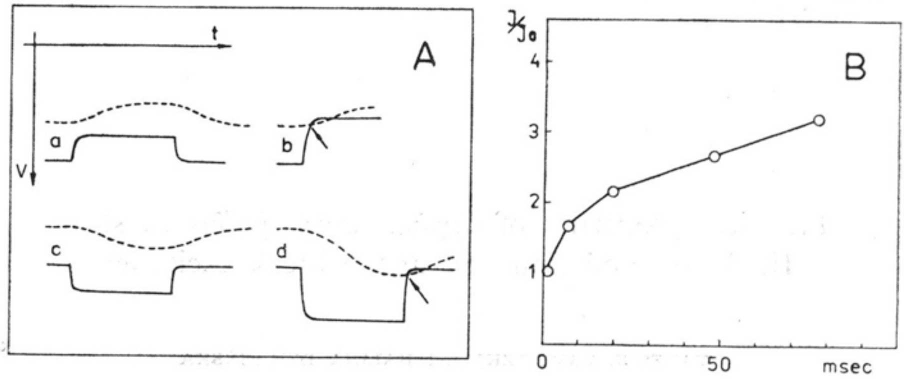

Fig. 1. A. Accommodation in response to constant currents. In each case the continuous line shows the membrane potential and the dotted line the threshold membrane potential. a and b: depolarizing current. In b the current is strong enough to cause excitation after it is switched on, at the point marked by the arrow. c and d: hyperpolarizing current. In d the current is strong enough to cause "anode break excitation" after it is switched off, at the point marked by the arrow (Aidley, 1971). B. Accommodation curve for motor fibres. Common type with successively diminishing slope. Figure from paper by Skoglund (1942).

hence that the threshold voltage is lower for rapidly rising voltage than for a slowly rising one (Aidley, 1971).

Accommodation is an essential parametre characterizing nerve excitation. This problem has been extensively treated in the literature (Hill, 1936, Solandt, 1936, Skoglund, 1942, Stoney and Machne, 1969, Tsintsadze, Kukushkin, and Sakson, 1974). Accommodation occurs both in the isolated nerve (e.g. sciatic-gastrocnemius preparation of frog) and in intact nerves (human ulnar nerve). Skoglund (1942) applied linearly rising electrical stimuli. He demonstrated that nerves of various types (motor, sensory) exhibit various degrees of accommodation. An example of accommodation curve is shown in Fig. 1B.

In higher plants accommodation has so far not been studied. The present investigations were undertaken to give a characteristic of accommodation and of the "anode break excitation" phenomenon in the stem of *Lupinus*. The present work is based on the investigations of Solandt (1936) and Skoglund (1942).

MATERIALS AND METHODS

The material (40-70-day-old plant of *Lupinus angustifolius* L.), apparatus and methods are described in the preceding paper (Paszewski and Zawadzki, 1973). The rheobase value (rectangular stimulus — lowest effective voltage) was determined, and then the threshold values were established for 3-4 successive stimulations by means of stimuli with various rate of rise (voltage gradient) and the rheobase measurement was repeated as control. The experiments were replicated for step stimuli and linearly rising ones. Changes of potential were recorded from the stem surface close to the cathode (cathodal depression). Between the successive stimulations there was a 90-min interval. The anode break excitation phenomenon was observed close to the anode, when exclusively rectangular stimuli were applied. Illumination (daylight, about 2-4 $W \cdot m^{-2}$) was continuous and the temperature was within the range of 21-23°C. The experimental arrangement is shown schematically in Fig. 2 and Fig. 4.

RESULTS AND DISCUSSION

Typical AP records obtained upon application of slowly increasing and rectangular stimuli are shown in Fig. 2. The records were chosen from two measurement series in which step and linearly rising stimuli were used (Fig. 2A and B, respectively). Series of measurements were performed on different *Lupinus* plants. The threshold value of the slowly rising stimulus is higher than the rheobase value (Fig. 2A). This is true in the case when the stimulus rises both step-wise and linearly. When there are several stimuli with different rates of rise (Fig. 2B), the threshold stimulus is higher for a slowly rising stimulus than for a rapidly rising one. This is manifested by a higher threshold value of the stimulating voltage and by a higher threshold depolarization close to the cathode (cathodal depression) evoked by the stimulus. No major differences were found in the accommodation phenomenon, when stimuli rising step-wise or linearly were applied, provided the successive voltage steps for step stimulation were small in relation to the rheobase. The presented results bring evidence of the existence of accommodation in the stem of *Lupinus*.

Fig. 3A shows curves characterizing the increase of the stimulus threshold value in dependence on the time of rise of the stimulus and on the rheobase value. These curves are referred to as accommodation curves (Skoglund, 1942). Curve a correspond to a plant with a very low rheobase, that is a highly excitable one. Curve c concerns a plant with a relatively high rheobase. The general character of the accommodation curves is identical. It may be assumed that the accommodation curves are of approximately exponential character. It results from the slope of these curves and from the level they reach, that plants with a low rheobase have a wider accommodation range. An identical character of accommodation curves is shown by Skoglund (1942) in Fig. 1B and Tsintsadze et al. (1974) for nerves.

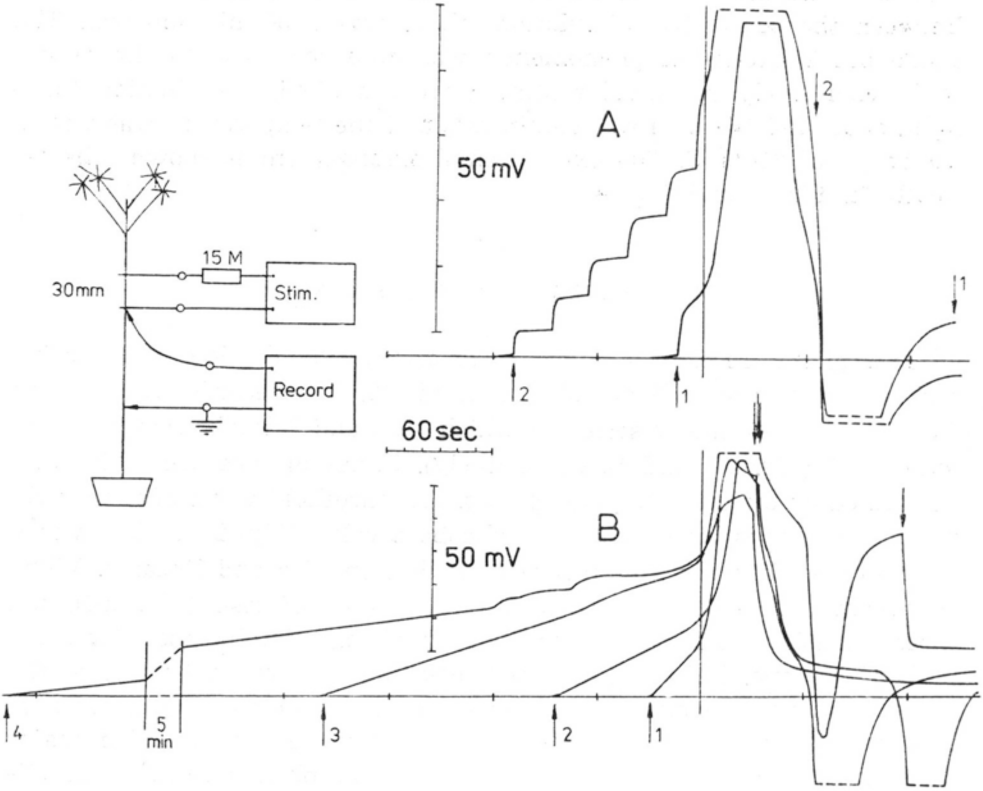

Fig. 2. Typical cathodal depression and AP records obtained from two *Lupinus* plants stimulated with step-wise (A) and linearly rising (B) stimuli at various rates of rise. Diagram of experimental arrangement on left side. Superimposed records. On both figures the moment of AP arising is adopted as the common point of reference and is marked by a vertical axis. The numbered arrows indicate the switching on and off the successive stimulations. A. Stimulation 1: rheobase — rectangular stimulus of 9V elicited a threshold cathodal depression of about 10 mV. Stimulation 2: the stimulus increases step-wise by 3 V at 20-sec intervals. Excitation appeared at 18 V and at threshold cathodal depression (threshold depolarization) about 40 mV. B. The rate of rise of the stimulus for the successive stimulations 1, 2, 3, and 4 is 15, 7, 3.4, and 1.7 $V \cdot min^{-1}$, respectively. Excitation occured successively at 8, 10.5, 14.5, and 18 V. The threshold cathodal depression was 15, 22, 38, and 41 mV, respectively (curve a in Table 1).

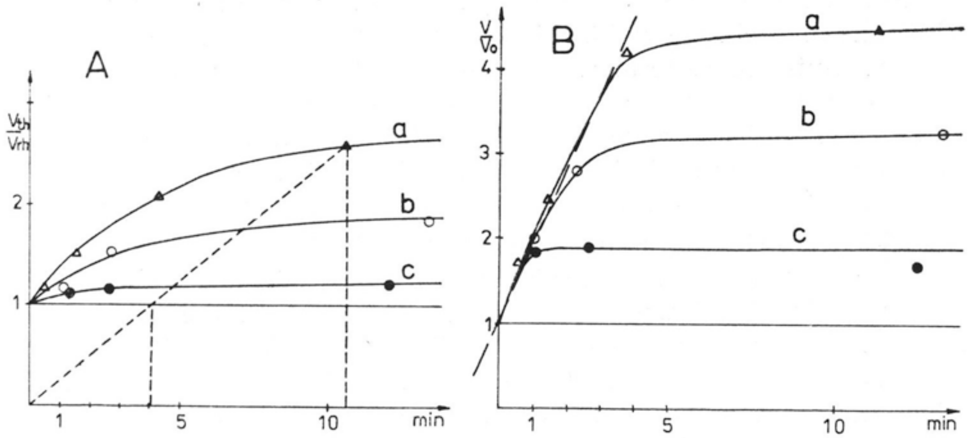

Fig. 3. Accommodation curves for *Lupinus* stem plotted for three plants with different excitability. Linearly rising stimuli at different rates. A. Curves *a*, *b*, and *c* characterize the rise of threshold value V_{th} in dependence on the time of stimulus rise (rising at different rates) and rheobase value (the rheobase proportions were 1.0 : 1.9 : 2.2, respectively). Ordinate — relative increase of threshold stimulus value (V_{th}) in reference to rheobase (V_{rh}) adopted as unity. The mode of plotting experimental points is shown by dotted lines on curve a. The stimulus rises at such a rate that the rheobase value is reached in ca. 4 min and excitation arises around 11 min when the stimulus reached 2.6 of the rheobase value. Experimental data included in Table 1A. B. Curves *a*, *b*, and *c* characterize the rise of the threshold cathodal depression V (threshold depolarization) in dependence on the time of depolarization rise resulting from the action of linearly rising stimuli at various rates of rise and on threshold cathodal depression values V_o (value corresponding to rheobase). The V_o value proportions were 1.0 : 1.7 : 2.2. Ordinate — relative increase of threshold cathodal depression (V) in reference to V_o adopted as unity. Method of experimental point plotting as in Fig. 3A. Dotted line represents the slope of curves at the phase of their rise. Experimental data included in Table 1B.

Table 1

Results obtained for three *Lupinus* plants in studies on accommodation. The results are graphically presented in Fig. 3A and B, respectively

A. Absolute values of threshold stimuli. V_{rh} — rheobase, V_{th} — threshold for linearly rising stimuli at rates of 15, 7, 3.4, and 1.7 $V \cdot min^{-1}$. High voltage values are due to the fact that for current stabilisation a resistance of 15 M was inserted in series with the plant into the stimulating circuit. B. V_0 — threshold cathodal depresion for rectangular stimuli (corresponds to rheobase), V — threshold cathodal depression for linearly rising stimuli at the same rate as in A.

A	Threshold stimulus intensity, (V)					B	Threshold cathodal depression (depolarization), (mV)				
curve	V_{rh}	V_{th}				curve	V_0	V			
a	7	8	10.5	14.5	18	a	9	15	22	38	41
b	13	15	19.5	—	24	b	15	30	42	—	49
c	15.5	17	18	—	18.5	c	20	37	38	—	33

Accommodation curves can be plotted for plants, the rheobase of which does not change within 10 — 12 h, that is the time during which measurements are performed. Changes in the rheobase in the course of one day (even 2 or 3 times) are a frequent finding and make it impossible to obtain a full accommodation curve for such a plant (Paszewski and Zawadzki, 1974, Zawadzki, 1979).

Fig. 3B shows curves characterizing an increase of the threshold of cathodal depression (threshold depolarization) in dependence on the time of rise of the cathodal depression and on the threshold cathodal depression value V_0. These results were obtained from the same three measurement series mentioned above (Fig. 3A and Table 1A). As shown in Fig. 2B, a certain value of cathodal depression corresponds to each stimulus. In the authors' opinion the accommodation curves plotted in Fig. 3B characterise better the accommodation phenomenon than those in Fig. 3A, because the membrane potential changes are better described by the value of cathodal depression than by the absolute value of the stimulating voltage applied from without. It results from the curves in Fig. 3B that there exists a limiting value of the membrane potential, to which the threshold potential of the membrane can rise. This is indicated by the plateau of the curves where accommodation does not occur. The range of accommodation extends within the area of rise of the curves. It has been frequently observed in the case of *Lupinus* stem that when stimulation is repeated with a given rate of rise of the stimulus, excitation may arise at various values of the stimulating voltage, but at the same value of cathodal depression (depolarization). In a complex system such as a plant stem (or a nerve) the action of a stimulating voltage applied from without may be resolved to a number of elements changing in time (e.g. resistance of the contact stimulating electrode — stem, resistance of dead and unexcitable tissues). There is a possibility that it is not the threshold, but the effective value of the stimulus acting on the excitable elements that undergoes change. The curves in Fig. 3A and B have similar courses because they correspond to the measurement series in which the rheobase did not change during the period of measurements.

When extracellular electrodes are used it is not possible to determine the absolute value of the cell membrane potential. Therefore a description of the accommodation phenomenon in the *Lupinus* stem may be attempted with the application of the definitions mentioned in the introduction, under the assumption that the happenings observed are derived from potential changes in the membranes of excitable cells (Sibaoka, 1962).

As example the curve a in Fig. 3B (Table 1B) may be considered. If the threshold potential is to be reached by the rectangular stimulus, it is necessary that the stimulus produce a depolarization of 9 mV.

Linearly rising stimuli produce linearly rising depolarization (Fig. 2B), and this in turn provokes an increase of the threshold membrane potential (the threshold "runs away" — Fig. 1A). If the rate of rise of depolarization is higher than that of the threshold, the membrane potential will reach after a certain time the threshold membrane potential. This is seen in the initial rising part of curve a. It may be concluded from the results presented in Table 1B that there exists a maximal depolarization value (ca. 40 — 50 mV) the attainment of which produces excitation even at very low rates of rise of the stimulus. It results therefrom that there exists a limiting membrane potential value to which the threshold membrane potential may shift.

The slope of the accommodation curves characterizing the rate of accommodation is expressed by the formula

$$\frac{\Delta U}{\Delta t} = \frac{1}{\alpha}$$

where: ΔU — increment of the threshold depolarization value (threshold depolarization value V_0 for the rectangular stimulus adopted as unity), Δt — time increment corresponding to $\Delta U = 1$, α — accommodation constant. The accommodation constant α introduced describes the time during which the threshold membrane potential will increase by the value of the threshold depolarization V_0. This parametre is close to the time constant of accommodation λ described by Hill (1936) and Solandt (1936). The accommodation constant α calculated for curves a, b, and c (Fig. 3B) in the range of the growing segment of the curves is independent (or only slightly dependent) on the value of V_0 and indirectly on the degree of excitability of the given plant. Its value amounts about 60 — 70 sec. In other words, the accommodation rate in *Lupinus* stem is constant. On the other hand, the range of accommodation is different and dependent on the difference between V_0 (or rheobase) and the threshold depolarization limiting value V (or limiting threshold). Hence plants with a low threshold exhibit a wider range of accommodation.

Fig. 4 shows typical records of AP arising close to the anode upon switching off the electrical stimulus. These records give the characteristic of the anode break excitation phenomenon in *Lupinus* stem. The record in Fig. 4A was obtained after application of a stimulus equalling the rheobase value. Excitation arises after complete subsidence of hyperpolarization. The record in Fig. 4B was obtained by applying a stimulus of 1.5 of the rheobase. Excitation arises after partial disappearance of hyperpolarization. It results from Fig. 4 that an appropriate rate of rise of the potential step with amplitude about 15 mV, appearing locally close to the anode as the response to switching off of the stimulus is a condition for the appearance of AP. It was also found that AP arises when the duration of hyperpolarization (from switching on to switching off) is equal or longer than the above determined accommodation constant α. The phenomenon of anode break excitation is the consequence of the existence of accommodation (Hill, 1936). This proves true in the case of *Lupinus*. It may also explain the phenomena referred to as Pflüger's contraction laws earlier described in *Lupinus* (Paszewski and Zawadzki, 1973).

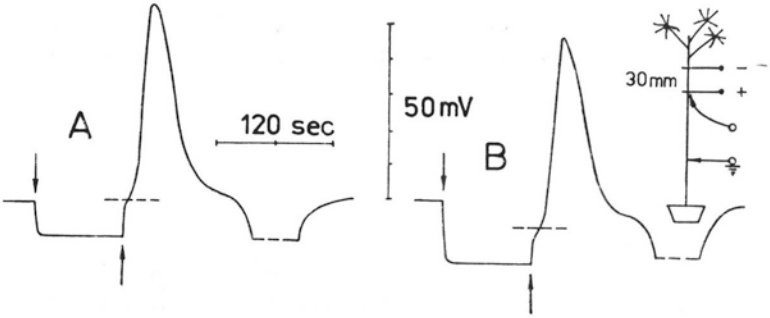

Fig. 4. Anode break excitation phenomenon in *Lupinus* stem. Typical records of two stimulations of the same plant. Arrows mark the moment of switching on and off of the stimulation. Diagram of the experimental arrangement on right side. Potential changes positive within the anode area during stimulations (hyperpolarization).

The characteristics of accommodation and anode break excitation here presented are identical with those of the same phenomena in nerves and axons, when measured by external electrodes. They only differ by a different, much longer (about 10^3—10^4 times) duration. These results confirm our suggestions and conclusions in previous papers (Paszewski and Zawadzki, 1973, 1974, 1976; Zawadzki, 1979) that excitation in plants is governed by the same laws as in animal excitable systems, that it can be described in terms and notions used in neuroelectrophysiology and that it probably occurs on the membranes of excitable cells (Sibaoka, 1962) which as a tissue may form an excitable system within the plant body.

Acknowledgments

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Elektryczne własności łodygi Lupinus angustifolius L.
II. Akomodacja i "anode break excitation"

Streszczenie

Przy elektrycznej stymulacji łodygi *Lupinus* zachodzą zjawiska akomodacji i "anode break excitation". Ich charakterystyka jest taka sama jak w aksonie lub nerwie. Jedynie czas trwania zjawisk jest około 10^3 — 10^4 razy dłuższy u roślin.

Wyznaczono stałą charakteryzującą szybkość akomodacji. Stwierdzono występowanie granicznej wartości progu, powyżej którego pobudzenie powstaje bez względu na szybkość narastania bodźca (gradientu napięcia stymulującego). Szybbość akomodacji jest w przybliżeniu stała, natomiast zakres akomodacji zmienia się i jest zależny od różnicy między wartością reobazy i wartością graniczną progu. Stąd rośliny o niskiej reobazie charakteryzują się szerszym zakresem akomodacji.

Przypuszcza się, że zmiany potencjału w tym także potencjał czynnościowy mierzone na powierzchni łodygi mają swoje źródło w zmianach potencjału błon komórkowych (Sibaoka, 1962).