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Electrical properties of *Lupinus angustifolius* L. stem
I. Subthreshold potentials

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Abstract

Subthreshold and action potentials in *Lupinus* stem in response to subthreshold and threshold stimuli (square constant current pulses) were studied. The occurrence of electrotonic potentials and local responses is demonstrated. The general characteristic of these responses and their amplitudes are the same as demonstrated for the isolated crab axons by Hodgkin (1939) and Hodgkin and Rushton (1946). The course of the phenomenon, however, is about 10^3-10^4 times slower in plants.

INTRODUCTION

Hodgkin (1939) and Hodgkin and Rushton (1946) gave a characteristic of the responses of axon to subthreshold electrical stimuli. The axon was stimulated with brief (60 μsec) electrical shocks or square constant current pulses (15 msec), and the response was recorded in the vicinity of the stimulating electrodes. Stimulating and recording electrodes were of extracellular type. The results of typical experiments are shown in Fig. 1.

In both cases the phenomena are of the same character. Anodic or weak cathodic currents seem to affect only the passive charging process; the curves (in Fig. 1A or 1B) have the same shape and their amplitude is roughly proportional to the applied current. The reason for this is that the nerve membrane behaves electrically as a resistance and capacitance in parallel, and the voltage change is caused by the passive charge or discharge of the membrane capacitance through the membrane resistance. This type of electrical change in axon is known as an electrotonic potential. For cathodal stimuli of intensity above about 0.5 the threshold level the resulting potentials differ in their time course from the responses to anodal stimulation of the same intensity because these

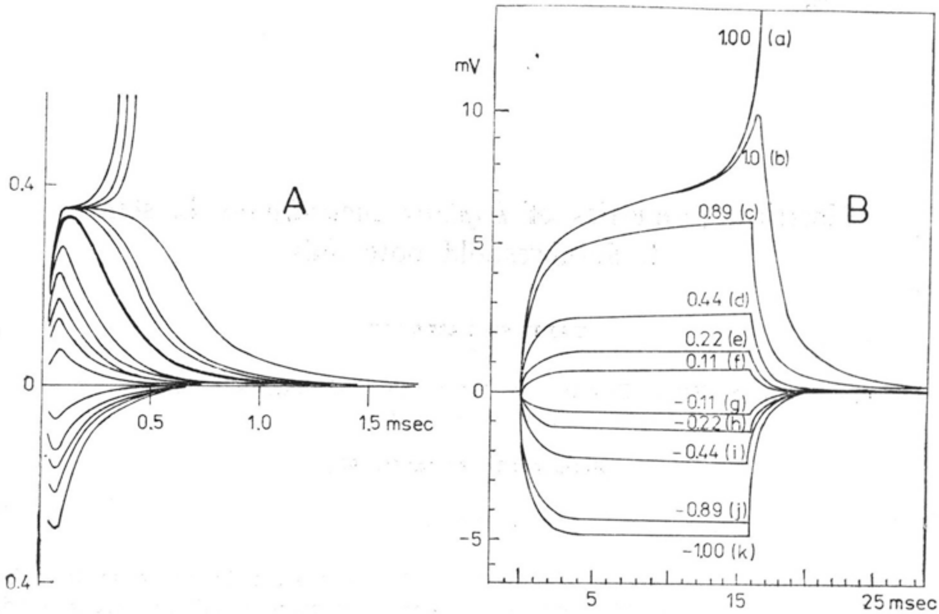

Fig. 1. Subthreshold responses and APs recorded extracellularly from a crab axon in the vicinity of the stimulating electrodes (figures and descriptions from papers of Hodgkin, 1939, and Hodgkin and Rushton, 1946. Fig. 1A modified).

A. Electrical changes at stimulating electrode produced by shocks (60 μ sec) with relative strengths, successively from above, 1.00 (upper 6 curves), 0.96, 0.85, 0.71, 0.57, 0.43, 0.21, —0.21, —0.43, —0.57, —0.71, —1.00. The ordinate scale gives the potential as a fraction of the propagated spike, which was about 40 mV in amplitude. The 0.96 curve is thicker than the others, because the local responce had begun to fluctuate very slightly at this strength. The width of the line indicates the extent of fluctuation. B. Response of *Carcinus* axon to rectangular waves of current of different intensity; recorded at a polarizing electrode of width about 200 μ. The numbers on each record give the strength of current relative to threshold. Depolarization of the nerve is shown as positive.

responses are composed of a local response added to the electrotonic potential. The time course of the local response can be obtained by subtracting the expected electrotonic potential from the whole response (Hodgkin 1939).

When the cathodal response crossed a particular level a propagated action potential (AP) appears. This critical level of membrane potential is called the threshold membrane potential and the stimulus which brought on this state — the threshold stimulus. The definitions used here are commonly used in electroneurophysiology (Aidley, 1971).

Subthreshold responses in plants are but little known. Auger and Fessard (1935) noted the appearance of a subliminal response when investigating the excitable cells of *Chara*. They observed potentials in the stimulated region and were able to record a small wave-like response, which grew up to AP when it reached a certain size. Hodgkin (1939) starting his investigations on axons mentions these phenomena in algae. An identical phenomenon is described by Sibaoka

(1954, 1973) in the case of electrical stimulation of *Mimosa pudica* and *Biophytum* sp., and he calls the changes in potential local responses. The concept of local potential in higher plants is used by Umrath (1959).

Excitation in *Lupinus* stem is fully subject to such laws of excitability as the all-or-nothing law, strength-duration relation or refractory periods (Paszewski and Zawadzki, 1973, 1974, 1976a). The present paper is an attempt at demonstration that subthreshold responses in *Lupinus* stem are of the same character as those in the axon. The work is based on the investigations of Hodgkin (1939) and Hodgkin and Rushton (1946).

MATERIALS AND METHODS

The material (40-70-day-old plant of *Lupinus angustifolius* L.), apparatus and method are described in the preceding paper (Paszewski and Zawadzki, 1973). The measuring electrodes were applied to the stem surface: one at a distance of 0-1 mm and the other (earthed) at a distance of 5-15 cm counting along the stem from the point of insertion of the stimulating electrode (lower one). The layout of the experimental arrangement is presented in Fig. 2.

Stimuli (square constant current pulses of 0.3 sec and 25-30 sec) of increasing intensity were given successively. Resistance of 5 M was inserted in series in the stimulating circuit in order to stabilise the current (Hodgkin and Rushton, 1946). The amplitude of the stimulus provoking AP (threshold stimulus) was adopted as unity. The time intervals between the successive stimulation were: with cathodal and anodal stimuli up to about 0.5 the threshold — 15 min, with stimuli above this value — 45 to 90 min (Paszewski and Zawadzki 1976a). Illumination (white light, 2-4 $W \cdot m^{-2}$) was continuous and the temperature was within the range of 21-23°C.

RESULTS AND DISCUSSION

Typical records from three measurement series are shown in Fig. 2. The results were obtained on three different *Lupinus* plants. Application of successive stimuli of increasing intensity causes changes in the potential of the cathodal area (known as cathodal depression). Their characteristic corresponds 1° to electrotonic potentials and local responses and 2° to membrane depolarization phenomena. When the stimuli are weak, the responses increase and decline exponentially, and their amplitude stabilises at a certain level and is proportional to the stimulus intensity. With stronger stimuli, and particularly those approaching the critical value at which excitation occurs, the responses at first show

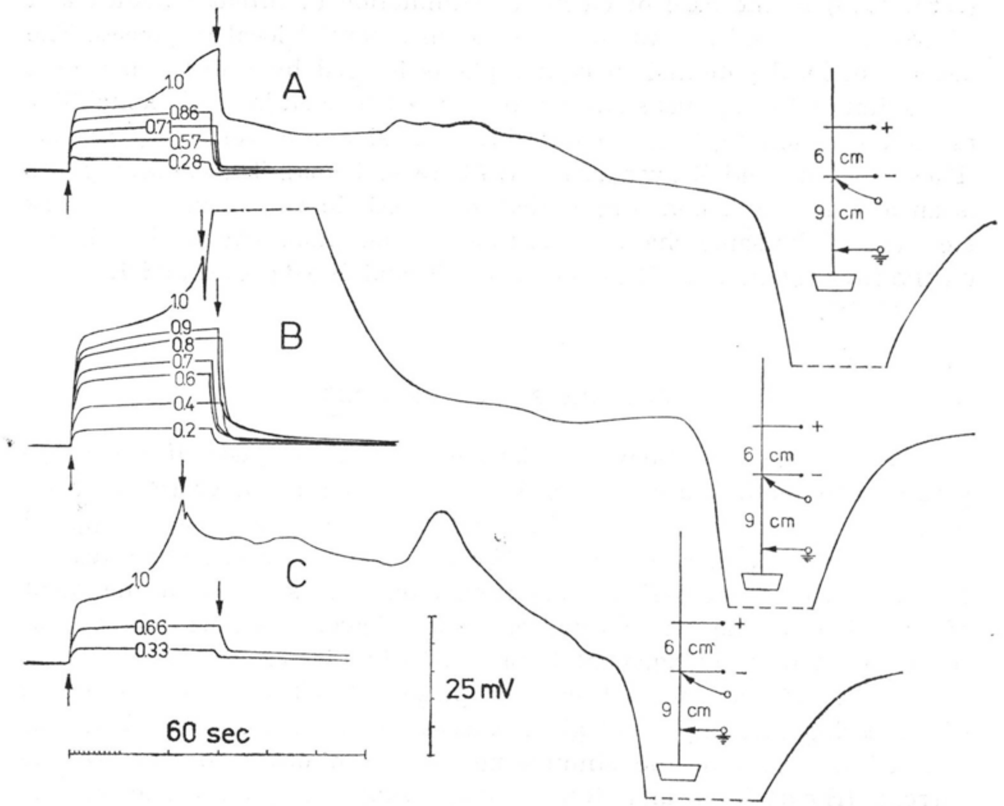

Fig. 2. Subthreshold responses and APs recorded extracellularly from *Lupinus* stem. A, B, C — three selected exemplary measurement series on three different plants. Diagrams of the experimental arrangement on right side. The figures on each record give successively the stimulus intensity in relation to threshold. The arrows indicate the moment of switching on and off of the stimulus. Electrotonic potentials and local responses developing in AP under a threshold stimulus (1.0) can be seen.

an exponential rise, and after reaching a certain value the amplitude continues to increase. Switching off of stimulation produces a slow fall of the potential.

As mentioned by Hodgkin and Rushton (1946), a precise determination of the threshold stimulus value (rheobase) is not possible. This is due to the following reasons. The intensity of the threshold value is different for individual *Lupinus* plants. The values may differ 2-3 times. In a single plant the threshold may change in the course of a day, and even between successive stimulations (2-h period). At best only slight variations of the threshold occur and the records here presented were obtained from such plants (Paszewski and Zawadzki, 1974). The threshold stimulus may evoke excitation recorded in the cathode area (Fig 2B). It may, however, produce a response which in the cathode area is of local character, but at a distance of several centimetres from this area develops into a full AP as shown in Fig. 2A and C (Hodgkin, 1939).

In the examples illustrated in Fig. 2A, B, C the values of the threshold potential were adopted as units. The proportions of their absolute values were 1.2:1.7:1.0, respectively.

We shall now consider the amplitudes of the successive responses. The character of these responses corresponds to the membrane depolarization phenomenon. It may be assumed that excitation will arise when the amplitude of the response reaches a given value. In investigations on axons this value is connected with the threshold potential of the membrane and characterizes the intensity of the threshold stimulus. In Fig. 2A, B, C the amplitudes of maximal local responses amount to about 12, 18, and 10 mV, respectively, this being in agreement with the above mentioned proportion of absolute stimulus values. To put it otherwise, a condition for excitation is the attainment of local "depolarization" (area with lowered potential as compared with that of the remaining stem area) of the value of 10 to 20 mV. Attention should be called here to the good agreement between the electrotonic potential amplitudes and local response amplitudes on the surface of *Lupinus* stem and of the crab axon (Fig. 1B).

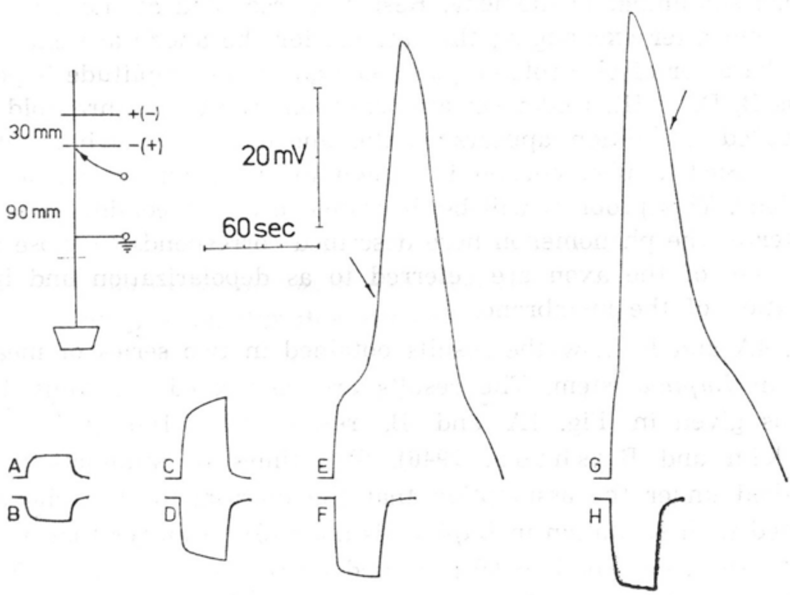

Fig. 3. Subthreshold responses and APs recorded extracellularly from *Lupinus* stem in the vicinity of the stimulating electrodes. Stimulus intensity: A and B — 0.3, C and D — 0.8, E and F — 1.0, G and H — 1.3 of the threshold value. Records A, C, E, G at cathode, B, D, F, H at anode. Arrows at E and G indicate the moment of switching off the stimulus. Diagram of experimental arrangement on left side.

Exemplary records of potential changes with cathodal and anodal stimulation in one series of measurements are shown in Fig. 3. The responses were chosen with stimuli of 0.3, 0.8, 1.0, and 1.3 of the threshold value (cf. H o d g k i n, 1939). Records A and B are symmetrical. The potential grows when the stimulus is switched on and decays exponentially when it is switched off, with amplitude directly proportional to the stimulus intensity. This is a typical example of electrotonic potentials. Records C and D are no more symmetric. The amplitude of the response obtained at the cathode (record C) at first grows exponentially and in the first few seconds is of the character of an electrotonic potential. When the amplitude of the response exceeds a certain value, the response develops further. Record C is an example of a response composed of a local response added to the electrotonic potential. In record E (stimulus 1.0) the response appears at first as an electrotonic potential, it grows (local response) and, when the amplitude exceeds the value of about 15 mV, it develops into AP. When the stimulus exceeds the threshold value (record G) the amplitude of the response reaches more rapidly its critical value. The duration of the electrotonic potential and local response is shortened, thus the response develops quicker into AP. With strong stimuli (e.g. 10-fold threshold value) AP appears so soon that it is difficult to distinguish on the record the above described subliminal phenomena. Responses recorded at the same point of the stem after exchanging the cathode for the anode are exclusively of the character of electrotonic potentials and their amplitude is positive (records B, D, F, H). Under certain conditions when the threshold value is exceeded, excitation appears at the anode after switching off the stimulus. Such a phenomenon is known in the axon as "anode break excitation". This problem will be discussed in a forthcoming paper. The character of the phenomenon here described corresponds to those which in the case of the axon are referred to as depolarization and hyperpolarization of the membrane.

Fig. 4A and B show the results obtained in two series of measurements on *Lupinus* stem. The results are eleborated according to the diagrams given in Fig. 1A and B, respectively (H o d g k i n, 1939, H o d g k i n and R u s h t o n, 1946). The times of stimulations were established under the assumption that the duration of the phenomena connected with excitation in *Lupinus* is about $5 \cdot 10^3$ longer than in axon. Thus 0.3 sec correspond to 60 µsec and 30 sec correspond to 15 msec. According to the strength-duration relation, the absolute value of stimuli lasting 0.3 sec in respect to the corresponding stimuli lasting 30 sec had to be several times higher. Therefore stimuli lasting 0.3 sec may be described as "electrical shocks" (H o d g k i n, 1939). This presentation of the results allows to follow the above described regularities

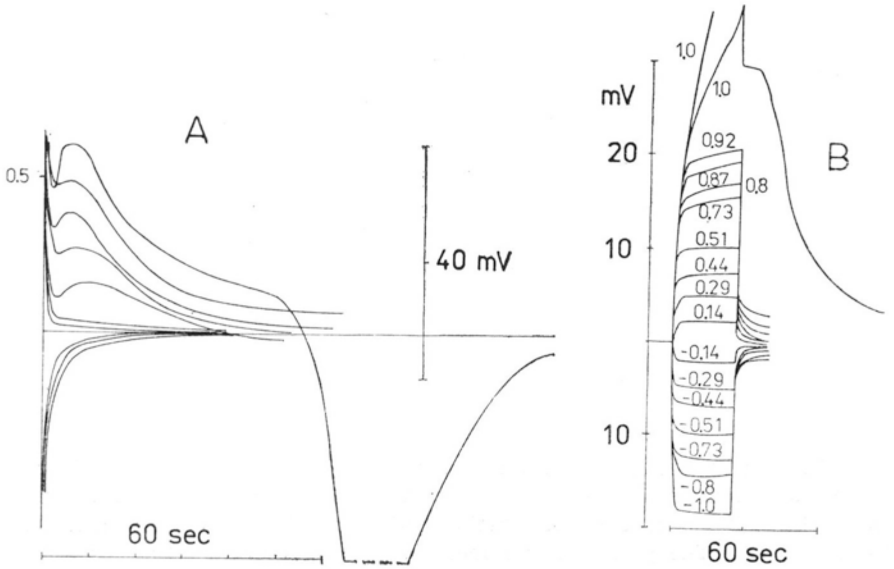

Fig. 4. Subthreshold responses and APs recorded extracellularly from *Lupinus* stem in the vicinity of the stimulating electrodes. Two typical measurement series. Square constant current stimuli. Elaboration of results as in Fig. 1A and B, respectively. A. Electrical changes produced by stimuli (0.3 sec) with relative strengths, successively from above, 1.0, 0.95, 0.88, 0.82, 0.7, 0.53, 0.23 (cathodal stimuli), —0.53, —0.82, —0.95 (anodal stimuli). The ordinate scale gives the potential as fraction of the AP amplitude, which was about 55 mV. B. Electrical changes produced by stimuli (about 25 sec) of different intensity. The figures on each record give the strength of the stimulus relative to threshold. AP amplitude was about 60 mV. Anodic stimulation denoted by minus sign.

and to compare them with the results obtained on axons. Electrotonic potentials in the vicinity of the anode and cathode up to a stimulus of about 0.5 of the threshold value, and local responses in the cathode area developing with rising stimulus intensity up to AP are distinctly visible. The curves in Fig. 1A and B, and Fig. 4A and B, respectively, show an identical character and close amplitude value but the duration of the phenomena in plants is prolonged about 10^3-10^4 times.

Fig. 5 characterizes the subthreshold responses in *Lupinus* stem without taking time into account (H o d g k i n, 1939). For anodal and for cathodal stimuli of intensities below about 0.5 of the threshold value the relation between the stimulus and the response is straight line, so that the behaviour of the system is in accordance with Ohm's law. Above this value, for cathodal stimuli, it is not so, and it has to be assumed that certain properties of the system (in the case of axon — the resistance and/or capacitance of the membrane) change during local response and AP.

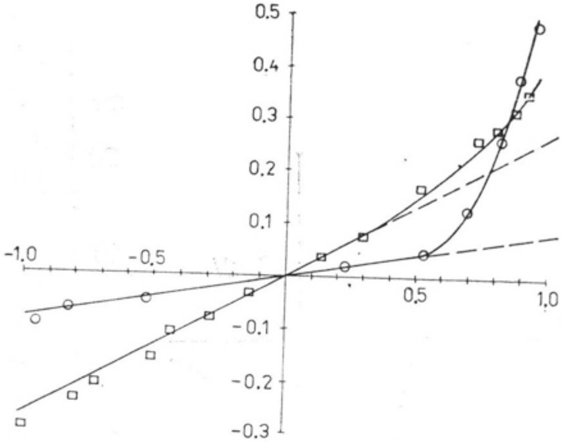

Fig. 5. Relation between stimulus and responses in *Lupinus* stem, derived from Fig. 4A and B. Ordinate — potential as fraction of AP amplitude. Abscissa — stimulus intensity as fraction of threshold stimulus. Curve ○ corresponds to results in Fig. 4A. The potential was measured 5 sec after the stimulus. Curve □ corresponds to the results in Fig. 4B. The potential was measured about 20 sec after switching on the stimulus.

The here presented results may be summed up as follows. Under experimental conditions analogous as in the case of axon there appear subthreshold responses in *Lupinus* stem. Their characteristic is identical as that in axon. The slower course (10^3 to 10^4 times) of the excitation processes in plants (e.g. AP, propagation velocity, refractory periods) as compared with that in axons or muscle fibres as well as the different rates of these processes in various types of axons or muscle fibres seem to be a general feature of excitation in various biological systems and they result from their different resistance-capacitance properties. It may be assumed that from the electrophysiological point of view the description of the phenomenon of excitation in higher plants is quite possible with the use of neuroelectrophysiological nomenclature and notions (Paszewski and Zawadzki, 1973, 1974, 1976a). There are good grounds to suppose that the passive behaviour of *Lupinus* stem may be described by the equations of the cable theory, as has been done for the axon by Hodgkin and Rushton (1946). The main difficulty lies, however, in the fact that at present the structure of tissues in the stem responsible for excitation cannot as yet be indicated (Sibaoka, 1966, Paszewski and Zawadzki, 1976b). Therefore an electrophysiological model of the stem cannot be suggested as has been done for axon. The solution of this problem would be equivalent in the author opinion, with the demonstration of the existence of an excitable system in higher plants.

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Elektryczne własności łodygi Lupinus angustifolius L.
I. Potencjały podprogowe

Streszczenie

Badano potencjały podprogowe i czynnościowe w łodygach *Lupinus* jako odpowiedzi na podprogowe i progowe bodźce (prostokątne impulsy prądu stałego). Wykazano występowanie potencjałów elektrotonicznych i odpowiedzi lokalnych. Ogólna charakterystyka potencjałów podprogowych oraz wielkości ich amplitud są takie same, jak w przypadku wyizolowanych aksonów kraba (Hodgkin, 1939; Hodgkin i Rushton, 1946). Przebieg zjawiska jest jednak około 10^3-10^4 razy wolniejszy u roślin.