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"Action Potentials" in Nematode-Induced Plant Transfer Cells

Brief Report

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With 1 Figure

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Summary

In a study of membrane potential properties of giant cells induced in the roots of *Impatiens balsamina* by a rootknot nematode, trains of action potential-like fluctuations were recorded. Giant cells are multinucleate transfer cells, and it is suggested that the occurrence of action potentials may be characteristic of transfer cells.

1. Introduction

The occurrence of electrical events in membranes of non-nervous, non-muscular cells ("neuroid" activity) probably arose before the evolution of nerve cells (MACKIE 1970). In cells of lower plants action potentials (AP) have been recorded from *Noctiluca* (ECKERT 1965), *Acetabularia* (NOVAK and BENTRUP 1972), *Chara* and *Nitella* (SIBAOKA 1966). The occurrence of AP's in higher plants is well documented for the highly specialized group which exhibit rapid leaf movements—*Dionea, Drosera, Mimosa,* and *Biophytum* (SIBAOKA 1969, WILLIAMS and PICKARD 1972). They have also been reported from the sensitive stigmas of *Incarvillea* (SINYUKHIN and BRITIKOV 1967) and nectaries of *Tilia* (MOLOTOK, BRITIKOV, and SINYUKHIN 1968). Trains of AP-like fluctuations in potentials of surface recordings from non-motile plants thought to originate from intracellular propagated AP's have been reported from several widely separated genera; this work has been reviewed by PICKARD (1973), who stresses the possibility that electrical control mechanisms in cells less specialized than those of animal nerve cells may play significant co-ordinating roles.

We have been studying transmembrane potential properties of giant cells induced in *Impatiens balsamina* by the root knot nematode (*Meloidogyne*

incognita) (JONES, NOVACKY, and DROPKIN 1973). Giant cells are multinucleate transfer cells that possess typical wall ingrowths, lack a central vacuole, and evidently have an extremely active metabolism (JONES and NORTHCOTE 1972, PATE and GUNNING 1972). In our material giant cells are 100–350 μm long by 100 μm wide. We report here the first description of AP-like fluctuations in the membrane potential of clearly identified transfer cells.

2. Material and Methods

Root segments of dwarf balsam (*Impatiens balsamina*) with 10–15 day old galls containing giant cells were mounted in a plexiglass holder in a chamber with flowing bathing solution. The solution, 10 × (ETHERTON 1968), contained in mM: KCl, 10; $Ca(NO_3)_2$, 10; $MgSO_4$, 2.5; NaH_2PO_4, 9.04; Na_2HPO_4, 0.48; pH 5.5; 1 × solution contained one-tenth the concentration of these solutes. The glass micropipette (tip diameter < 0.5 μm) was filled with 3 M KCl, and the reference pipette (diameter 1 mm) was filled with 3 M KCl solidified with 2% agar. The pipettes were connected via Ag/AgCl electrodes to a Keithly 604 electrometer amplifier (10^{14} Ω input resistance). The micropipette was advanced into the tissue by a micromanipulator, and the position of the tip was observed through a microscope by transmitted light. During an experiment the electrometer output was continuously recorded on a Gould Brush 220 chart recorder. Balsam was chosen as host plant since it has translucent roots in which the giant cells can clearly be identified.

3. Results and Discussion

The intracellular membrane potential recordings we obtained represent the potential across the plasmalemma alone, and not the combined potentials of the tonoplast and plasmalemma as measured in most higher plant studies. In this case the tip of the micropipette comes to rest in the cytoplasm. Recordings from giant cells are much more stable than those from normal higher plants cells and can be maintained for many hours.

As illustrated in Fig. 1 *A*, which shows part of a train of 64 fluctuations, irregular trains of from 3 to over 100 transient depolarizations have been recorded from different giant cells. Although the time separating individual fluctuations or groups of fluctuations in a train is irregular, the time course and level of depolarization is remarkably constant (Fig. 1 *B*). The average depolarization of the fluctuations shown in Fig. 1 *A* is 24 ± 2.5 mV. Although there is little variability within a given train, for different trains the depolarization may range from 18 to 30 mV. The position of the micropipette tip in the cell probably does not affect the magnitude of depolarizations. Before the depolarization occurs there is usually a characteristic waviness in the record of 1–3 mV for about 3 seconds (Fig. 1 *C*). The main depolarization follows, often after a slight shoulder. The depolarization (10–90%) takes about 0.1 second and is followed by a slight repolarization (3 mV), then a plateau of variable length before the potential gradually increases to the original resting value. The total recovery time varies from 5–60 seconds, but in most cases takes

between 15 and 30 seconds. Frequently new depolarizations occur before recovery of the resting potential is completed (Fig. 1 *B*). However, the minimum value is the same as if the resting potential had been reached before such a depolarization. After the last depolarization of a train the resting potential is recovered again, and the trace shows no sign of instability.

The trains of fluctuations have mainly been recorded in 10× solution and were only found in 2 out of over 100 recordings from cells in 1× solution. The fluctuations may be a response of the cells to high concentrations of ions in the bathing solution. About one cell in six in 10× solution exhibits the

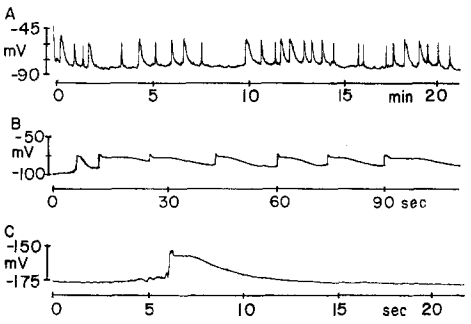

Fig. 1. Membrane potential recordings from giant cells. *A.* Start of a train of fluctuations. *B.* Expanded time scale to show the constant time course of depolarization and variable recovery phase. *C.* Time scale expanded further to show the characteristic waviness of the trace before depolarization, the slight repolarization after depolarization, the plateau and the recovery phase

phenomenon. The excitability does not seem to depend on a particular membrane potential value, since fluctuations may occur with resting potentials from — 50 to — 185 mV. (The low resting potential values were obtained from cells immediately after excising the root segment, and higher values after incubating the segment in aerated bathing solution [aging] for 20 hours—this will be reported in a subsequent paper.) Trains of fluctuations were usually initiated mechanically—by insertion or vibration of the micropipette and also by increasing the ionic concentration of the bathing solution from 1 to 10×. However, we are unable to induce these trains at will; it appears that the cell must be in a particular physiological state before excitability can be exhibited. AP-like fluctuations were not recorded from normal cells of the same root.

Membrane potential fluctuations were recorded from giant cells associated with 2nd-stage nematode larvae, moulting larvae, and young adults. Since moulting larvae shed their stylets and cannot feed for a period of about 3 days (BIRD 1959), the fluctuations are not associated with nematode feeding.

The regularity and repeatability of recorded fluctuations from giant cells, and the fact that they may be induced by changing the ionic composition of the bathing medium during a recording, strongly suggest that the fluctuations are not artifacts caused by regular plugging and unplugging of the micropipette tip (SLAYMAN 1965) but reflect membrane properties of giant cells. The propagated AP's recorded from the surface of the sensitive plant *Biophytum* (SIBAOKA 1973) have remarkably similar form to giant cell fluctuations. Furthermore, MOLOTOK *et al.* (1968) have made intracellular potential recordings from the vascular bundles and associated secretory cells of the nectaries of lime (*Tilia cordata*). They reported that after stimulation of the nectaries a train of AP's, with similar characteristics to giant cell fluctuations (amplitude 60–80 mV in the phloem cells and 30–40 mV in the secretory cells) was followed by secretion of nectar. Presumably the cells which produce nectar are secretory transfer cells (PATE and GUNNING 1972). In which case this type of membrane potential fluctuation may be characteristic of both secretory and absorptive transfer cells. Whilst the function of AP's in secretory cells is correlated with nectar secretion, a similar functional correlation in giant cells has yet to be found. Further studies of the function of AP-like fluctuations in giant cells are in progress.

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