

CHANGES IN ELECTRICAL CONNECTION DURING CELL FUSION IN THE HELIOZOAN, *ECHINOSPHAERIUM AKAMAE*

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SUMMARY

The electrophysiology of cell fusion in the heliozoan, *Echinospaerium akamae*, was studied by intracellular recording from two unicellular organisms undergoing fusion. Fusion was preceded by the electrical connection of axopodia of each cell to the cell body of the other. In the early stages of the fusion process, spikes evoked in one cell body failed to invade the other, but electrotonic potentials (subthreshold depolarizations) did pass to the other cell. When background depolarizing currents were injected into the organism into which the potentials had invaded, these potentials developed into spikes. In advanced stages of fusion, spikes were transmitted in both directions from one organism to the other, in the absence of polarizing current. At this time, application of appropriate hyperpolarizing currents to either of the two organisms prevented spikes produced in one from invading the other. These results suggest that in the early stage of fusion, relatively few axopodia were bridged by membrane fusion between paired cell bodies, and that the number of such bridged axopodia increased as fusion proceeded, allowing spikes to be transmitted between the two organisms.

INTRODUCTION

A unicellular heliozoan, *Echinospaerium*, composed of a globular cell body from which radiate hundreds of needle-like axopodia, characteristically shows bicellular fusion, termed plasmogamy. According to Shigenaka & Kaneda (1979), cell fusion can be divided into at least two stages; the early stage, in which reciprocal axopodia react either with each other or with the surface of the partner's cell body, and the late stage, in which paired cell bodies react with each other. It has been suggested that these axopodial reactions in the early stage are necessary for fusion of cell bodies (Shigenaka, Maruoka & Toyohara, 1978; Toyohara, Maruoka & Shigenaka, 1977).

Key words: heliozoan, cell fusion, electrical connection.

However, it has not been clarified whether such an axopodial reaction is associated with functional changes between membranes of the reciprocal axopodia or between those of the axopodia and the cell body. Heliozoans are capable of producing action potentials, and their membranes are periodically hyperpolarized in conjunction with contractions of their contractile vacuole (Nishi, Kobayashi & Shigenaka, 1986).

In the present study, the electrical coupling between two organisms undergoing cell fusion was investigated by intracellular recording from the cell bodies. Preliminary results of some of these experiments have been presented previously (Nishi *et al.* 1988).

MATERIALS AND METHODS

The *Echinospaerium* species used in the present experiments was cultured as described previously (Nishi *et al.* 1986). Several organisms were placed in about 2 ml of physiological saline in a chamber set on the stage of an inverted microscope. Cell fusion occurred spontaneously in the normal saline, which was composed of 3 mmol l^{-1} each of KCl, CaCl_2 and MgCl_2 , and 5 mmol l^{-1} Tris, pH 7.2.

Intracellular recordings were made simultaneously from the cell bodies of two organisms undergoing fusion. The electrodes were glass microelectrodes filled with 3 mol l^{-1} KCl (resistance 15–30 M Ω). Current was applied through each electrode *via* a bridge circuit. The indifferent electrode was an agar–saline bridge. The methods used to supply current and to measure its intensity have been described previously (Nishi *et al.* 1986). Electrical signals were displayed on an oscilloscope, stored in a data recorder (Sony, DFR 3515), and redisplayed on an inkwriting pen-recorder. All experiments were carried out at room temperature, 18–23°C.

RESULTS

Electrical connections during cell fusion

Cell fusion between two organisms takes 2–3 h to complete. Shigenaka & Kaneda (1979) have divided the fusion process into the following four stages; stage A which lasts until tip-to-tip axopodial contact; stage B which lasts from then until axopodial tip contact with the partner's cell surface; stage C which lasts until cell-to-cell contact; and stage D which lasts from then until completion of fusion (see Fig. 1). The time required for each stage varied markedly from one pair of organisms to another. The series of experiments which follow (Figs 2–4) was designed to investigate the electrical connection that occurred between the organisms during these different stages.

Membrane potentials showed spontaneous hyperpolarizations (15–20 mV in amplitude, 0.1–1 s in duration), which correlated with contraction of the contractile vacuole, and action potentials (50–70 mV in amplitude), which were either spontaneous or occurred on the rebound from a hyperpolarization. The hyperpolarizations are termed H-CV (Nishi *et al.* 1986). In simultaneous recordings from the two

cell bodies during stage A, spikes and H-CV produced in one cell could not be detected in its partner. A similar situation was observed during the early part of stage B, i.e. at a stage similar to that shown in Fig. 1A, although the tips of axopodia were in contact with those of the other cell.

During the middle of stage B, however, action potentials produced in one cell body could be detected as electrotonic depolarizations (less than 5 mV in amplitude) in the other cell (Fig. 2Ai,Bi). To investigate the efficiency of such propagation, one cell's steady-state hyperpolarization response (V_1) to injected current was compared with the hyperpolarization (V_2) which spread as a result to the other cell (Fig. 2Aii,Bii), yielding an electrical coupling ratio between the two organisms with a value of 0.1–0.15. The coupling ratio between H-CVs had a similar value.

During the early part of stage C, roughly at the stage shown in Fig. 1B, a few spikes evoked in one cell body spread to the other as depolarizing deflections of about 10 mV in amplitude (Fig. 3), larger than those shown in Fig. 2. Moreover, other spontaneous and rebound spikes after H-CVs elicited in either cell body were

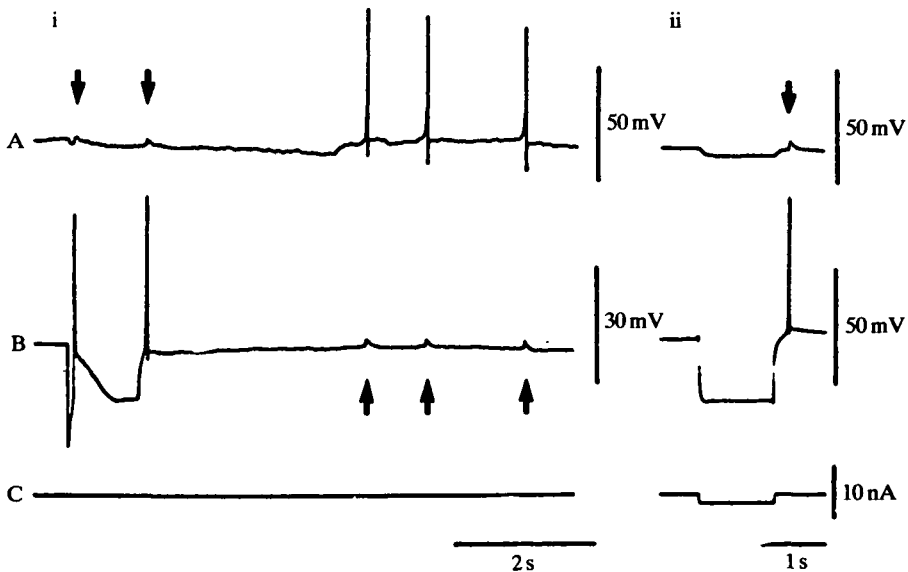


Fig. 2. Simultaneous recordings from a pair of heliozoan cell bodies at a stage of the fusion process between those shown in Fig. 1A and 1B, showing electrical connection. Traces A and B are membrane potentials from the two cells, showing spontaneous spikes and rebound spikes after H-CV (shown in B). Hyperpolarizing currents (shown in C) were applied to measure the coupling ratio between the organisms (ii). The coupling ratio was also measured using H-CV. In this and all subsequent figures one electrode was used for both current injection and potential recording by means of a bridge circuit. Thus the steady-state hyperpolarizing response to current injection was overestimated owing to unbalanced compensation for potential changes occurring in the bridge circuit. Arrows show depolarizing deflections spreading into one of the paired organisms, occurring as a result of spontaneous or rebound spikes evoked in its partner. Note that records A and B were taken at different amplifications. Initial resting membrane potential was -35 mV in A and -40 mV in B.

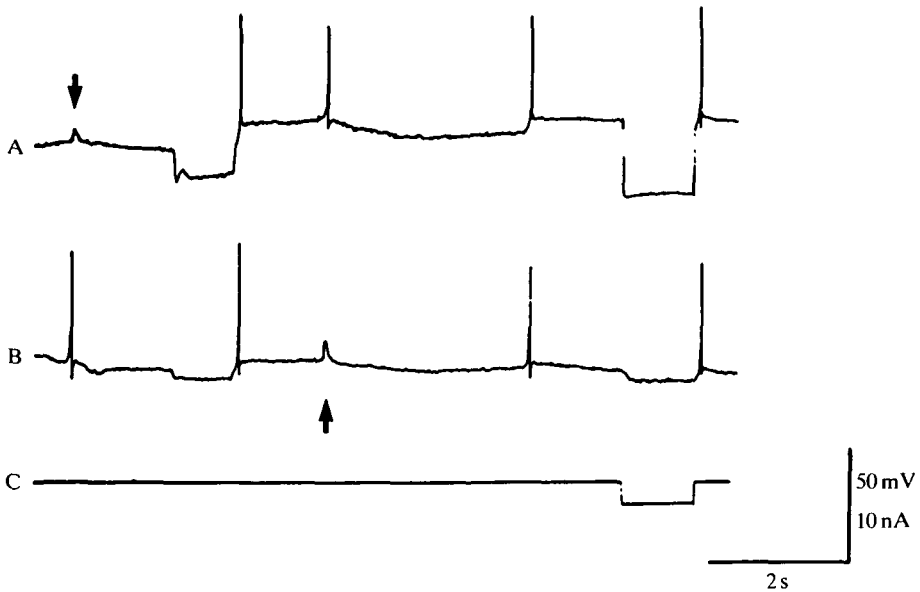


Fig. 3. Simultaneous recordings from two heliozoan cell bodies (A,B) at a stage of the fusion process similar to that in Fig. 1B. All symbols are the same as in Fig. 2. Note that depolarizing deflections shown by arrows are larger than those in Fig. 2. Initial resting potential was -35 mV in both A and B.

followed by spikes in the partner's cell body. Recordings taken at faster speeds revealed a constant delay between spikes in paired cells of about 40 ms, suggesting their active conduction from one cell to the other (not shown, but see Fig. 5). At this time, the coupling ratio of the electrotonic potential was 0.2–0.3 (Fig. 3). Similar experiments during this stage showed that the coupling ratio was not affected by the direction of the potentials.

These results suggest that electrotonic depolarization of spikes developed into action potentials when they reached a critical amplitude, thus allowing the spikes to be conducted through the axopodia from one cell body to the other. When cell fusion proceeded further, to the late part of stage C (as shown in Fig. 1C), the electrical coupling ratio reached 0.4–0.5, regardless of the direction of spreading, and almost all action potentials were conducted to the partner's cell (Fig. 4). In this process, a constant delay of about 10 ms existed between a spike appearing in one cell and its subsequent appearance in the other (not shown, but see Fig. 5), suggesting that spikes still conduct between the paired cells. Finally, in stage D (Fig. 1D), the coupling ratio reached 1.0, so that spikes and H-CVs were synchronized in the fused cell body just as if it were a single cell (data not shown).

These data support previous observations that during cell fusion, the cytoplasm of some axopodia in one cell is connected with the cytoplasm of axopodia or cell body of the other cell by membrane fusion before the direct connection of paired cell bodies (Nishi *et al.* 1988).

Effect of membrane polarization on electrotonic and action potentials

The above results suggest that in organisms undergoing cell fusion both electrotonic and action potentials were able to propagate through the axopodial membrane to the partner's cell body before their bodies began to fuse.

If depolarizing deflections recorded in a heliozoan cell body are a reflection of axopodial spikes, blocked in the axopodia at some distance from the cell body by the loading effects of the resting non-excited cell body membrane (Tauc, 1962; Tauc & Hughes, 1963), then these deflections should develop into full spikes when this loading effect is weakened by application of a depolarizing current to the cell body membrane. To investigate this, recordings were obtained from paired cell bodies in the middle of stage B (Fig. 5A,B). A spike evoked in one cell body (B) became a small depolarizing deflection in the other (A), as already shown in Fig. 2. When the cell body recorded in A was depolarized, however, the amplitude of this deflection increased slightly (Aii), and further depolarization resulted in the production of a full spike, indicating a discharge by the cell body itself (Aiii).

Conversely, propagated spikes could be diminished by applying a hyperpolarizing current to the membrane. Fig. 5C,D shows the effect of background hyperpolarization on spikes propagated from one cell body (D) to the other (C) during the early part of stage C. Characteristically, a prominent inflection was observed on the rising phase of a propagated spike (Fig. 5Cii), probably representing dissociation of an axopodial spike from a cell body spike. Further hyperpolarization prevented the cell body discharge, leaving only a small depolarizing deflection (Fig. 5Ciii), and indicating blockage of axopodial spike propagation.

The amplitude of depolarizing deflections occurring as a result of axopodial spikes was larger at later stages of fusion (Figs 3, 5C) than at earlier ones (Figs 2, 5A),

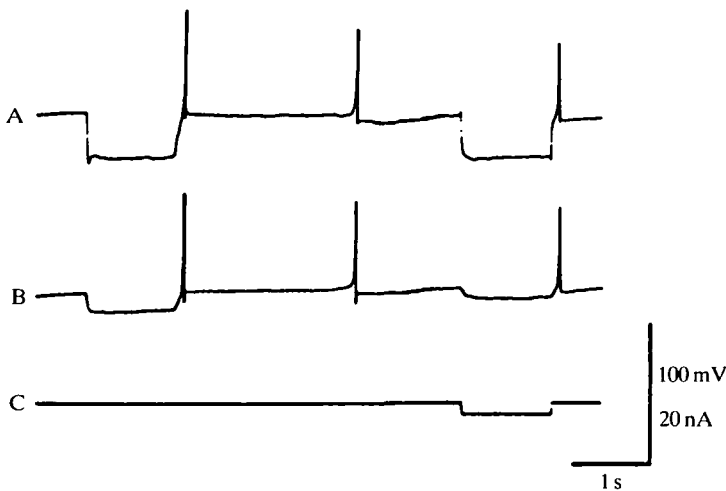


Fig. 4. Recordings from paired heliozoan cell bodies (A,B) at a stage of the fusion process similar to that in Fig. 1C. All symbols are the same as in Fig. 2. Initial resting potential was -40 mV in A and -38 mV in B.

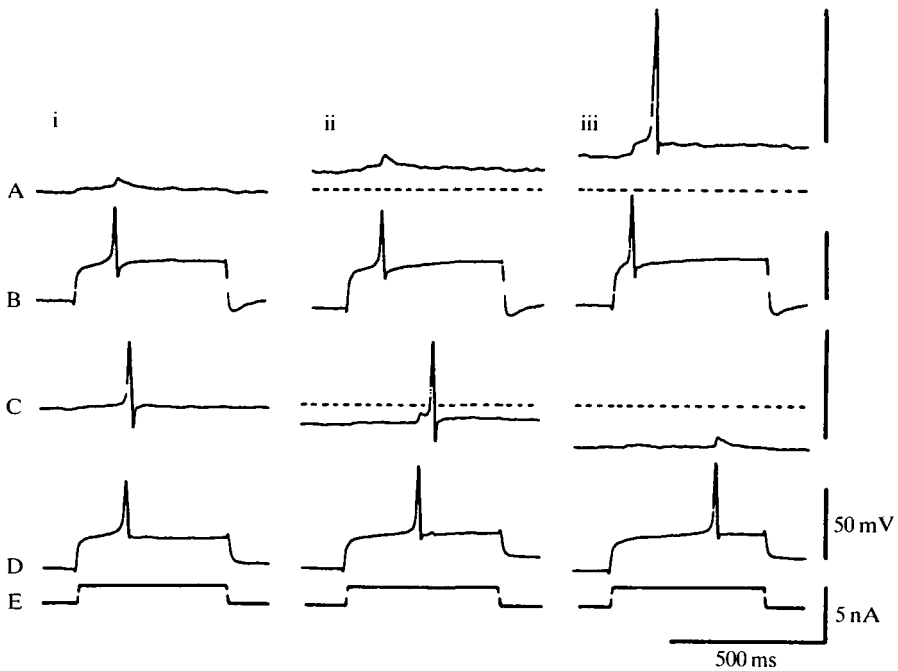


Fig. 5. (A,B) Effect of background depolarization on the electrical deflections spread from one cell body (B) to the other (A) at a stage of the fusion process between those of Fig. 1A and 1B. (C,D) Effect of background hyperpolarization on spike propagation from one cell body (D) to the other (C) at a stage of the fusion process nearly the same as that in Fig. 1C. In records A and C cell bodies were progressively depolarized and hyperpolarized from normal (-40 mV in Ai; -30 mV in Ci) to Aii, Aiii and Cii, Ciii, respectively. Dotted lines indicate the control membrane potential. All spikes in cell bodies (B and D), with initial resting membrane potentials of -45 mV in B and -32 mV in D, were evoked by the depolarizing current pulses shown in record E.

though the paired organisms' resting potentials and membrane resistances did not change during fusion.

DISCUSSION

The results of the present study suggest that during the fusion of two heliozoan cells, changes in electrical coupling and spike propagation are related to the morphology of the axopodia and cell bodies.

At the start of cell fusion, stage A and early stage B, electrical changes in a cell body could not be detected in its partner. At slightly more advanced stages, however, electrical coupling between paired organisms appeared, and the coupling ratio gradually increased from 0.1–0.15 in the middle of stage B, to 0.2–0.3 in early stage C, though no changes in external morphology were observed. A previous preliminary electron-microscopic study (Nishi *et al.* 1988) suggests that such coupling ratios

resulted from membrane fusion of reciprocal axopodia resulting in bridges between the cell bodies. Such membrane fusion seems to be compatible with the data showing that electrical coupling occurred independently of the direction of spike or electrotonic potential.

When the coupling ratio was only 0.1–0.15, spikes evoked in one organism failed to invade its partner, only a small depolarizing deflection (due to blockage of propagating axopodial spikes) reaching it. A rise in coupling ratio to 0.2–0.3 often made possible conduction of the spike to the partner's cell body. In these cases, even if the spikes were not able to invade the partner's cell, the depolarizing deflection recorded in the partner (due to spread of axopodial spikes) was always larger than when the coupling ratio was lower. When the coupling ratio increased to 0.4–0.5 (stage C), all spikes induced in one organism were conducted to the other.

These results suggest an analogy with the propagation of spikes between axonal branches and soma in *Aplysia* giant neurones (Tauc, 1962). Tauc & Hughes (1963) also suggested that the amplitude of the depolarizing deflection or inflection in the soma depended upon the size of the axonal branch. This suggestion has been confirmed for the axonal branches of *Onchidium* neurones visualized by intracellular cobalt dye injection (Gotow, 1975). Thus, the analogy between propagated spikes in *Aplysia* or *Onchidium* neurones and paired heliozoans allows the following explanation for the data obtained in the present study to be put forward.

In the present study, the diameter of the axopodia (2–3 μm) showed little change during cell fusion, implying that an increase in fused area must correspond to an increase in the number of axopodia fused with the opposite cell body. Thus, it is probable that when only relatively few axopodia are bridged between paired cell bodies, axopodial spikes are blocked at some distance from the cell body, and can be detected only as small depolarizing deflections in the opposite cell. When the number of bridged axopodia increases as cell fusion progresses, each deflection summates to overcome the loading effect of the non-excited cell body and to develop into spikes.

Okada *et al.* (1975) have reported that membrane potential and resistance of cultured FL cells are transiently lost during fusion with Sendai virus. However, in this study with a heliozoan, both were unchanged throughout cell fusion. Thus, the mechanism of cell fusion is unlikely to be similar in heliozoan and FL cells. Electrophysiological studies of cell fusion have also been carried out using rat and chick myogenic cells (Rash & Fambrough, 1973) and adult mollusc *Helisoma* neurones (Bulloch, 1985), though these studies did not provide information concerning the propagation of action potentials between cells.

The function of bidirectional spike propagation during cell fusion is not yet clear. However, when the action potentials of heliozoans were blocked by saline containing 10 mmol l^{-1} KCl, the two organisms often failed to fuse (unpublished observations). Therefore, the transmission of spikes between them may have carried important signals for cell fusion.

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