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Factors Responsible for Oscillations of Membrane Potential Recorded with Tight-Seal-Patch Electrodes in Mouse Fibroblasts

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Summary. In giant fibroblastic L cells, penetration of a conventional microelectrode brought about marked decreases in the membrane potential and input resistance measured with a patch electrode under tight-seal whole-cell configuration, and repeated hyperpolarizations were often observed upon penetration. Therefore, the question arose whether such leakage artifact is a causal factor for generation of the membrane potential oscillation even in giant L cells. During whole-cell recordings, however, regular potential oscillations were observed in the cells that had not been impaled with a conventional microelectrode, as far as the Ca^{2+} buffer was not strong in the pipette solution. Oscillatory changes in the intracellular potential were detected by extracellular recordings with a tight-seal patch electrode in the cell-attached configuration. Thus, the potential oscillation occurs even in the absence of penetration-induced leakage or without rupture of the patch membrane. Withdrawal of a micropipette from one cell was often found to induce marked cell damage and elicit oscillatory hyperpolarizations in a neighboring cell with a certain time lag. The longer the distance between the injured and recorded cells, the greater was the time lag. Application of the cell lysate on the cell surface also gave rise to oscillatory hyperpolarizations. After repeated applications of the lysate, the membrane became unresponsive (desensitized), suggesting the involvement of receptors for the lysate factor. The lysates of different cell species (mouse lymphoma L5178Y cells or human epithelial Intestine 407 cells) produced similar effects. The effective component was heat stable and distinct from ATP. Lysate-induced hyperpolarizations were inhibited by deprivation of extracellular Ca^{2+} and by application of a Ca^{2+} channel blocker (nifedipine) or a K^+ channel blocker (quinine) in the same manner as spontaneous oscillatory hyperpolarizations. It is concluded that the mouse fibroblast exhibits membrane potential oscillations, when the cell was activated, presumably via receptor systems, by some diffusible factors released from damaged cells.

Key Words membrane potential oscillation · whole-cell recording · cytosolic factors · wound · fibroblast

Introduction

Conventional intracellular recordings have shown that L-strain mouse fibroblasts exhibit repeated hyperpolarizations, displaying membrane potential oscillations (Okada et al., 1977). This behavior has been attributed to repeated activation of Ca^{2+}-dependent K^+ channels (Nelson & Henkart, 1979; Okada, Tsuchiya & Inouye, 1979; Okada, Tsuchiya & Yada, 1982). In fact, cyclic changes in the cytosolic Ca^{2+} concentration observed by means of Ca^{2+}-selective microelectrodes coincided with the membrane potential oscillation (Ueda, Oiki & Okada, 1986). Similar potential oscillations were also observed in normal diploid fibroblasts derived from human skin (Okada et al., 1984).

Recently a significant membrane leakage has been noted upon impalement with a conventional microelectrode in large L cell homokaryons produced by X-irradiation (Ince et al., 1984). Thus, Ince et al. (1984) raised the possibility that potential oscillations may result from Ca^{2+} leaked into the cell around the impaled microelectrode. However, no substantial increase in the intracellular free Ca^{2+} level was associated with penetration of a conventional microelectrode in giant L cells produced by polyethyleneglycol-mediated cell fusion (Ueda et al., 1986). In the present study we have examined whether penetration with a microelectrode does indeed induce a significant membrane leakage even in giant L cells produced by cell fusion, thereby causing oscillatory hyperpolarizations.

Our previous study has suggested that the potential oscillation in L cells is an event intrinsic to the cell (Okada et al., 1977). In contrast, Ince et al. (1984) concluded that membrane potentials do not oscillate prior to microelectrode impalement in L cells or macrophages. The tight-seal patch-electrode technique (Sakmann & Neher, 1983) can eliminate most of the leakage artifact expected to be produced by impalement with conventional microelectrodes. Therefore, we have attempted to explore whether oscillations of the membrane poten-

tial are present in nonimpaled L cells under whole-cell recordings or extracellular recordings with giga-seal patch electrodes.

In the course of these experiments, we have incidently found that some cytosolic factor(s) released from the injured cells triggers the potential oscillation in the remaining cells. Possible physiological functions of membrane potential oscillations are also discussed.

A preliminary account of some of these results has been given in abstract form (Oiki, Ohno-Shosaku & Okada, 1985).

Materials and Methods

The cell culture and electrophysiological techniques were identical with those described previously (Okada et al., 1977). Studies with conventional microelectrodes filled with 3 M KCl (resistance 30–50 MΩ) were performed with a pre-amplifier (input impedance 10^{11} Ω: WPI KS-700) in multinucleate giant L cells (containing 30–100 nuclei; cell diameter 30–70 μm) produced by cell fusion (3 to 12 hr after the treatment with 50% polyethyleneglycol 6000 for 1 min). The cells were incubated in a control saline composed of (in mM) 4.2 K^+, 145.5 Na^+, 144.5 Cl^-, 0.9 Ca^{2+}, 0.5 Mg^{2+}, 14 HEPES and 20 mannitol (pH 7.3). The input membrane resistance was monitored by injecting a constant current of 0.3 nA (duration about 0.5 sec) via a bridge circuit after compensating for the electrode resistance. To stimulate the cell, an outward current of 10 nA (duration 0.5–1 sec) was injected into the cell or a micro air bubble was applied to the cell surface, when necessary. The fast transients of recording potentials occurring within 50 msec after the microelectrode impalement were monitored on a storage oscilloscope (Tektronix 5113). In these experiments, changes in the input resistance were monitored by passing brief constant currents of 0.5 nA (duration 5 msec) at 25-msec intervals.

Both giant L cells and normal single L cells (cell diameter about 7 μm) incubated in the control saline were subjected to studies with patch electrodes (resistance around 5 MΩ) filled with a solution composed (in mM) of 147 KCl, 10 Na-HEPES, 10 HEPES (pH 7.3), 1 $MgCl_2$, 0.004–5.0 EGTA and an appropriate amount of $CaCl_2$ (pCa 8, 7, 6.5 and 6). The whole-cell configuration was produced by increasing negative pressure to 100–200 cm H_2O within the pipette immediately after attaining the giga-seal cell-attached mode under a negative pressure of less than 50 cm H_2O (Hamill et al., 1981). Zero-current potentials were measured under tight-seal whole-cell recordings using the above conventional pre-amplifier or a high-input impedance preamplifier (input resistance 10^{15} Ω: WPI FD-223) after adjusting the gate leakage current to around zero (far below 10^{-14} A). Zero-current potential measurements were made mainly in giant L cells, because the high intrinsic resistance of small L cells, and consequently large leak currents from the patch-electrode seal render such measurements inaccurate (Hosoi & Slayman, 1985). In some experiments, both membrane current measurements at a certain holding potential and zero-current potential measurements were alternatingly made using the voltage-clamp mode and the current-clamp mode of a patch-clamp amplifier (input impedance 10^{12} Ω: Nihon Kohden Kogyo S-3666). Extracellular recordings of changes in the intracellular potential were made with a patch pipette in the cell-attached configuration (Fenwick, Marty & Neher, 1982) using the above conventional pre-amplifier after nullifying the gate-leakage current. The input membrane resistance was monitored by injecting 20 or 1 pA (duration 1 sec) at constant intervals. A salt bridge was employed in the bathing solution to eliminate changes in the reference electrode potential during electrophysiological experiments. Electrophysiological experiments were performed at room temperature (22–26°C).

Cell lysates of L cells, mouse T-lymphoma L5178Y cells and human epithelial Intestine 407 cells were prepared as follows: The cell pellets obtained by centrifugation (900 rpm, 10 min, twice) were diluted with a control saline of the two- to threefold volume and were then disrupted by giving several freeze-thaw cycles at −20 and +37°C. The suspension was centrifuged at 3,000 rpm for 10 min and the supernatant collected. In some experiments, the cell lysates were employed after boiling at 100°C for 5 min. Cell lysates and ATP (Sigma Chemical) were focally applied by pressure to the cell surface through a blunted micropipette which was placed 200–300 μm from the cell (local application) or were administered directly to the bathing medium (bath application). Quinine (Nakarai Chemicals) and nifedipine (a gift from Bayer Yakuhin Ltd.) were dissolved in DMSO and then diluted with a control saline (×100–500). The vehicle at doses used herein did not affect the electrical properties of L cells.

All data are expressed as the mean ± SE of the mean (n: number of observations). Negative potentials (hyperpolarizations) and negative (inward) currents are plotted upward in the chart records presented herein.

Results

Leakage Artifact Due to Penetration with a Conventional Microelectrode

Fast negative-going peak-shaped potential transients observed upon microelectrode penetration are known to be indicative of leakage induced by incomplete sealing between the membrane and the electrode (Lassen et al., 1971). Ince et al. (1984) observed such transients in large L cells and macrophages produced by X irradiation. We first examined whether the leakage artifact is brought about by microelectrode penetration even in much larger giant L cells produced by polyethyleneglycol-mediated cell fusion. As shown in Fig. 1 (A–C), peak-shaped potential transients were actually observed in almost all the (32 of 35) cells upon microelectrode impalement: the recorded potential reached a peak value (about −36 mV) within several msec and decayed to a certain level (about −21 mV) in about 50 msec (Table 1). Subsequently (with a delay of several sec), the cells usually started to exhibit repeated hyperpolarizations (up to −38.2 ± 1.8 mV, n

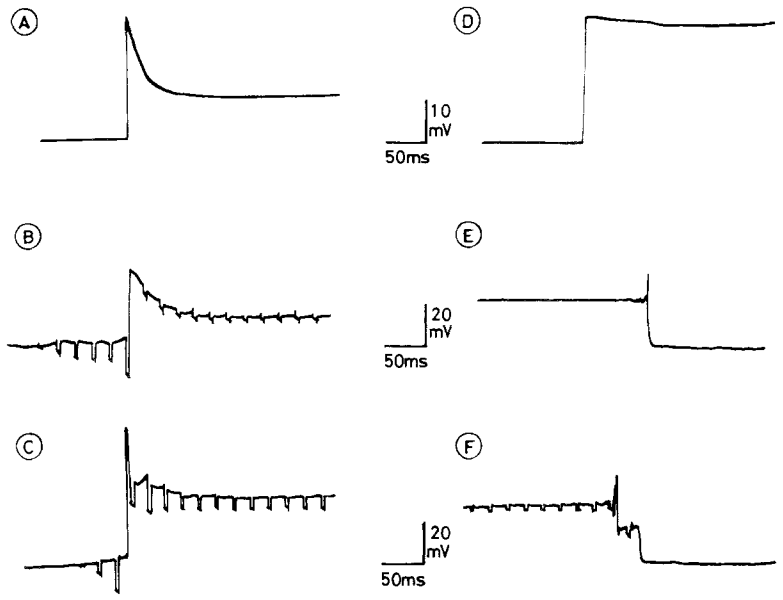

Fig. 1. Fast potential transients recorded in giant L cells with conventional microelectrodes upon their impalement (*A–D*) and withdrawal (*E,F*). Note that the input resistance (monitored by brief currents of 0.5 nA) progressively increased after pressing against the cell surface with a microelectrode and progressively decreased after the insertion (*B,C*)

Table 1. Peak transient potentials and steady resting potentials recorded in giant L cells with conventional microelectrodes upon their impalement and withdrawal

	Potential (mV)	*n*
Upon impalement		
peak transient	−36.4 ± 1.4	32
steady[a]	−20.7 ± 1.2	32
Upon withdrawal		
steady[b]	−22.5 ± 5.9	16
peak transient	−28.1 ± 2.2	16

[a] Recorded 100–200 msec after microelectrode impalement.
[b] Recorded about 100 msec before microelectrode withdrawal.

Table 2. Membrane potentials and resistances measured by whole-cell recordings and by conventional intracellular recordings in giant L cells

	Membrane potential (mV)	Input resistance (MΩ)	*n*
Whole-cell recordings[a]	−33.4 ± 0.7[b]	303.8 ± 3.6	96
Conventional recordings	−15.5 ± 0.2	36.2 ± 0.9	407

[a] Whole-cell recordings were made with patch electrodes filled with solutions of *p*Ca 6–7 containing 1–5 mM EGTA.
[b] This value should be corrected for the junction potential (about 7 mV, *see* text).

= 29) displaying oscillations of the membrane potential. These transients often showed an exponential decay (Fig. 1*A,B*), but occasionally did not (Fig. 1*C*). Irrespective of the form of the transient decay, the input resistance monitored by passing brief currents (0.5 nA, 5 msec) was usually progressively decreased during the transient potential changes (Fig. 1*B,C*), except for a few cases. The peak transient was often (16 of 35 observations) observed also upon withdrawal of a microelectrode (Fig. 1*E,F*; Table 1). This suggests that the peak transients may not merely reflect leakage artifact.

In large L cells and macrophages produced by X irradiation, Ince et al. (1984) always observed such a peak-shaped transient, but never a step-shaped one, which might be expected to occur during hyperpolarizations induced with a large increase in the membrane conductance. In our case, step-shaped transients were observed in 3 of 35 giant L cells examined (Fig. 1*D*).

The effects of impalement with a conventional microelectrode were examined by whole-cell recordings with a tight-seal patch electrode. Both the membrane potential and the input resistance were found to decrease, to a great extent, upon penetration (Fig. 2). Similar penetration-induced decreases in the membrane potential have recently been observed in combination with whole-cell current-clamp measurements in cultured human monocytes (Ince et al., 1986). Because of junctional potentials at the tip of patch pipettes, the values of the membrane potential measured with patch electrodes were smaller (by 7.3 ± 0.5 mV, $n = 24$) than those simultaneously recorded with conventional microelectrodes (Fig. 2). As shown in Table 2, the membrane potential measured by whole-cell current

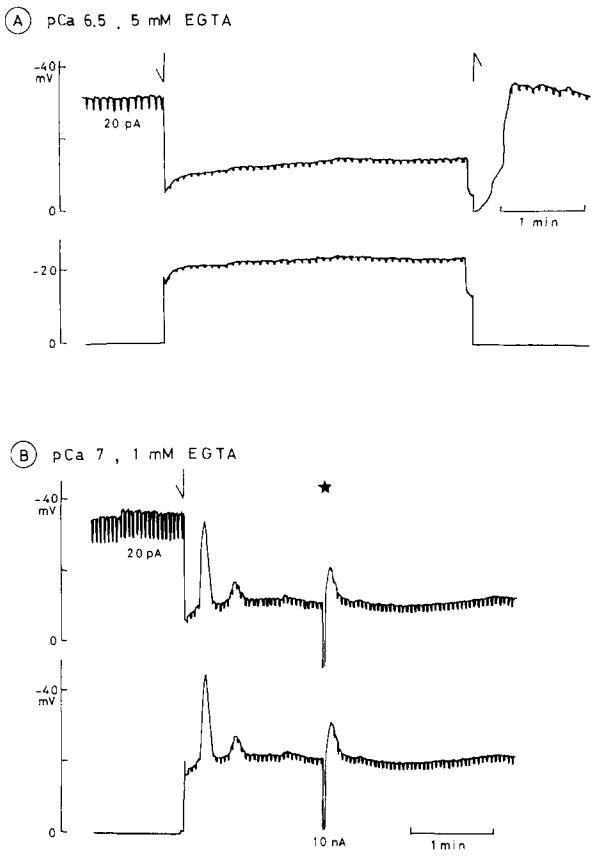

Fig. 2. Whole-cell zero-current potential measurements with tight-seal patch electrodes (upper traces) in combination with intracellular recordings with conventional microelectrodes (lower traces). A conventional microelectrode was impaled into (downward arrow) and withdrawn from (upward arrow) a giant L cell under whole-cell recordings with a patch pipette. The Ca-buffer conditions within the patch pipette are indicated. To monitor the input membrane resistance, outward current pulses of 20 pA were applied via a patch electrode at regular time intervals. To stimulate the cell, an outward current of 10 nA was injected via a conventional microelectrode (star)

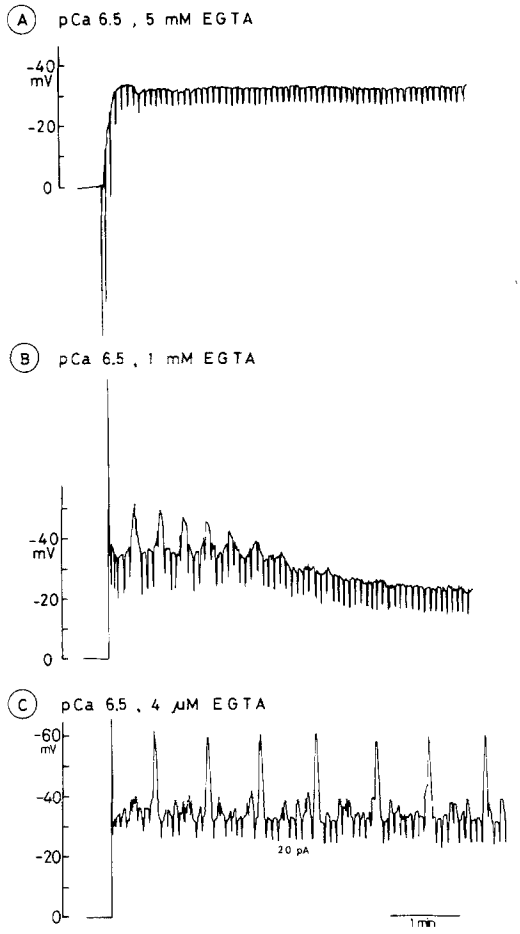

Fig. 3. Effects of the intracellular Ca-buffer capacity on potential profiles observed in giant L cells immediately after attaining the whole-cell configuration. The employed Ca-buffer conditions are indicated. Regular downward deflexions produced by injections of 20-pA pulses reflect the input resistance

clamp recordings (about −40 mV after correction for junctional potential) was higher than that obtained by conventional recordings (about −16 mV). Upon withdrawal of a conventional microelectrode from the cell under whole-cell recordings, the membrane potential and resistance decreased to zero, but showed a gradual recovery (Fig. 2*A*). These results indicate that leakage artifact is induced even in giant L cells by microelectrode penetration and more intensely by the withdrawal.

Endogenous Potential Oscillations Measured with Tight-Seal Patch Electrodes

Under whole-cell recordings using pipette solutions containing 1 to 5 mM EGTA, long-lasting potential oscillations were never observed (Figs. 2*A*,*B* and 3*A*), though single or a few hyperpolarizations could often be induced by penetrating the cells with a microelectrode (Fig. 2*B* at arrow). Similar transient hyperpolarizations were also evoked by mechanical stimulation, such as touching with a micropipette (*data not shown*), or by electrical stimulation (Fig. 2*B* at star). However, under moderate Ca^{2+}-buffering conditions using 0.1 to 1 mM EGTA, irregular long-lasting potential fluctuations (*data not shown*) or regular damped potential oscillations (Fig. 3*B*) were frequently observed after attaining the whole-cell configuration even without any stimulation. Furthermore, under weaker Ca^{2+}-buffering conditions (with EGTA less than 10 μM), sustained potential oscillations were consistently observed in giant L cells (Fig. 3*C*). These results show that potential oscillations are not necessarily associated with leakage produced by microelectrode penetration.

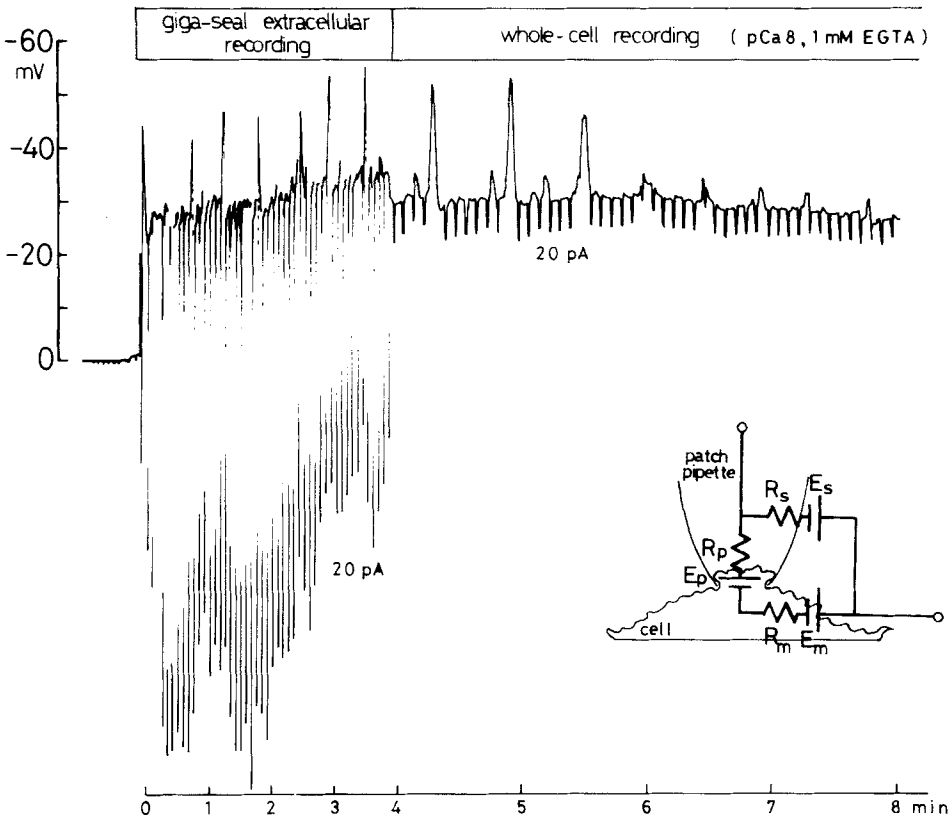

Fig. 4. Comparison between potential oscillations observed by extracellular recordings in the cell-attached configuration and by whole-cell recordings with a tight-seal patch electrode. The Ca-buffer condition in a patch pipette is indicated in parentheses. The input resistance is reflected in the downward deflections produced by regular 20-pA pulse injections (3–6 GΩ in extracellular recordings and about 0.3 GΩ in the resting state). Note the difference in time scale before and after attaining the whole-cell configuration. *Inset:* Equivalent circuit model during the zero-current potential measurements in the cell-attached mode with a tight-seal patch electrode. E, R, s, p and m designate the electromotive force (electrical potential), the resistance, the seal shunt pathway, the patch membrane and the cell membrane, respectively. The recorded potential (V) can be expressed by the following equation, since R_m is negligibly small compared with R_s and R_p in a giant cell:

$$V = (R_s E_m + R_s E_p + R_p E_s)/(R_p + R_s).$$

This equation can be reduced to $V = E_m$, provided that the patch membrane resistance R_p is much smaller than the seal resistance R_s and that the patch membrane potential E_p is approximately zero. The former situation might have been produced by some local damage in the membrane patch during the gigaseal formation (Fenwick et al., 1982). The latter situation might be induced by the relatively high conductance of nonselective pathways due to the local damage in the patch membrane. Alternatively, in the present experimental condition, the latter situation might be accomplished by a number of open K^+ channels in the patch membrane, which was interposed by two solutions containing similar K^+ concentrations

There remains a possibility that such potential oscillations recorded by whole-cell recordings might be related to the rupture of patch membranes and/or to perturbation of cytosolic conditions induced by dialysis with the pipette solutions. To test this possibility, extracellular recordings of intracellular potential changes were made with patch electrodes in the cell-attached configuration, according to Fenwick et al. (1982). As shown in Fig. 4, regular oscillatory potential changes were recorded from a giant L cell, immediately after attaining the cell-attached mode. If the resistance across the patch membrane (R_p) was much smaller than the seal resistance (R_s), as shown by Fenwick et al. (1982) in bovine chromaffin cells, and if the potential difference across the patch membrane (E_p) interposing extracellular and intracellular solutions with similar K^+ concentrations was negligibly small, extracellular recordings with a patch electrode could approximately record the intracellular potential (*see* Fig. 4, *Inset* and legend). In fact, immediately after rupturing the patch membrane, a quite similar oscillation of the intracellular potential was recorded under the whole-cell mode (Fig. 4). Amplitudes of the potential oscillation gradually diminished, presumably because of intracellular Ca^{2+} buffering by 1 mM

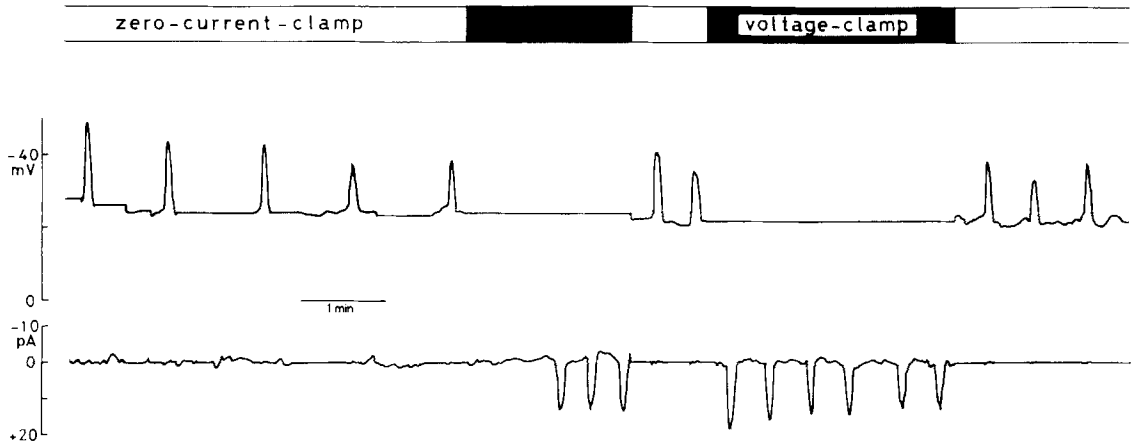

Fig. 5. Regular potential oscillations and current oscillations in a nonfused small L cell. The zero-current potential and the membrane current under voltage clamp were measured by whole-cell recordings in a single small L cell found in the polyethyleneglycol-treated monolayer containing giant L cells. The patch pipette contained 1 mM EGTA (*p*Ca 7)

EGTA in the pipette solution. These results clearly indicate that the potential oscillation is not the result of cell perturbation suffered from microelectrode penetration or from the rupture of patch membranes, but is inherent in the fibroblasts.

Induction of Repeated Hyperpolarizations by Cytosolic Factors

Regular potential oscillations were not often observed in small L cells (without the polyethyleneglycol treatment or X irradiation), as noted previously (Okada et al., 1977). This was also true under cell-attached extracellular recordings or whole-cell intracellular recordings. However, single (not giant) L cells found in the monolayer culture treated with polyethyleneglycol often exhibited regular long-lasting oscillations of zero-current membrane potentials or current oscillations under voltage-clamp conditions (Fig. 5). On the day after the polyethyleneglycol treatment, a number of giant L cells in culture dishes were found to die or exhibit low resting potentials and no halo under a phase-contrast microscope. Therefore, there is a possibility that the presence of damaged cells may cause the generation of potential oscillations in the remaining cells.

As shown in Fig. 2*B*, penetration with a conventional microelectrode could bring about several oscillatory hyperpolarizations in the cell under whole-cell recordings. An impalement of one cell with a microelectrode often induced oscillatory hyperpolarizations also in an adjacent cell under whole-cell recordings (Fig. 6*A*). No evidence for electrical coupling has been obtained in the present monolayer system (Y. Okada, *unpublished observation*). In fact, the patterns of oscillations observed in two adjacent cells were different from each other. Furthermore, touching (without impaling) one cell with a microelectrode, which mechanically evoked a hyperpolarizing response in the cell, failed to induce hyperpolarizations in the adjacent cells. Such impalement-induced responses could not be observed in the cells located at a distance of about 150 μm from the impaled cell (Fig. 6*B*). However, the withdrawal of a microelectrode could cause oscillatory responses in such cells with a certain time delay (Fig. 6*B*). Induction of oscillatory responses by impalement and withdrawal of a microelectrode apparently depended upon the distance between the impaled and recorded cells (Fig. 6*C,D*). The multiple strokes, which induced irreversible cell damage (evidenced by the loss of halo and membrane potential) could bring about responses even in a further distant cell (350 μm; Fig. 6*D*). The longer the distance between the damaged and recorded cells, the longer was the time lag for the onset of responses. These observations suggest that the spatial gradient of some diffusible cytosolic factor(s) released from damaged cells may be responsible for the generation of potential oscillations.

To examine directly whether any cytosolic factors can induce hyperpolarizing responses, effects of cell lysates on the membrane potential were observed by applying focally on the cell surface. The cell lysate of L cells could actually induce hyperpolarizing responses (Fig. 7*A* at *L*). The cell lysates of an epithelial cell line (Intestine 407) and a lymphoma cell line (L5178Y) were also effective in inducing hyperpolarizing responses (Fig. 7*B,C* at *I*

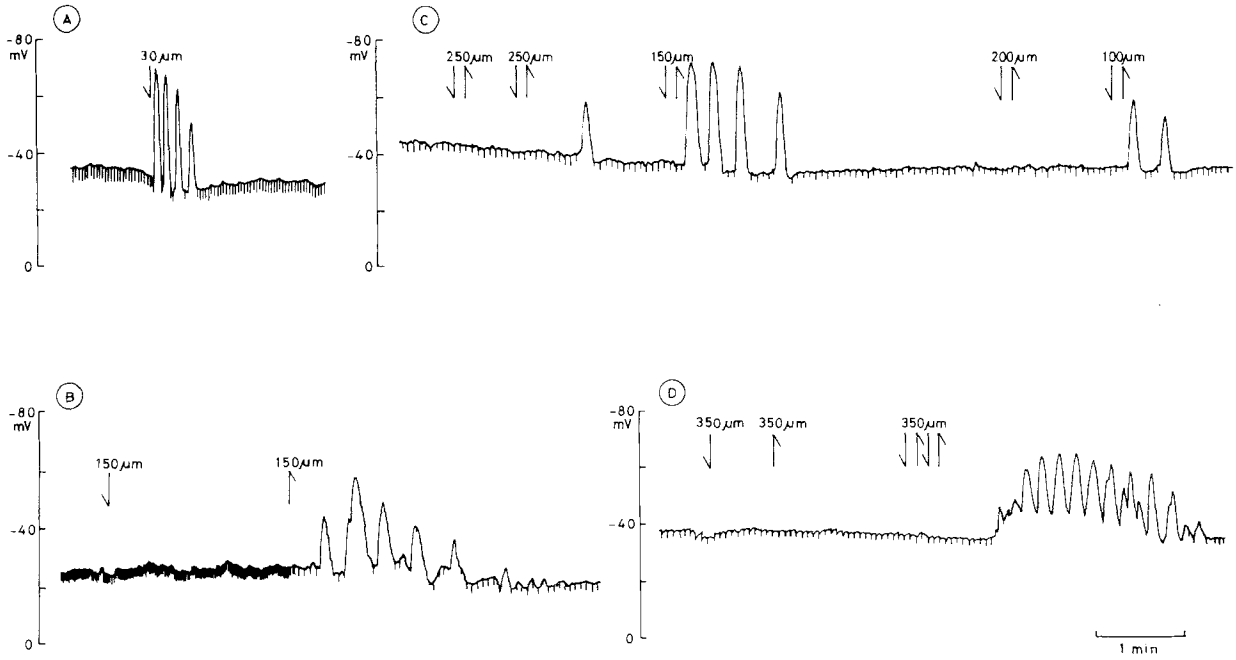

Fig. 6. Oscillatory hyperpolarizations recorded in one giant L cell under whole-cell recordings in response to the microelectrode penetration (downward arrow) into and/or withdrawal (upward arrow) from another giant L cell. *A* to *D* were recorded in different cultures. The distances between the two neighboring cells are indicated above the arrows. Changes in the input resistance were regularly monitored by injecting 20-pA pulses

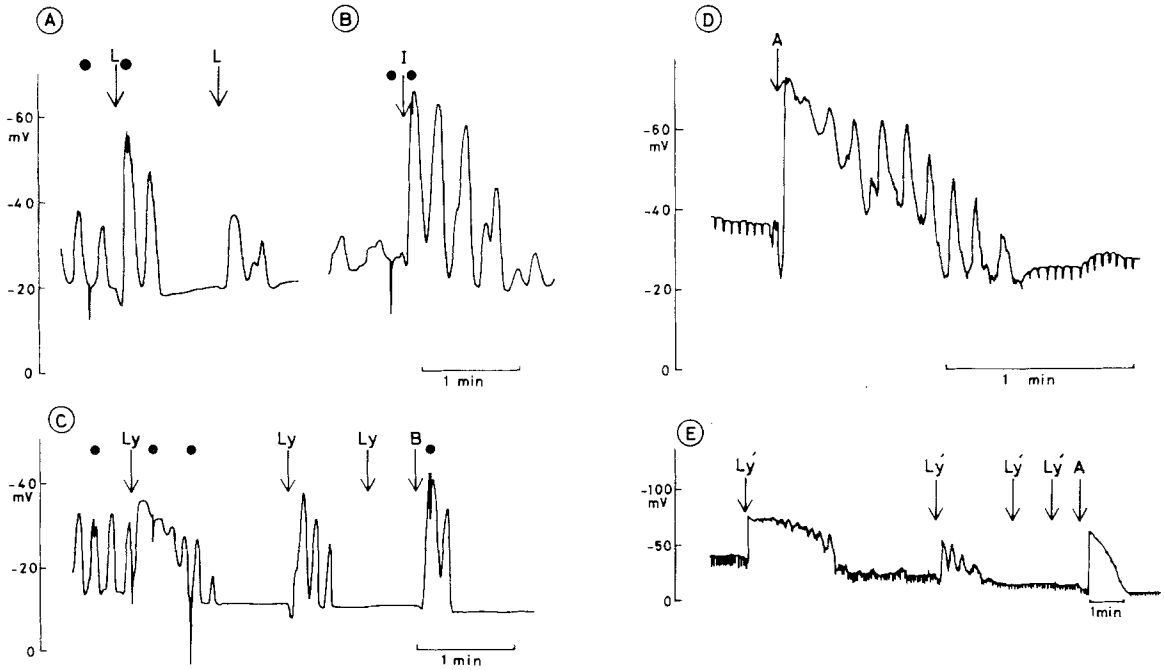

Fig. 7. Oscillatory hyperpolarizations in response to local applications of cell lysates (*A–C,E*; at arrows) or bath applications of ATP (*D,E*; at arrows). Conventional recordings were made in fused giant L cells (*A–C*). Spontaneous oscillations of the membrane potential were observed prior to the lysate application. Depolarizing currents of 0.3 nA were applied to monitor the input resistance (dots). Whole-cell recordings were made in a nonfused single L cell (*D,E*) using patch electrodes filled with a solution containing 1 mM EGTA and *p*Ca 7. Depolarizing currents of 1 pA were regularly injected via a patch electrode to monitor the input resistance. Cell lysates were prepared from mouse fibroblastic L cells (*L*), human epithelial Intestine 407 cells (*I*), and mouse lymphoma L5178Y cells (*Ly*). Boiled lymphoma lysates (*Ly′*) were also effective. Note that mechanical stimulation with a micro air bubble applied to the cell surface (*B*) and chemical stimulation with ATP (*A*) evoked hyperpolarizing responses even after desensitization to cell lysates (*C,E*)

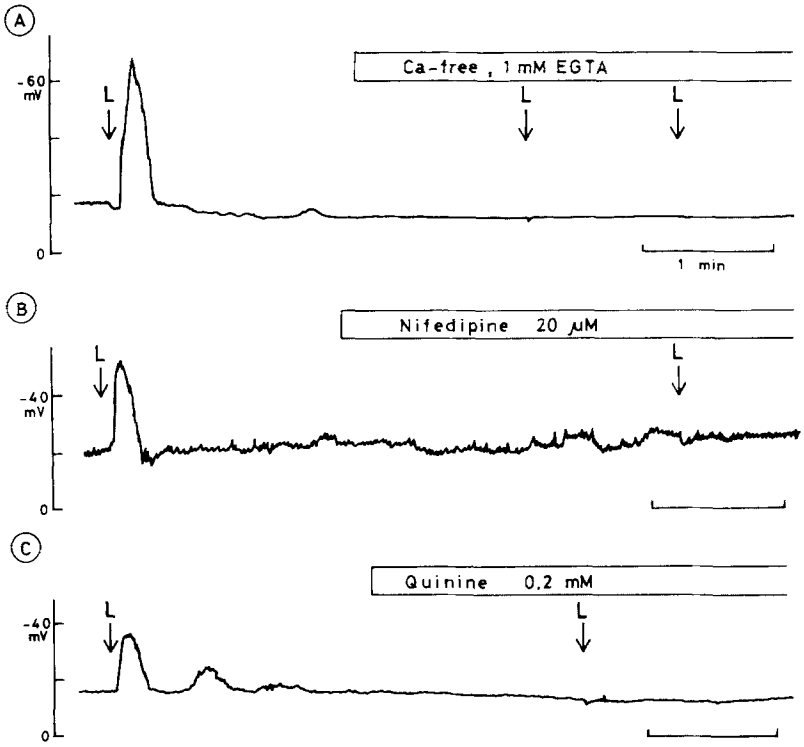

Fig. 8. Inhibition of the hyperpolarizing responses to cell lysate by deprivation of extracellular Ca^{2+} (with 1 mM EGTA added to a Ca^{2+}-free saline) and by application of nifedipine (20 μM added to a control saline) or quinine (0.2 mM added to a control saline). Whole-cell recordings were made in nonfused single L cells using patch electrodes filled with a solution containing 1 mM EGTA and *p*Ca 7. Cell lysates prepared from L cells were administered by bath applications (arrows). Using cell lysates prepared from L5178Y cells, similar data were obtained with EGTA, nifedipine and quinine

and *Ly*). After repeated applications of cell lysates, the cell membrane became unresponsive. Since the application of a mechanical stimulus could still elicit the hyperpolarizing response during this period (Fig. 7*C*), it appears that such unresponsiveness to cell lysates was not due to nonspecific cell damage. After desensitization to the lysate derived from one of these cell species, the cell could not respond to that from the other cell species (*data not shown*). ATP, which is known to interact with its own receptors in fibroblasts (Okada et al., 1984), was thought to be a candidate for the common effective component of all the lysates. In fact, ATP induced similar hyperpolarizing responses not only in fused giant L cells (Okada et al., 1984) but also in nonfused small L cells (Fig. 7*D*). However, even after desensitization to cell lysates, ATP could still be capable of inducing the response (Fig. 7*E*). Therefore, it is likely that the cytosolic factor-induced response involves receptor systems which are independent of ATP receptors. The effective component is heat stable, since the boiled lysate still induced responses (Fig. 7*E* at *Ly'*).

Lysate-induced hyperpolarizing responses were inhibited by deprivation of extracellular Ca^{2+} (Fig. 8*A*) or application of a Ca^{2+} channel inhibitor, nifedipine (Fig. 8*B*) in the same manner as spontaneous oscillatory hyperpolarizations (Okada et al., 1982). Quinine, which block the Ca^{2+}-activated K^+ conductance and spontaneous hyperpolarizing oscillations in L cells (Okada et al., 1982), also inhibited the hyperpolarizing response to cell lysates (Fig. 8*C*).

Discussion

One of the limitations of conventional intracellular recordings is that incomplete sealing at the site of microelectrode penetration may introduce a significant shunt, thereby providing a source of error in membrane potential measurements. Taking a peak transient potential recorded upon the microelectrode penetration as an indicator for leakage through the membrane-to-glass seal (Lassen et al., 1971), Ince et al. (1984) argued that a leakage artifact could be induced by an impalement with a microelectrode even in X-irradiated large L cells or macrophages of about 10 to 30 μm in diameter. In the present study, similar peak transients were also observed even in fused giant L cells of about 30 to 70 μm in diameter (Fig. 1; Table 1). Measurements of zero-current potentials and input resistances by patch electrodes in the whole-cell configuration also evidenced a significant leakage around an impaled microelectrode (Fig. 2; Table 2).

Recently it has been claimed that potential oscillations of L cells and macrophages result from leakage (especially of Ca^{2+} ions) introduced by impaled conventional microelectrodes (Ince et al.,

1984). However, several lines of circumstantial evidence have argued against this possibility. For example, (i) in a number of records, the potential oscillation could be initiated at any phase, suggesting that the oscillation is endogenous in nature (Okada et al., 1977); (ii) an increase in the intracellular free Ca^{2+} level was little observed by means of Ca^{2+}-selective microelectrodes upon penetration with a conventional microelectrode (Ueda et al., 1986); (iii) a variety of Ca^{2+} channel blockers inhibited the oscillation without impairing Ca^{2+}-activated K^+ channels (Okada et al., 1979, 1982); and (iv) a number of drugs such as cytochalasin B, local anesthetics and metabolic inhibitors blocked the oscillation without inhibiting Ca^{2+}-activated K^+ conductance (Okada et al., 1977, 1981; Tsuchiya et al., 1981*a*). In the present study, three pieces of direct evidence were given for the endogenous nature of potential oscillations in L cells. (i) Upon impalement of giant L cells with a conventional microelectrode, the membrane potential change occurred occasionally in a step-shaped manner (rather than a peak-shaped fashion) (Fig. 1*D*), which is expected during a high-conductance hyperpolarization of the pre-impaled cell; (ii) regular potential oscillations were actually observed by the whole-cell current-clamp technique with giga-seal patch electrodes, as far as Ca-buffering capacity was not very strong in the pipette solution (Fig. 3*C*); and (iii) regular cyclic potential changes were also recorded by extracellular recordings with a patch electrode in the cell-attached configuration (Fig. 4).

Oscillatory hyperpolarizings were elicited in an L cell under whole-cell recordings in response to microelectrode penetration of an adjacent cell (Fig. 6*A*). Similar observations were also made by conventional intracellular recordings by Nelson and Peacock (1973). They interpreted that these electrical responses can be transmitted from one L cell to another. This inference, however, is at variance with the following observations: (i) the patterns of oscillatory hyperpolarizations recorded in the two cells were different; and (ii) touching one cell with a microelectrode without impalement did evoke a hyperpolarizing response in the cell but failed to induce hyperpolarizations in adjacent cells. Withdrawal of a microelectrode from a cell was even more effective in triggering the electrical response in a distant cell than microelectrode penetration (Fig. 6*B*). Furthermore, several strokes of penetration and withdrawal, by which the cell was injured to suffer irreversible damage (thus to exhibit no electrical responses), could elicit oscillatory responses in another cell (Fig. 6*D*). Therefore, it appears that the responses were induced by some diffusible substance(s) released from injured cells, though the nature of the factor(s) is unknown. In fact, cell lysates focally applied to the cell surface could induce oscillatory hyperpolarizing responses (Fig. 7). Cell lysates derived from three different cell species were effective in the same manner, and, during desensitization to the lysate prepared from one cell species, the response to lysates from the other cell species was also blocked. Therefore, some common factor(s) appears to be involved in lysate-induced responses. The effective component(s), which was heat stable, would be distinct from ATP, since the cell membrane responded to ATP even after desensitization to cell lysates (Fig. 7*E*). It is known that oscillations of the membrane potential are less frequently observed in small single L cells than in nondividing giant L cells (Okada et al., 1977, 1981). This could be related to the fact that the population of nondividing giant cells treated with polyethyleneglycol or X-ray contains a number of injured cells that may release their cytosolic factors.

It is known that the fibroblasts migrate toward the site of injury and inflammation and they play essential roles in wound healing (van Winkle, 1967). Mouse fibroblastic L cells are known to have specific receptors to ATP, which should be released from injured cells, to respond with oscillatory hyperpolarizations to ATP and to show chemotaxis toward ATP (Okada et al., 1983, 1984). Cytosolic constituents other than ATP should also be delivered from injured cells residing in wound tissues. Thus, there is a possibility that not only ATP but also some cytosolic factors released from a variety of cell species in the wound are chemotactic factors for fibroblasts and regulatory mediators for activation of the fibroblast function.

It has been reported that potential oscillations may be related to Ca^{2+}-dependent membrane-mobile activities of fibroblasts such as endocytosis (Okada et al., 1981; Tsuchiya, 1981*b*) and chemotaxis (Okada et al., 1983, 1984; Oiki & Okada, 1988). Thus, the membrane potential oscillation and/or the responsible oscillation of intracellular free Ca^{2+} are likely to be endogenous in nature in activated fibroblasts participating in wound healing.

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