J. Membrane Biol. 35, 319–335 (1977)

Oscillations of Membrane Potential in L Cells

I. Basic Characteristics

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Received 30 August 1976; revised 3 March 1977

Summary. The membrane potentials and resistances of L cells were measured using a standard electrophysiological technique. The values obtained in physiological media were around −15 mV and 37 MΩ, respectively. Almost all the large nondividing L cells (giant L cells) showed spontaneous oscillations of the membrane potential between around −15 and −40 mV. Application of an appropriate electrical or mechanical stimulus was also capable of eliciting responses but such were usually induced only once. The total membrane conductance increased significantly and in parallel with such a hyperpolarizing response. Cooling of the cells and application of metabolic inhibitors to the cells completely blocked the spontaneous oscillation despite the fact that the electrically induced hyperpolarizing response remained. Intracellular K^+, Na^+ and Cl^- concentrations were measured by means of a flame photometer and a chloridometer, and the equilibrium potential for each ion was estimated.

Electrical and ionic diffusion properties of the membrane of L cells, an established cell line of mouse fibroblastic origin, were studied by Lamb and MacKinnon (1971*a, b*) who reported that Na^+ and K^+ permeabilities across the cell membrane were almost equal, and that the measured resting potentials of these cells were small (about −15 mV). They also demonstrated that the Cl^- permeability was about ten times larger than permeabilities of Na^+ and K^+, and that Cl^- was passively distributed across the membrane. Additional interesting evidence was obtained by Nelson and collaborators (Minna, Nelson, Peacock, Glazer & Nirenberg, 1971; Nelson, Peacock & Minna, 1972; Nelson & Peacock,

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1972; 1973): L cells respond to a mechanical or an electrical stimulus with a prolonged 20–30 mV increase (hyperpolarization) in the membrane potential, with a concomitant decrease in the membrane resistance.

In our experiments with large nondividing L cells (giant L cells) obtained by X-ray irradiation, we reproduced the same results; however, we also observed in almost all these cells spontaneous and stable oscillations of the membrane potential between about -15 and -40 mV. This report mainly concerns such spontaneous oscillations of the membrane potential and some characteristics observed in giant L cells are also discussed.

Part of the results has been presented in short form (Okada, Doida, Irimajiri, Tsuchiya & Inouye, 1975).

Materials and Methods

Cells

L cells maintained uncloned were used exclusively and were cultured in Fischer culture medium containing 10% bovine serum $+100$ μg streptomycin and 63 μg K-penicillin G per ml. For electrophysiological studies, the cells were plated on the glass bottom (the cover slip) of a glass chamber such as was used previously (Okada, Ogawa, Aoki & Izutsu, 1973). Sometimes glass petri dishes (9 cm in diameter) were used in place of the glass chamber. A monolayer of nondividing giant L cells can be obtained by X-ray irradiation (2000 rads), applied 2 days after plating. These giant cells were subjected to electrophysiological studies 5–12 days after X-ray irradiation. Because of their large size (30–100 μm in diameter), these cells are favorable for electrophysiological study as reported by Nelson and Peacock (1972). Some electrophysiological studies were done with nearly confluent cultures of nonirradiated L cells in which a small percentage of giant cells appeared spontaneously. For ion analyses, L cells were subcultured for 2 days in Falcon bottles (250 ml) taking care to have approximately the same cell number. Ion contents within the cells were analyzed 5–7 days after X-ray irradiation.

Electrical Measurements

Glass microelectrodes were filled with 3 M KCl by the glass fiber method as described previously (Okada & Inouye, 1976). These electrodes had resistances and tip potentials ranging from 10 to 20 MΩ and from 0 to -5 mV, respectively. Membrane potentials were measured through a high input-impedance preamplifier (W-P, M 701) and recorded on a strip chart. This equipment could also be used to send constant current pulses through the recording electrode. By carefully measuring the electrode resistance before and after penetration, such could be subtracted from the total measured resistance while the electrode was still inside the cell, thereby giving an evaluation of the membrane resistance. Sometimes the membrane resistance was measured by compensating the electrode resistance with a bridge circuit in W-P, M 701 before penetration. Because the current-voltage curves with two microelectrodes were almost linear, at least in the range near the normal potential (Roy & Okada, *unpublished observation*), the membrane resistance measurements with a single electrode would be valid as long as the electrode resistance was carefully measured

and small pulses of current were used (usually 0.3 or 1 nA). Indeed, a few measurements made with two intracellular microelectrodes showed the same results as those obtained with a single electrode. Because of the complexity of surface membrane structure (Lamb & MacKinnon, 1971*a*), no attempt was made to evaluate the specific membrane resistance.

Using acetylcholine (0.2 g/ml) and carbamylcholine (1 mM/ml) electrodes, iontophoresis of these drugs was performed by the same method as reported by Nelson and Peacock (1972).

All the electrophysiological measurements were made on an inverted phase microscope (Chiyoda Type T-2) under a magnification of 1000× or 600× as described previously (Okada *et al.*, 1973). To maintain the temperature of the cell chamber, the temperature of the microscope stage was kept at 37±1 °C by circulating warm water with a pump. The circulating warm water was sometimes stopped and cells were exposed to room temperature (15±1 °C). To reduce the temperature of cells (3–5 °C), a cell chamber (or a Petri dish) was kept in the refrigerator (2 °C) for 5 min and then placed on the microscope stage pre-cooled to 3±1 °C by circulating ice-cold water.

Ion Analyses

Intracellular K^+, Na^+ and Cl^- concentrations were estimated by the "sheet method" described by Burrows and Lamb (1962) with slight modification. After the culture medium was replaced by a phosphate-buffered saline, the cells were incubated for 10–30 min at 37 °C. This medium was then decanted and the cells were washed for a total time of 2 min in four changes of an ice-cold Ca-sorbitol solution (0.3 M sorbitol +10 mM Tris-SO_4+0.6 mM $CaSO_4$; pH=7.3±0.1), and were extracted in 5 ml of 0.1 N HNO_3 with shaking for 1–2 days at room temperature. Ion contents were measured in duplicate by flame photometry and chloridometry.

The cells were counted using a hemocytometer and the diameter was measured under phase microscopy (1000×). Applying the value (0.82) for the ratio of cell water to total volume of L cells reported by Lamb and MacKinnon (1971*a*), the intracellular ion concentrations were expressed as mEquiv/liter of cell water.

Solutions

Fischer culture medium was employed for cell cultures. In order to prevent changes in pH, Fischer-HEPES culture medium or phosphate-buffered saline (K^+=4.2, Na^+=143.0, Cl^-=132.5 mEquiv/liter and pH=7.3±0.1) was used during electrophysiological studies and ion analyses. Fischer-HEPES culture medium (K^+=5.4, Na^+=144.2, Cl^-=144.5 mEquiv./liter and pH=7.3±0.1) was prepared by replacing 13 mM bicarbonate in Fischer culture medium with 10 mM HEPES. The detailed ionic composition of the phosphate-buffered saline (PBS) was described previously (Okada, Sato & Inouye, 1975).

All data presented here were given as means ±SE.

Results

1. Membrane Potential in L Cell

On impaling the irradiated giant L cells in the Fischer-HEPES culture medium with a microelectrode, there usually appeared three phases of potential changes as exemplified in Fig. 1A; a sharp jump to a level

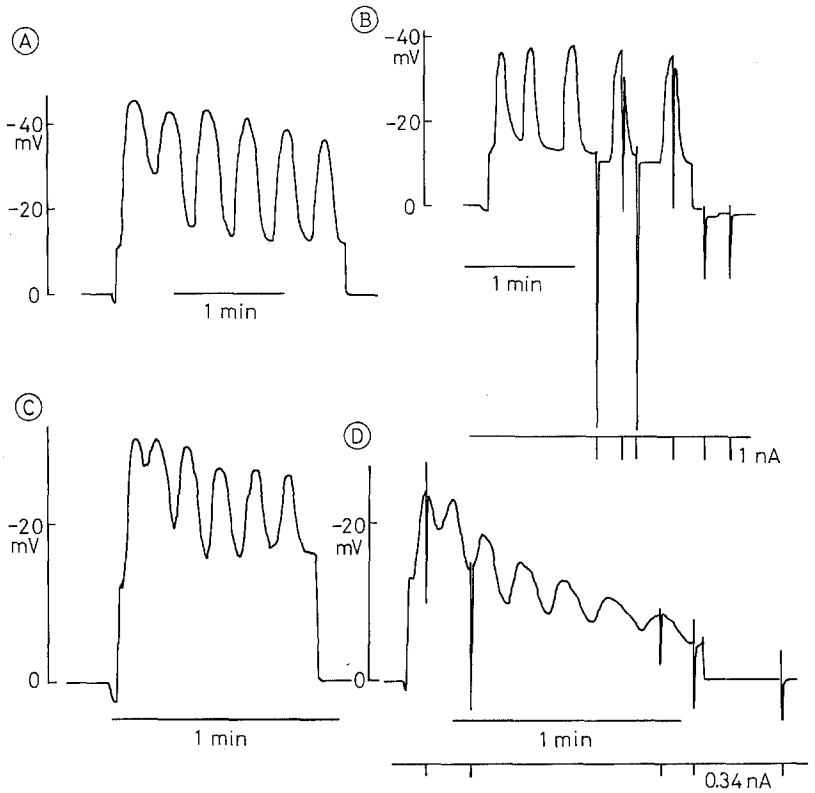

Fig. 1. Oscillations of membrane potentials in L cells. (*A* and *B*): Oscillations observed in large nondividing L cells (giant L cells). (*C* and *D*): Oscillations observed in normal L cells

of about -15 mV (the resting level; mean $= -16.1 \pm 0.3$ mV, $n = 183$), a further hyperpolarization after more or less a distinct break, which attains a level of -20 to -70 mV (the activated level; mean $= -34.6 \pm 0.9$ mV, $n = 102$) and returns to resting level. When a microelectrode was left within the cell, the potential of most giant L cells (80% or more of the impaled cells) oscillated between these two levels (sometimes for 1 hr or more without any significant decay), as shown in Fig. 1*A*. The frequency of such a stable oscillation was chiefly determined by the length of the pause period at the resting level and ranged between 1 and 7 (usually 3–4) cycles/min, being stable during one oscillation pattern obtained on a given cell. The potential oscillation was also observed in giant L cells appearing spontaneously (i.e. without X-ray irradiation) in a confluent normal L cell culture, suggesting that the irradiation is not related to the appearance of the potential oscillation.

Similar periodical changes in membrane potentials have also been found in macrophages (Gallin, Wiederhold, Lipsky & Rosenthal, 1975). Nelson and his co-workers (Nelson *et al.*, 1972) also found similar, but not so conspicuous, changes in membrane potentials only in a small number of giant L cells.

On the other hand, it was rather difficult to obtain a potential oscillation of the normal L cell in nearly a confluent state. Some cells (less than 10%) showed typical oscillations (Fig. 1*C*) just as observed in the large L cells or abortive forms (Fig. 1*D*), but the membrane potential of most cells remained at a steady level after a single hyperpolarization wave of various heights (-29.9 ± 1.5 mV, $n=54$) following the electrode penetration. Such a steady, so-called resting potential of the normal L cell in interphase was -15.5 ± 0.4 mV ($n=73$) in Fischer-HEPES culture medium, a value in good agreement with that of the resting level of the giant cell as well as with the resting potential of the normal L cell in Krebs medium reported by Lamb and MacKinnon (1971*b*; -15.4 ± 0.5 mV, $n=78$).

The mean membrane resistance of giant L cells at the resting level in Fischer-HEPES medium was 36.8 ± 1.2 MΩ ($n=183$) and that at the hyperpolarized level was 22.7 ± 1.1 MΩ ($n=97$). Thus it is obvious that spontaneous hyperpolarization is associated with a decrease in the membrane resistance (Fig. 1*B*). The mean resting membrane resistance of normal L cells in Fischer-HEPES medium was slightly higher than that of the giant cells (42.5 ± 2.1 MΩ, $n=73$). The cell diameter of the giant cell is about five times greater than that of the normal cell, therefore the latter should have a much higher (about 25 times) resistance if the specific membrane resistance is the same as that of the giant cell. Our results suggest that the giant cell has a higher specific resistance and/or that the effect of leakage produced by electrode penetration in the resistance measurement is far greater in the normal cell than in the giant one. Indeed, as seen in Fig. 1*D*, the membrane resistance measured in most normal cells rapidly decreased. Such a greater leakage depresses or abolishes the oscillation of the potential, if present, in the normal cells. These results suggest that the electrical properties of the membrane of irradiated nondividing giant L cells are not so different from those of nonirradiated normal L cells.

In the PBS medium, the giant L cells showed quite the same behavior as in the Fischer-HEPES culture medium. Fig. 2 summarizes the distributions of the resting potentials and activated potentials as well as the frequencies of oscillations observed in PBS. The membrane resistance

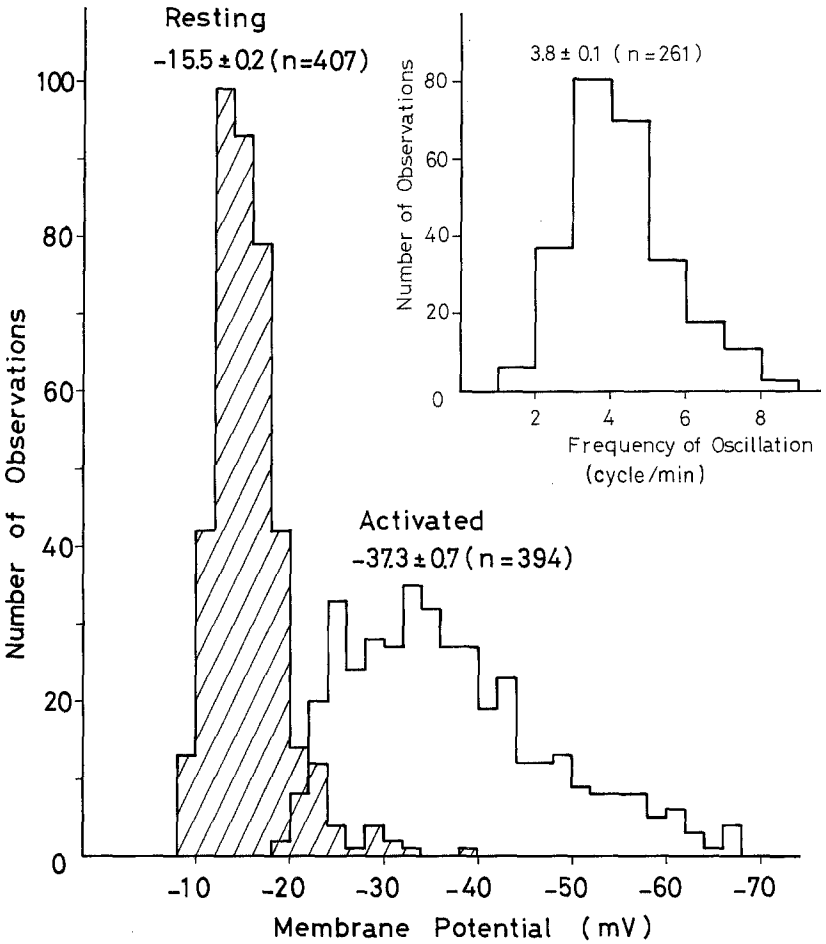

Fig. 2. Distribution of the resting membrane potentials in giant L cells in control phosphate-buffered saline. Hatched column: the resting membrane potentials. Open column: the activated hyperpolarizations. Insert: distribution of the frequencies of oscillations of membrane potentials in giant L cells in control phosphate-buffered saline

obtained in PBS was 36.2 ± 0.9 MΩ ($n=407$) in the resting state and 20.9 ± 0.8 MΩ ($n=388$) in the activated state. These values are of the same order of magnitude as those obtained in the Fischer-HEPES culture medium.

2. *Hyperpolarizing Response: Effects of Electrical, Mechanical and Chemical Stimuli*

When the membrane potential of the giant L cell remained in the resting state (i.e. at a pause period of oscillations, or in the nonoscillating

state after the decay of oscillations), it is possible to elicit a hyperpolarization with a large inward current pulse (usually 5–10 nA, 0.5–1 sec). This activated hyperpolarization response is quite similar to the spontaneous oscillatory hyperpolarization in amplitude (-33.7 ± 1.2 mV, $n=59$, in Fischer-HEPES medium) and resistance variation (18.7 ± 1.0 MΩ, $n=59$, in Fischer-HEPES medium), but the time course of the former is somewhat slower than the latter. Such electrically induced hyperpolarizing responses have been reported by Nelson *et al.* (1972) in giant L cells and by Gallin *et al.* (1975) in macrophages. The threshold current for eliciting this activated response showed considerable variation among the cells tested and ranged from 2.5 to 20 nA. Rarely was the current up to 20 nA ineffective in eliciting responses. On the other hand, electrical stimuli (up to 20 nA and lasting 1 sec) elicited no responses superimposed thereon when the cell was in the hyperpolarized state, irrespective of whether the hyperpolarization originated from spontaneous potential oscillation or from the previous electrical stimulation. A single electrical stimulation usually produced only one activated hyperpolarization, but there were a few occurrences of a train of hyperpolarizing responses but such were short-lasting and ceasing after 2–4 oscillations. Sometimes, an outward current pulse was also capable of inducing such a hyperpolarization response, but the threshold for eliciting the response was higher than that for an inward current pulse applied to one and the same cell. On some giant L cells which showed a quite stable potential, the relationship between the strength of stimuli and of responses elicited thereby was examined. The results suggested that the amplitude of the response was independent of the intensity of the stimulating current (i.e. all-or-none character).

In order to determine possible mechanical effects such as were reported by Nelson and Peacock (1972), an additional electrode was positioned close to the cell membrane while the membrane potential was being recorded by another electrode. When touching the surface, a hyperpolarizing response as reported by Nelson and Peacock (1972) was evoked in some cells in the nonoscillating state; however regular oscillations were not produced. Moreover, in most cells in the oscillating state, touching the surface and even penetrating the cell resulted in a cessation of the oscillation.

Contrary to the finding of Nelson and Peacock (1972), not only acetylcholine but also carbamylcholine applied locally on the cell surface was ineffective in eliciting hyperpolarizing responses. This discrepancy may be the result of differences in the experimental and/or cell culture

Table 1. Effect of acetylcholine, carbamylcholine and atropine on the electrical membrane properties of L cells

	Condition	Resting state	Spontaneously activated state	Electrically activated state
Membrane potential (mV)	control	-14.9 ± 0.5 (45)	-36.5 ± 1.5 (43)	-36.5 ± 2.1 (18)
	Ach[a]	-15.8 ± 1.1 (14)	-34.8 ± 2.3 (14)	-30.7 ± 2.0 (6)
	Cch[b]	-17.0 ± 1.3 (7)	-38.5 ± 4.4 (6)	-32.6 ± 1.0 (3)
	atropine[c]	-14.5 ± 0.6 (35)	-39.6 ± 2.4 (24)	-37.6 ± 2.1 (23)
Membrane resistance (MΩ)	control	34.3 ± 2.2 (45)	22.7 ± 1.8 (43)	17.7 ± 1.8 (18)
	Ach	38.7 ± 2.6 (14)	25.5 ± 2.7 (14)	24.0 ± 4.7 (6)
	Cch	34.1 ± 3.9 (7)	22.6 ± 3.6 (6)	21.1 ± 6.2 (3)
	atropine	37.1 ± 2.5 (35)	17.2 ± 1.6 (24)	20.1 ± 2.0 (23)
Frequency (cycles/min)	control	–	4.5 ± 0.3 (44)	–
	Ach	–	4.4 ± 0.4 (14)	–
	Cch	–	4.3 ± 0.6 (6)	–
	atropine	–	4.3 ± 0.5 (24)	–

[a] Acetylcholine, 5×10^{-4} M, 1~30 min after addition.
[b] Carbamylcholine, $7\times10^{-6}\sim7\times10^{-5}$ M, 4~30 min after addition.
[c] Atropine sulfate, $10^{-5}\sim2\times10^{-4}$ M, 1~50 min after addition.

Table 2. Effect of low temperature on the electrical membrane properties of L cells

	Condition	Resting state	Spontaneously activated state	Electrically activated state
Membrane potential (mV)	37 °C	-16.1 ± 0.3 (183)	-34.6 ± 0.9 (102)	-33.7 ± 1.2 (59)
	13~18 °C	-15.8 ± 0.6 (37)	-33.4 ± 2.0 (15)	-32.6 ± 2.9 (10)
	3~5 °C	-14.8 ± 0.6 (11)	–	-23.2 ± 0.9 (11)
Membrane resistance (MΩ)	37 °C	36.8 ± 1.2 (183)	22.7 ± 1.1 (97)	18.7 ± 1.0 (59)
	13~18 °C	41.2 ± 2.5 (37)	26.5 ± 2.3 (15)	21.3 ± 2.6 (10)
	3~5 °C	42.0 ± 0.5 (11)	–	20.5 ± 0.4 (11)
Frequency (cycles/min)	37 °C	–	3.2 ± 0.1 (75)	–
	13~18 °C	–	1.4 ± 0.1[a] (26)	–
	3~5 °C	–	0 ± 0 (11)	–

[a] Significantly different from the value obtained at 37 °C with $p<0.05$.

conditions. Acetylcholine (5×10^{-4} M), carbamylcholine (7×10^{-6} to 7×10^{-5} M) and atropine sulfate (10^{-5} to 2×10^{-4} M) added directly to the bathing fluid had no effect on the oscillations and the electrically induced hyperpolarizing responses, as presented in Table 1.

At the present stage of our investigations, therefore, the mechanical effects of the recording microelectrode as well as some substances having

muscarinic action appear to be excluded from stimuli which generate the spontaneous hyperpolarizing response.

3. Characteristics of Membrane Potential Oscillation

a) *Dependency on metabolic activities: Effects of temperature and metabolic inhibitors.* The effect of temperature on the potential oscillation is shown in Table 2. At 3 °C, the spontaneous oscillation was completely blocked (frequency = 0), despite evidence of electrically activated responses of a smaller size. Such a finding also suggests that the spontaneously repetitive hyperpolarization is not due to a mechanical stimulation of the microelectrode impalement. When the cells were re-warmed to 37 °C, both the spontaneous oscillation and electrically activated hyperpolarization were fully recovered. At room temperature (13–18 °C), the spontaneous potential oscillation was always observed, whereas the resting membrane potential as well as the spontaneously or electrically activated hyperpolarization were slightly smaller than those at 37 °C. Such small differences are ascribed only to the simple thermodynamic temperature effect (RT/F). In contrast, the frequency of the oscillation remarkably decreased at room temperature and the value of Q_{10} derived from these two temperature levels was about 1.5. Such a finding suggests that a process generating the spontaneous repetitive hyperpolarization is far more dependent on temperature than the magnitude of hyperpolarization resulting therefrom. As summarized in Table 3, cyanide and DNP completely abolished the spontaneous potential oscillation without any significant effects ($p > 0.05$) on the electrically activated hyperpolarizing response. These findings indicate that metabolic energy and certain chemical processes take part in the generating mechanism of the oscillation, but not in that of the electrically induced hyperpolarization. On the other hand, ouabain up to 10^{-3} M was found to show no significant effects ($p > 0.25$) on either the spontaneous or the electrically induced hyperpolarizing responses (Table 3). Thus it is quite unlikely that the (Na + K)-ATPase contributes to the generation of the spontaneous potential oscillation as well as of the hyperpolarizing response induced by appropriate stimuli.

b) *Superposition of hyperpolarizing responses.* Occasionally appearance of oscillations composed of two or more waves was observed, suggesting the possibility of superposition of hyperpolarization responses. Indeed,

Table 3. Effects of metabolic inhibitors on the electrical membrane properties of L cells

	Condition	Resting state	Spontaneously activated state	Electrically activated state
Membrane potential (mV)	control	-15.4 ± 0.5 (41)	-38.0 ± 1.5 (37)	-36.0 ± 1.6 (34)
	KCN 10^{-3} M[a]	-14.8 ± 0.5 (25)	–	-35.0 ± 1.5 (22)
	DNP 10^{-4} M[b]	-14.8 ± 0.3 (36)	–	-30.4 ± 1.2 (29)
	DNP 10^{-3} M[c]	-15.2 ± 0.8 (35)	–	-25.1 ± 2.0 (13)
	ouabain 10^{-3} M[d]	-14.8 ± 0.3 (51)	-34.6 ± 1.4 (42)	-34.4 ± 1.7 (32)
Membrane resistance (MΩ)	control	38.6 ± 2.7 (41)	22.2 ± 1.9 (37)	17.8 ± 1.6 (34)
	KCN 10^{-3} M[a]	36.1 ± 2.7 (25)	–	14.2 ± 1.4 (22)
	DNP 10^{-4} M[b]	56.1 ± 4.0[e] (36)	–	31.6 ± 2.9[e] (29)
	DNP 10^{-3} M[c]	32.2 ± 2.6 (35)	–	28.2 ± 2.7[e] (13)
	ouabain 10^{-3} M[d]	39.5 ± 2.7 (51)	23.2 ± 1.6 (42)	22.1 ± 1.4 (32)

[a] Measured 10–36 min after addition of 10^{-3} M KCN to the bathing fluid.
[b] 5–60 min after addition of 10^{-4} M 2,4-dinitrophenol.
[c] 1–30 min after addition of 10^{-3} M 2,4-dinitrophenol.
[d] 10–100 min after addition of 10^{-3} M G-strophanthin.
[e] Significantly different from the control values ($p < 0.05$).

on rapidly adding drops of PBS to the bathing PBS solution at the peak of an oscillation, further hyperpolarization of the membrane was often induced and superimposed upon the previous hyperpolarization. Since touching or pressing on the oscillating cell surface with an additional microelectrode did not produce any significant effects on the pattern of oscillation, whereas impalement usually evoked temporary or permanent stoppage of the oscillation, the effect of adding drops cannot be attributable to one of mechanical stimuli. Levine (1960) reported that washing in saline solution changed the permeability of HeLa cell membrane, therefore movement of bathing fluid produced by a rapid addition of drops may affect the properties of the cell membrane. Another possibility is that changes in the ionic composition of the stagnant fluid layer attached to the membrane surface would result in changes in the potential level.

On the other hand, an electrical stimulation applied at (or near) the peak of a hyperpolarization wave was ineffective in eliciting a hyperpolarization response superimposed thereon. When the stimulation was applied at the resting level of oscillations, a hyperpolarizing response greater than the original was elicited and was followed by several smaller oscillatory responses, as if summation of both the spontaneous and electrically activated hyperpolarization responses occurred at the moment.

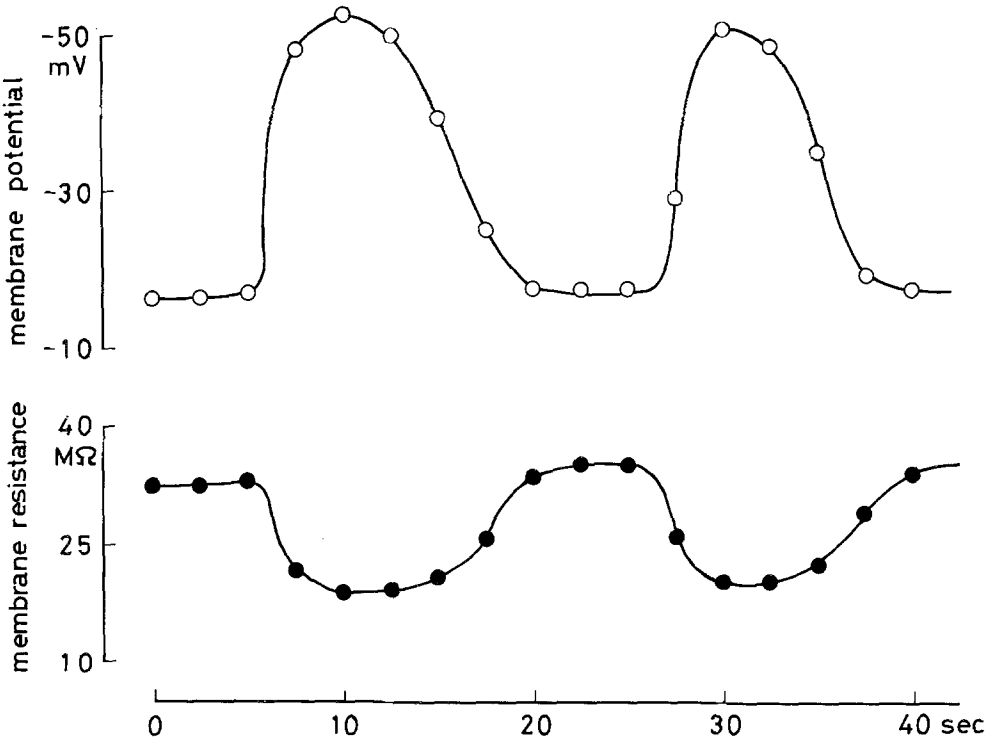

Fig. 3. Synchronization of oscillatory changes in the membrane potential with those in the membrane resistane observed in giant L cells

But the frequency of oscillations was not affected by such an electrical stimulus, and amplitudes of oscillatory responses were also retrieved after such modifications of amplitudes of several responses. Therefore the electrical stimulus seems to be having nothing to do with the cause of oscillations.

These results suggest that superposition of the oscillation is possible under certain conditions despite the presence of an apparent refractory period in the electrical stimulation.

c) *Correlation with the resistance changes.* As shown in Fig. 1 and Tables 1 and 2, the effective membrane resistances decreased remarkably in the hyperpolarized state irrespective of whether the state was attained spontaneously or by appropriate stimuli. Such decreases in the membrane resistance during a spontaneous oscillation synchronized completely with increases in hyperpolarization of the membrane potential as exemplified in Fig. 3. To determine the dependency of the membrane resistance on

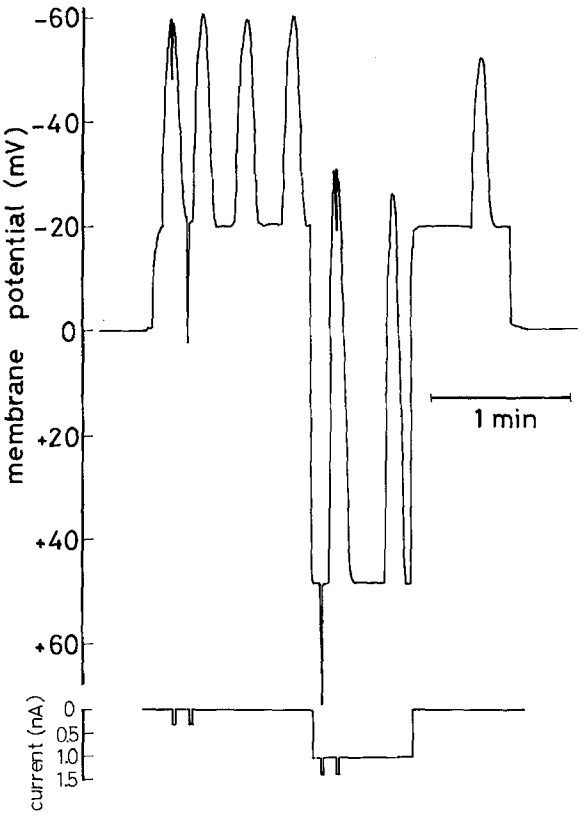

Fig. 4. Effects of constant current application on the pattern of oscillations of the membrane potential and on the membrane resistance

the potential level, constant currents of different magnitude were applied to the giant L cells in such a way that the membrane potential could be brought to different positive or negative levels. An example of the results obtained with such a procedure is illustrated in Fig. 4. It is apparent from this Figure that the oscillation had amplitudes which were different and dependent on the membrane potential level, but kept the same regular pattern with a frequency identical with that prior to current application, while the resistances in both the resting and activated states did not change significantly with application of the constant current. This result indicates that changes in membrane resistance in L cells were not dependent on the magnitudes of the membrane potential, a finding in sharp contrast with the case of excitable cell membranes. Decreases in membrane resistance in the activated state may not be related to dependency of the membrane resistance on the magnitude

of membrane potentials, but rather to increases in the ionic permeabilities across the cell membrane. Similar results were obtained by Nelson and his co-workers for the hyperpolarizing response (Nelson *et al.*, 1972).

4. Intracellular Monovalent Ion Concentrations

The values of intracellular K^+, Na^+ and Cl^- concentrations in giant L cells incubated in PBS for 20–40 min at 37 °C were 151 ± 4.4, 27.7 ± 2.7 and 46.6 ± 4.6 mEquiv/liter of cell water ($n=12$), respectively. These values are in good agreement with those hitherto reported on normal L cells (Lamb & MacKinnon, 1971*a*, *b*; Quissell & Suttie, 1973).

Assuming that all these ions within the cells have the same activity coefficient as in the extracellular PBS, the equilibrium potential of each ionic species (E_i) was calculated by the Nernst equation as follows: -95.7 mV for E_K, $+43.8$ mV for E_{Na} and -27.9 mV for E_{Cl}. Lamb and MacKinnon (1971*b*) showed that Cl^- was distributed passively in the normal L cells. If such is also the case in the giant cells having an oscillating membrane potential between -15 mV and around -37 mV, in PBS (Fig. 2), the computed E_{Cl} is expected to be the average of these two levels ($\simeq 26$ mV). Indeed, coincidence between the calculated value and the expected one is satisfactory, therefore Cl^- would be distributed passively in giant L cells also. This view was supported by direct experimental evidence (Okada, Roy, Tsuchiya, Doida & Inouye, 1977).

Discussion

The most important aspect of the potential measurements in these giant L cells is the stable and long-lasting oscillation of the membrane potential. Since the spontaneously occurring hyperpolarization is quite similar to the electrically or mechanically induced hyperpolarizing response, it is naturally assumed that such an oscillation might result from a mechanical stimulation elicited by impalement with the electrode. As discussed above in detail, however, such a possibility is quite unlikely. Moreover, we obtained some records in the course of this study, in which the potential oscillation could be initiated at any phase, as illustrated in Fig. 5. As shown by the experiment with two microelectrodes, the penetrating microelectrode often resulted in a temporal cessation

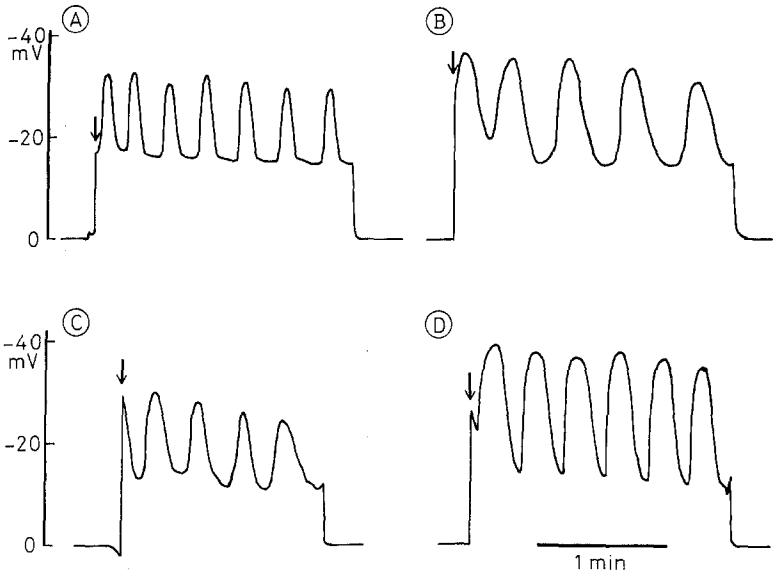

Fig. 5. Various initiating patterns of the recording of the oscillations

of the oscillation. Thereby the oscillation recorded in most cells started from the resting level (Fig. 5*A*). However, records like those of Fig. 5*B*, *C* and *D* clearly demonstrate that oscillations are not triggered by electrode penetration but are endogeneous, even though these records were not often obtained.

Nelson and Peacock (1973) reported that the electrically activated response can be transmitted from one L cell to another. In view of the regularity of the oscillation pattern, however, it is most unlikely that the oscillations originate from propagation of hyperpolarizations randomly produced somewhere in the cell population. Recording of the potential on two adjacent cells with two microelectrodes showed that their oscillations were not in the same phase, and the frequency also was different. Moreover, we observed that some cells having no morphological contact with others under phase microscopy (1000 ×) also showed potential oscillations. Thus the possibility of cell-to-cell transmission as the origin of the spontaneously occurring oscillation can be safely ruled out.

On the other hand, the potential oscillation was abolished in the cold, as well as in the presence of metabolic inhibitors. Under these conditions, however, the giant cells remained responsive to electrical stimulation with hyperpolarization. Such a finding strongly suggests that

hyperpolarization of the cell membranes is repetitively triggered by a process or processes which largely depend on the cellular metabolic activity, but the hyperpolarization itself is a membrane process of rather physical nature. When the oscillation was recorded by two microelectrodes penetrated at the same time in the same cell, the oscillations thus recorded were in the same phase and completely identical. Therefore, heterogeneous potential distribution within the cell produced by protoplasmic streaming or other local events inside the cell could hardly be responsible for these oscillations. At present, nothing is known of the nature of the biochemical process(es) underlying repetitive generation of the membrane current. Our recent experiments suggest that intracellular Ca^{2+} may play the role of trigger in membrane hyperpolarization (Tsuchiya & Okada, *unpublished observations*).

The results presented in Figs. 1 and 2, as well as in Tables 1, 2 and 3 strongly suggest that the hyperpolarization of the membrane triggered by such a metabolism-dependent process is identical in nature with the electrically induced one. Nelson *et al.* (1972) suggested that the electrically induced hyperpolarization of L cells resulted from an increase in K^+ permeability of the membrane. It was also reported that the same mechanism underlies the electrically induced and spontaneously appeared hyperpolarization found in macrophages (Gallin *et al.*, 1975). Thus it is quite likely that the spontaneous potential oscillations described above also rest on the same ionic basis. Further detailed experiments are being designed in an attempt to validate this postulate. This problem will be discussed in detail in the succeeding paper (Okada *et al.*, 1977).

The last point to be discussed is whether the spontaneous potential oscillation actually occurs in normal cells. It was difficult to obtain regular oscillations in normal L cells. Because of their smaller size, however, it is quite natural to expect that impalement of the normal L cells with a microelectrode produces leakage effects more serious than that of the giant L cells. The fact that their effective membrane resistance was far lower than that expected supports this possibility. Moreover, the changes in ionic concentrations inside small cells as a result of leakage would also be far more rapid; a leakage current of around 1 nA would produce serious changes of the ionic milieu inside a spherical cell of 15 μm diameter within 10–20 sec, which in turn would affect the magnitude of the potential. However, 20–30 min is required to produce the same effect on a cell having five times this diameter and this length of time is sufficient to observe many oscillations with a period of around 15 sec without any significant effects of intracellular ion concentrations

upon the membrane potential. When the normal L cell was impaled with a microelectrode, we obtained almost always single hyperpolarizing responses, but of various sizes. It seems quite probable that the size of the response chiefly depends upon the leakage effect. Indeed, we occasionally observed a complete response followed by regular oscillations as seen in Fig. 1 *C*, probably because of fortuitously achieved small leakage. At present, therefore, we favor the theory that generation of the spontaneous potential oscillation observed on the nondividing giant L cells is an intrinsic property of L cells associated with their activities and shared with the normal dividing cells.

Gallin *et al.* (1975) found such an oscillation and a hyperpolarizing response induced mechanically or electrically in guinea pig and mouse peritoneal macrophages and human macrophages. We confirmed this in macrophages migrated from the primary culture of a mouse liver tissue (Okada, Doida & Tsuchiya, *unpublished observation*). It seems quite possible, therefore, that many so-called nonexcitable cells, especially of mesenchymal origin, might have such an electrical "excitability", whose properties are more or less different from so-called excitable cells. The physiological function of this "excitability" is under current investigation.

Thanks are due to M. Ohara, Kyoto University, for assistance with the manuscript.

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