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Oscillations of Membrane Potential in L Cells

II. Effect of Monovalent Ion Concentrations and Conductance Changes Associated with Oscillations

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Summary. Oscillation and activated hyperpolarizing responses induced by electrical stimuli (H.A. responses) were studied in large nondividing L cells (giant L cells) under a variety of ionic conditions. When Cl^- in the bathing fluid was partially replaced with SO_4^{2-} at fixed external Na^+ and K^+ concentrations, the membrane potential depolarized transiently, but recovered to the original potential level after about 10 min. Under such a steady state in a low-Cl^- medium, the amplitudes of oscillations and H.A. responses remained almost identical with those in the control medium. On exposure to a low-Na^+ medium, both membrane potentials in the resting and hyperpolarized states were slightly hyperpolarized, but the pattern and the amplitude of oscillations and H.A. responses remained much the same. Changes in external K^+ concentrations remarkably affected the amplitudes of oscillations and H.A. responses: the amplitudes decreased with increases in external K^+ concentration. Calculation of the changes in K^+, Na^+ and Cl^- conductances during oscillations and H.A. responses under these various ionic conditions showed that the change in K^+ conductance is the only factor responsible for the oscillation and the H.A. response. The reversal potential for the potential oscillation is about -94 mV under normal conditions, this value being quite close to that of the equilibrium potential of K^+. The reversal potentials in various external K^+ concentrations satisfied the Nernst equation for a K^+ electrode. Valinomycin induced remarkable hyperpolarization of the resting potential, resulting in an inhibition of oscillations. The level of valinomycin-induced hyperpolarization of the resting potential required to inhibit H.A. responses was the same as that of the peak potentials of the oscillation and H.A. response. In the light of these observations, it is concluded that the spontaneous potential oscillation and the H.A. response are caused solely by increase in the K^+ conductance of the cell membrane.

Nelson, Peacock and Minna (1972) found that L cells responded to an electrical, mechanical or chemical stimulus by producing a hyper-

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polarizing activated response (an H.A. response). They suggested that such an H.A. response results from an increase in potassium permeability, on the basis of their observation of the reversal potential of the H.A. We observed in almost all our recordings stable oscillations of membrane potentials occurring spontaneously in addition to the H.A. response in large nondividing L cells (Okada, Doida, Roy, Tsuchiya, Inouye & Inouye, 1977). Reported herein are data, which lend further support to our thesis, concerning the effects of changes in external K^+, Na^+ or Cl^- concentrations together with the effects of certain drugs on the oscillation and the H.A. response. Based on these results, the ionic mechinism of oscillating potential changes and H.A. responses is discussed.

Materials and Methods

The cell culture and electrophysiological techniques employed herein were identical to those described in the preceding paper (Okada *et al.*, 1977). In this work, a monolayer of large nondividing L cells (giant L cells) obtained by X-ray irradiation were subjected to electrophysiological studies.

A phosphate-buffered saline (PBS) containing 20 mM mannitol was used as the control medium throughout the present study. In order to change K^+, Na^+ and Cl^- concentrations in the bathing fluid, the control PBS was modified as illustrated in Table 1. Each of the modified buffered salines had the same pH (7.3 ± 0.1) and tonicity (except

Table 1. Ionic composition of the control phosphate-buffered saline and modified buffered salines

	Control PBS	Cl^--free PBS	Low-Cl^- PBS	Low-Na^+ PBS	K^+-free PBS	Na^+-free PBS	222.2-K^+ PBS	Control TBS	TEA^+ TBS
K^+	4.2	4.2	4.2	4.2	–	147.2	222.2	4.2	4.2
Na^+	143.0	143.0	143.0	28.6	147.2	–	–	143.0	–
Cl^-	132.0	–	69.0	143.4	132.5	132.5	207.5	163.5	157.5
Mg^{2+}	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ca^{2+}	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
$Tris^+$	–	–	–	111.2	–	–	–	13.5	13.5
TEA^+	–	–	–	–	–	–	–	–	137.0
$CH_3SO_4^-$	–	129.7	–	–	–	–	–	–	–
SO_4^{2-}	–	–	31.8	–	–	–	–	–	–
HPO_4^{2-}	8.0	8.0	8.0	1.6	8.0	8.0	8.0	–	–
$H_2PO_4^-$	1.5	1.5	1.5	0.3	1.5	1.5	1.5	–	–

PBS and TBS represent a phosphate-buffered saline and a tris-buffered saline, respectively. All solutions contained 20 mM mannitol. In addition, an appropriate amount of mannitol (about 80 mM) was added to the low-Na^+ PBS to maintain the total tonicity constant. Concentrations are mEquiv/liter.

for 222.2 K^+-PBS). In some experiments, another buffered saline (TBS) with tris(hydroxymethyl)-aminomethane ($Tris^+$) was employed (Table 1). Tetraethylammonium ions (TEA^+) were added in this saline in place of Na^+ (Table 1).

Valinomycin and tetrodotoxin, obtained from Sigma Chemical Co., were dissolved in ethanol at the concentrations of 4×10^{-4} M and 10^{-3} M, respectively, for stock solutions, and small aliquots of these concentrated solutions were added to the bathing fluid. The addition of ethanol, the vehicle for these drugs, up to 2% did not affect the electrical membrane properties of L cells.

All data presented here are the means ±SE.

Results

Effect of Low Cl^- *Concentrations*

On removal of all the external Cl^- by replacement with methylsulfate, a long-lasting depolarization of the resting potential (E_m^r), sometimes reaching a positive one, was observed as shown in Fig. 1*A*. Despite such a significant depolarization of E_m^r, the amplitude, ΔE_m ($=E_m^a - E_m^r$; E_m^a =the potential at the activated hyperpolarizing state), observed in the spontaneous oscillation did not appreciably change as exemplified in this same Figure.

Changes in potential profiles on suddenly reducing the external Cl^- from 132.5 to 69.0 mM by partially replacing Cl^- with SO_4^{2-} were similar to those observed with total removal of Cl^- stated above as seen in Fig. 1*B*. Statistically significant depolarization in both E_m^r and E_m^a was evident for about 10 min after reducing external Cl^-, but the changes in ΔE_m were slight and statistically insignificant (Table 2). The effective membrane resistance in the resting state (R_m^r) also decreased slightly from 42.0 ±4.2 ($n=20$) MΩ to 39.0±2.2 (31) MΩ, but such small decreases were statistically insignificant because of considerable variations in R_m^r among the cells tested. On the other hand, with an exposure of more than 10 min to this low-Cl^- medium, the original levels of E_m^r (around −15 mV, Table 2) and R_m^r (45.0±2.8 (32) MΩ) were reverted to. The effective membrane resistance in the activated state (R_m^a) was not so remarkably affected by low-Cl^- (around 20 MΩ). The membrane potential continued to oscillate in the medium having 69 mM Cl^- just as in the control PBS, the frequency (Table 3) as well as the amplitude, ΔE_m, of oscillations and of H.A. responses (Table 2) remaining practically unaffected. These results strongly suggest that the external chloride ions play no role in the mechanism of generation of the oscillation and the H.A. response.

This transient depolarization with reduction of the external Cl^- has already been reported in muscle fibers (Hodgkin & Horowicz, 1959), in

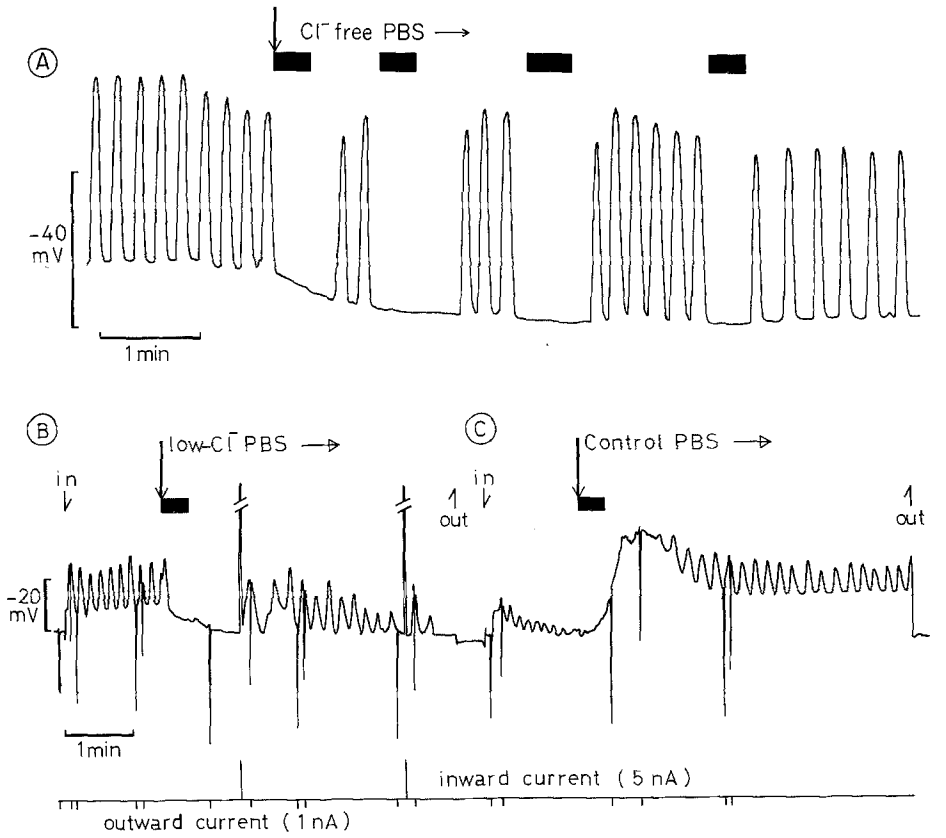

Fig. 1. Effect of low-Cl^- on the membrane potential. (*A*) Effect of the Cl^--free medium on the resting potential and the potential oscillation in a giant L cell. (*B*) Effect of suddenly reducing $[Cl]_o$ from 132 to 69 mM on the resting potential and the oscillation in a giant L cell. (*C*) Effect of suddenly increasing $[Cl]_o$ from 69 to 132 mM on the resting potential and the oscillation in the same cell as *B* (re-impalement). 'In' and 'out' indicate the time of entry and exit of the recording microelectrode, respectively. It should be noted here that the oscillation stopped during rapid flow of bathing fluid (■) produced by flushing a new saline. This effect may be attributed to changes in the ionic milieu of the stagnant fluid layer attached to the membrane surface (*see* Okada *et al.*, 1977)

intestinal epithelial cells (Okada, Irimajiri & Inouye, 1976) and in normal (nonirradiated) L cells (Lamb & MacKinnon, 1971). Moreover, on washing with the control PBS, the values for E_m^r and E_m^a returned to original levels after a transient hyperpolarization, as seen in Fig. 1*C*. Thus, it would appear that the chloride is passively distributed across the membrane of a giant L cell.

Table 2. Effect of the low-Cl^- medium on the membrane potential

		Resting potential (mV)	Spontaneously activated potential (mV)	Electrically activated potential (mV)
Control	PBS	-15.9 ± 1.1 (20)	-41.0 ± 2.8 (14)	-40.7 ± 4.8 (10)
Low-Cl^-	< 10 min [a]	-9.4 ± 0.5 (31) [$p<0.05$]	-32.4 ± 2.3 (22) [$p<0.05$]	-32.7 ± 2.4 (11) [$p<0.05$]
	> 10 min [b]	-15.4 ± 0.6 (32) [$p>0.50$]	-41.7 ± 2.2 (24) [$p>0.05$]	-39.5 ± 2.6 (20) [$p>0.05$]

[a] Observed for 0 to 10 min after introducing the low-Cl^- (69.0 mM-Cl^-) solution.
[b] Observed thereafter.
Numbers in parentheses and square brackets indicate number of observations and the significant difference from the control, respectively.

Table 3. Effect of ionic concentrations on the frequencies of oscillations

Solution	Number of observations	Frequency (cycles/min)	Significant difference from control
Control PBS	261	3.8 ± 0.1	–
Low-Cl^- (69.0 mM-Cl^-)	38	3.7 ± 0.1	$p>0.05$
Low-Na^+ (28.6 mM-Na^+)	26	4.0 ± 0.5	$p>0.10$
Low-K^+ (2.1 mM-K^+)	24	3.5 ± 0.2	$p>0.05$
High-K^+ (22.0 mM-K^+)	19	3.6 ± 0.3	$p>0.50$
High-K^+ (40.0 mM-K^+)	13	3.9 ± 0.3	$p>0.50$

Table 4. Effect of the low-Na^+ medium on the membrane potential (E_m) and the membrane resistance

	Solution	Resting state	Spontaneously activated state	Electrically activated state
E_m (mV)	Control PBS	-15.1 ± 0.6 (22)	-38.3 ± 2.8 (18)	-39.9 ± 4.8 (6)
	Low-Na^+ PBS	-23.9 ± 0.6 (42)	-41.2 ± 2.0 (26)	-41.7 ± 2.6 (11)
R_m (MΩ)	Control PBS	44.0 ± 3.8 (22)	23.2 ± 3.4 (18)	23.2 ± 4.1 (6)
	Low-Na^+ PBS	34.0 ± 2.6 (42)	22.0 ± 2.0 (26)	20.9 ± 1.6 (11)

Numbers in parentheses indicate number of impalements.

Effect of Low External Na^+, *Tetrodotoxin and Tetraethylammonium*

Replacing external Na^+ with tris(hydroxymethyl)aminomethane ($Tris^+$), the external Na^+ concentration was reduced to 20% of Na^+ in the control medium (Table 1). Such a low-Na^+, high-$Tris^+$ medium resulted in a hyperpolarization of the membrane in the resting state (Table 4). Contrary to our expectations based on the permeability coefficients of Na^+ and K^+ reported by Lamb and MacKinnon (1971), such a hyperpolarization was quite small (around 9 mV). Despite the removal of a large part of Na^+, the value of R_m^r decreased by around 20%. Replacement of external Na^+ with $Tris^+$ produced no significant changes in R_m^a (Table 4) or in the frequency of the potential oscillation (Table 3), and there was a slight hyperpolarization in E_m^a (Table 4). These results indicate that external Na^+ plays no essential role in the generation mechanism of oscillations and H.A. responses in L cells.

We next examined the effect of tetrodotoxin (TTX) which inhibits selectively increase in the Na^+ conductance during action potentials of various excitable cells (Narahashi, Deguchi, Urakawa & Ohkubo, 1960; Evans, 1972). Additions of this drug to the bathing media in doses up to 1.2×10^{-5} M, failed to produce significant changes in E_m^r and E_m^a, nor was the frequency of the spontaneous potential oscillation altered. This evidence further supports the view that the generation of oscillations and H.A. responses does not involve changes in the Na^+ conductance.

In contrast to TTX, a high dose (137 mM) of tetraethylammonium ions (TEA^+) added to the external TBS in place of Na^+ almost completely suppressed the oscillation and H.A. response (Table 5). In the

Table 5. Effect of tetraethylammonium ions (TEA^+) on the membrane potential (E_m) and the membrane resistance (R_m)

	Solutions	Resting state	Spontaneously activated state	Electrically activated state
E_m (mV)	Control TBS	-17.3 ± 0.7 (20)	-40.4 ± 1.9 (20)	-41.1 ± 2.5 (12)
	110-TEA^+, 28.6-Na^+ [a]	-24.2 ± 0.4 (24)	-29.6 ± 1.0 (16)	-28.8 ± 0.9 (13)
	137-TEA^+, Na^+-free [b]	-25.5 ± 0.8 (10)	-26.7 ± 1.0 (5)	-26.0 ± 0.7 (5)
R_m (MΩ)	Control TBS	34.7 ± 0.2 (20)	19.2 ± 1.9 (20)	18.8 ± 3.0 (12)
	110-TEA^+, 28.6-Na^+ [a]	34.8 ± 3.1 (24)	30.9 ± 3.7 (16)	27.3 ± 2.7 (13)
	137-TEA^+, Na^+-free [b]	30.9 ± 3.4 (10)	22.1 ± 5.0 (5)	27.9 ± 5.3 (5)

TBS represents a tris-buffered saline (Table 1).

[a] Observed in TBS containing 110 mEquiv-TEA^+ and 28.6 mEquiv-Na^+.

[b] Observed in TEA^+-TBS (137 mEquiv-TEA^+, Na^+-free) (Table 1).

Numbers in parentheses indicate number of impalements.

110 mM-TEA^+ TBS containing the same amount of Na^+ (28.6 mM) as the low-Na^+ PBS (Table 1), E_m^r and R_m^r were of the same order of magnitude as those in the low-Na^+ PBS (*see* Tables 4 and 5). On the other hand, amplitudes of the oscillation and the H.A. response were remarkably suppressed in this solution, as shown in Table 5. It has been demonstrated that a high dose of TEA^+ externally applied inhibits the potassium conductance during an action potential for various excitable cells without affecting the resting potential (Hagiwara & Saito, 1959; Koppenhöfer & Weymann, 1965; Schmidt & Stämpfli, 1966; Hille, 1967). These results would, therefore, suggest that the oscillation and the H.A. response were induced by increases in potassium conductances.

Effects of Changes in the External K^+ *Concentration*

Since reduction of the external Na^+ concentration was found to induce no pronounced changes in the pattern and amplitude of oscillations and H.A. responses, the external K^+ concentration ($[K]_o$) was modified by $K^+ - Na^+$ replacement with the control PBS, the K^+-free PBS and the Na^+-free PBS (Table 1), in order to determine whether or not these oscillations and H.A. responses were the result of changes in the K^+ conductance.

As seen in Fig. 2, the potential at both levels, resting (E_m^r) and activated spontaneously or electrically (E_m^a), are hyperpolarized on reducing $[K]_o$ but depolarized on increasing $[K]_o$. It should be noted here that the changes in E_m^a were more pronounced, varying linearly with $\log[K]_o$, than those in E_m^r. Oscillations as well as H.A. responses are negligibly small at 75.7 mM K^+ and completely disappeared at $[K]_o = 100$–150 mM. In 222.2 mM K^+ PBS prepared by adding 75 mM KCl to isotonic Na^+-free PBS (Table 1), the polarity of oscillations was reversed ($\Delta E_m = +1.6 \pm 0.3$ mV, $n = 22$), as shown in Fig. 2. The value of $[K]_o$ at the crossing of $E_m^a - \log[K]_o$ and $E_m^r - \log[K]_o$ lines (100–150 mM) is close to the intracellular K^+ concentration ($[K]_i$) measured in giant L cells (Okada *et al.*, 1976). These facts strongly suggest that the polarity of oscillations is dependent on the gradient between $[K]_o$ and $[K]_i$.

Changes in the effective membrane resistance are summarized in Table 6. The membrane resistances in the activated state (R_m^a) appear to show no systematic changes and are apparently independent on the external K^+ concentration. On the other hand, R_m^r tends to decrease gradually with increasing $[K]_o$. At any rate, the R_m^a values are smaller

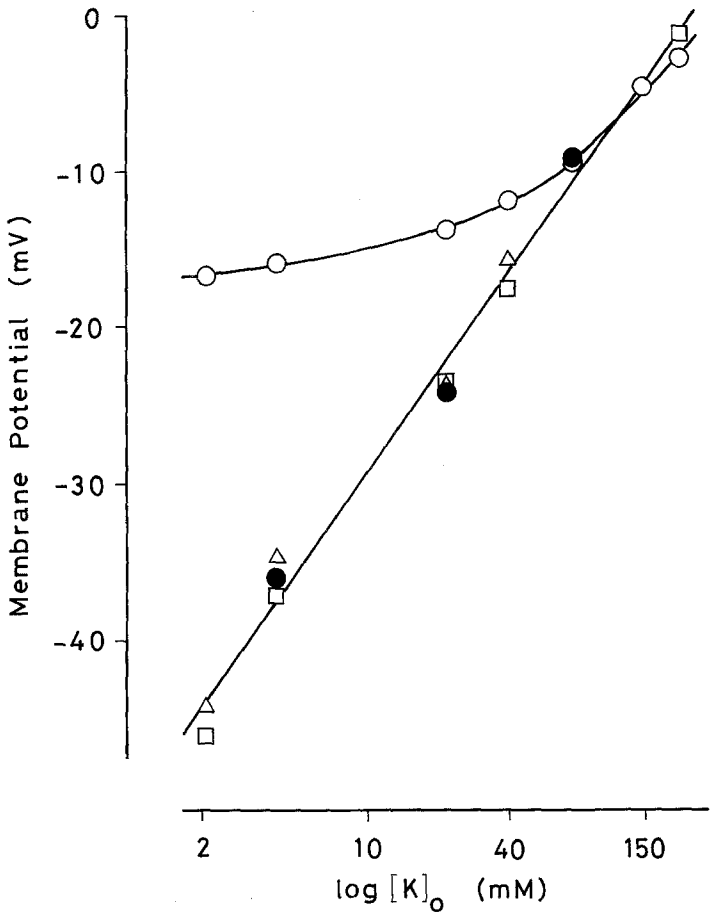

Fig. 2. Effect of $[K]_o$ changes produced by the KCl-NaCl substitution on the resting potential (○) and the spontaneously (□) or electrically (△) activated potential as well as on the steady hyperpolarization obtained on exposure to valinomycin (4×10^{-6} M) in the bathing fluid for more than 10 min (●). Each point represents the mean value of 222–17 observations with the standard error of the resting potential less than ± 0.5 mV and with that of the activated potential less than ± 3 mV

Table 6. Effect of K^+ concentration on the membrane resistance

K^+ concentration (mM)	Resting state (MΩ)	Spontaneously activated state (MΩ)	Electrically activated state (MΩ)
2.1	41.5 ± 2.8 (43)	20.7 ± 2.6 (20)	15.9 ± 2.2 (19)
4.2	36.8 ± 1.1 (222)	25.1 ± 1.2 (90)	18.0 ± 1.2 (42)
22.0	33.7 ± 1.8 (86)	25.6 ± 2.4 (26)	18.3 ± 2.3 (20)
40.0	29.3 ± 1.6 (55)	17.9 ± 1.5 (17)	17.7 ± 1.7 (18)
75.7	27.8 ± 2.9 (37)	–	–
147.2	22.5 ± 1.7 (39)	–	–
222.2	28.8 ± 2.0 (22)	21.8 ± 1.6 (22)	24.3 ± 2.3 (4)

Numbers in parentheses indicate number of impalements.

than the R_m^r values in all cases, thus suggesting that increases in ionic conductances do occur during oscillations or H.A. responses.

Though the frequency of an oscillation did not change on exposure to low- or high-K^+ media (Table 3), the amplitude and the polarity of oscillations and H.A. responses were remarkably affected by changes in external K^+ concentrations, in contrast to the cases of low-Cl^- and low-Na^+ media at a constant K^+ concentration. Thus, the generating mechanism of oscillations and H.A. responses appears to be quite dependent on the K^+ concentration.

Effect of Valinomycin

Valinomycin (4×10^{-6} M) completely blocked the spontaneous potential oscillations within 2 min after addition to the bathing media as shown in Table 7. The resting potential was gradually hyperpolarized up to about 10 min after this addition and the cells were still capable of responding to electrical stimuli with hyperpolarizing activation (Table 7). The valinomycin-induced hyperpolarization attained the maximum level about 10 min after application of the drug and levelled off. After achieving such a full hyperpolarizing effect, electrical stimulation failed to produce H.A. responses (Table 7).

Table 7. Effect of valinomycin (4×10^{-6} M) on the membrane potential

K^+ concentration (mM)	Condition	Resting potential (mV)	Spontaneously activated potential (mV)	Electrically activated potential (mV)
4.2	Control	-15.9 ± 0.3 (222)	-37.5 ± 1.1 (94)	-36.5 ± 1.7 (44)
	Vali. 2–10 min [a]	-19.6 ± 0.7 (5)	–	-32.9 ± 4.4 (5)
	Vali. 10 min < [b]	-36.2 ± 1.5 (26)	–	–
22.0	Control	-13.8 ± 0.4 (86)	-23.5 ± 0.9 (26)	-23.7 ± 1.3 (20)
	Vali. 2–10 min [a]	-14.9 ± 1.2 (6)	–	-23.6 ± 1.8 (6)
	Vali. 10 min < [b]	-24.3 ± 3.2 (25)	–	–
75.5	Control	-9.4 ± 0.5 (37)	–	–
	Vali. 2–10 min [a]	-9.1 ± 0.6 (5)	–	–
	Vali. 10 min < [b]	-9.3 ± 1.7 (24)	–	–

[a] Observed for 2 to 10 min after addition of 4×10^{-6} M valinomycin to the bathing fluid.
[b] Observed thereafter.
Numbers in parentheses indicate number of observations.

When the external K^+ concentration was raised to 75.7 mM, valinomycin lost its ability to induce any significant hyperpolarization of the resting membrane. Responsiveness to electrical stimulation of the cells was hardly retained in such a high-K^+ medium independently of the presence or absence of the drug. Of particular interest was the observation that the level of a full hyperpolarization brought about by valinomycin was nearly the same as that achieved by the spontaneously or electrically activated hyperpolarization (Fig. 2). The macrocyclic antibiotic valinomycin is known to act as a carrier or an ionophore for K^+ in lipid bilayer membranes as well as in biological membranes (Harris & Pressman, 1967; Lev & Buzhinsky, 1967; Mueller & Rudin, 1967; Pressman, Harris, Jagger & Johnson, 1967; Tosteson, Cook, Andreoli & Tieffenberg, 1967; Hoffmann & Laris, 1974). The results obtained by administration of this drug indicate, therefore, that hyperpolarization responses appearing spontaneously as well as those elicited electrically result chiefly from increases in the K^+ conductance of the cell membrane.

Reversal Potentials

For a more direct verification of the hypothesis that the oscillations are produced solely by a change in the K^+ conductance, constant currents of different amplitudes were applied to depolarize or hyperpolarize the membrane. If changes in the K^+ conductance play a key role in the occurrence of oscillating potential changes, the oscillations should reverse with sufficiently high hyperpolarizing currents and plots of ΔE_m against E_m^a obtained by applying depolarizing or hyperpolarizing currents should intersect the abscissa at the value of $E_m^a = E_K$, the equilibrium potential for K^+.

Examples of such determinations of the reversal potential (E_{rev}) are illustrated in Fig. 3*A*. As seen in this Figure, the linear character of $\Delta E_m - E_m^a$ relationship is obvious in the region of ΔE_m less than 50 mV, which enables to estimate E_{rev} by inter- or extrapolation. The plot of ΔE_m *vs.* E_m^r also showed a linear relationship. Despite considerable variations of E_{rev} from cell to cell under a given external K^+ concentration, the mean value of E_{rev} in the control PBS was -94.2 ± 4.8 mV ($n=6$), a value very close to E_K estimated previously (-95.7 mV; Okada *et al.*, 1977). Moreover, about 10-fold increase in the external K^+ resulted in about 50 mV shift in E_{rev} (-42.5 ± 4.6 mV ($n=4$), in 40 mM-K^+ PBS) and this shift was in fairly good agreement with that expected from the Nernst equation for a K^+ electrode. Since E_{rev} observed in 10 mM-K^+ PBS was -69.8

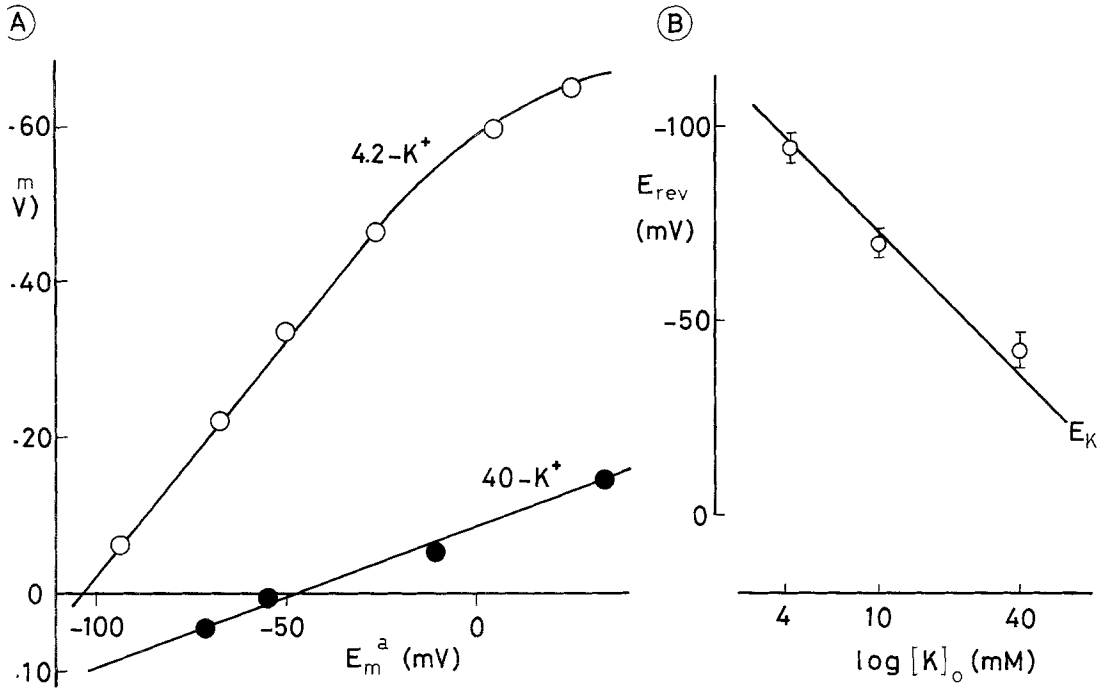

Fig. 3. Reversal potential of the oscillation. (*A*) Relationship between the activated potential level (E_m^a) and the amplitude of the oscillation (ΔE_m). The E_m^a value at $\Delta E_m = 0$ means the reversal potential (E_{rev}). ○, an example in the control PBS (4.2 mM K^+). ●, an example in 40 mM-K^+ PBS prepared by the NaCl-KCl substitution. (*B*) Effect on E_{rev} of $[K]_o$ changes produced by the KCl-NaCl substitution. Abscissa is given as $\log[K]_o$, and the straight line represents E_K calculated as $\log[K]_o/[K]_i$, by putting $[K]_i = 151$ mEquiv (Okada *et al.*, 1977). Each point represents the mean value of E_{rev} observed in 4–10 cells. Vertical bars represent standard errors on either side of the averages

± 3.7 mV ($n = 10$), indeed, changes in E_{rev} with increasing the external K^+ concentration well satisfy the Nernst equation, as shown in Fig. 3*B*. These results are in good accordance with the observations on E_{rev} of H.A. responses in L cells (Nelson *et al.*, 1972) and in macrophages (Gallin, Wiederhold, Lipsky & Rosenthal, 1975). Thus, the potential oscillations are no doubt the result of periodical changes in the K^+ conductance across the cell membrane.

Discussion

Observations of the reversal potential and the effect of valinomycin clearly show that the oscillations of membrane potentials in giant L cells are caused almost solely by an increase in the K^+ conductance across the cell membrane. Using the values of the membrane potential (E_m) and the membrane conductance ($G_m = R_m^{-1}$) measured in these experiments as

well as of the equilibrium potential of each ionic species (E_i), this concept can be supported by simple calculations for ionic conductances under plausible assumptions.

Now assume that K^+, Na^+ and Cl^- fluxes carry almost the total ionic current across the cell membrane, and these individual ionic currents (I_i) obey a simple current-voltage relation as given by

$$I_i = G_i(E_m - E_i) \tag{1}$$

where i refers to each ionic species and G_i is the conductance. Since the total ionic current across the membrane should be equal to zero at equilibrium, we have

$$\Sigma I_i = 0. \tag{2}$$

E_m is derived from Eqs. (1) and (2) as

$$E_m = (\sum_i G_i E_i)/G_m, \qquad G_m = \sum G_i. \tag{3}$$

Provided that the mean intracellular ionic composition is regarded as practically constant during the steady potential oscillation, the amplitude of the oscillation and the H.A. response (ΔE_m) is given as

$$\Delta E_m = \Sigma\{(E_i - E_m^a)\cdot \Delta G_i\}/G_m^r = \Sigma\{(E_i - E_m^r)\cdot \Delta G_i\}/G_m^a \tag{4}$$

where $\Delta G_i = G_i^a - G_i^r$ and $\Delta G_m = G_m^a - G_m^r$. It is assumed in Eq. (4) that the membrane potential is at equilibrium at the minima and maxima of oscillations and H.A. responses.

As clearly shown in Fig. 3, under normal ionic conditions or various $[K]_o$ conditions produced by the KCl—NaCl substitution, the reversal potential was equal to E_K (namely, when $\Delta E_m = 0$, $E_m^a = E_K$). Therefore, Eq. (4) can be rewritten as follows under these conditions, deleting the Na- and Cl-terms:

$$\Delta E_m = (\Delta G_K/G_m^r)(E_K - E_m^a). \tag{5}$$

The linear relationship between ΔE_m and E_m^a presented in Fig. 3*A* shows that $\Delta G_K/G_m^r$ remains constant, independently of E_m^a or E_m^r. Since a shift of the membrane potential induced by a current application did not produce a significant change in G_m^r, ΔG_K was constant on application of a limited constant current. Moreover, the direct calculation of ΔG_K using the ΔE_m and G_m^r values (Fig. 2 and Table 6) as well as the E_K value (Okada *et al.*, 1977) showed that ΔG_K was nearly constant (9 ~ 10 nmho), under the normal $[Cl]_o$ condition.

In the low-Cl^- medium (under the normal $[K]_o$ and $[Na]_o$ conditions), however, the Cl-term in Eq. (4) might not be feasibly deleted. Using the data of E_m (Table 2) and G_m^r (in the text) in the low-Cl^-

medium in the steady state (more than 10 min after reducing $[Cl]_o$), such a ΔG_{Cl} contribution can be estimated from Eq. (4) keeping the K- and Cl-terms and making the plausible assumption that ΔG_K does not change in the low-Cl^- medium. The values of ΔG_K and ΔG_{Cl} thus estimated for an oscillation in the low-Cl^- medium are +10.9 and +0.5 nmho, respectively, and those for an H.A. response are +10.7 and +2.1 nmho, respectively. In the low-Na^+ medium (under the normal $[K]_o$ and $[Cl]_o$ conditions), the ΔG_{Na} contribution can be estimated by a similar treatment with Eq. (4) keeping the K- and Na-terms. The values of ΔG_K and ΔG_{Na} thus estimated using the data in Table 4 are +9.1 and −0.2 nmho for an oscillation, respectively, and +10.1 and +0.5 nmho for an H.A. response, respectively. These results strongly suggest that the change in G_K is the only important factor producing the activated potential irrespective of whether it is a spontaneous oscillation or an electrically activated response, and this increase in G_K is almost constant (9–10 nmho) independently of the voltage, under the conditions of various monovalent ion concentrations.

In view of these results, it seems quite natural that valinomycin induced a remarkable hyperpolarization and completely blocked the oscillation and the H.A. response. As shown in Table 7, however, dissociation was observed between the action of inhibiting the oscillation and the H.A. response in the early stage of its application (2–10 min after addition). Valinomycin may penetrate through the cell membrane and directly affect the mitochondria, acting as an uncoupler of oxidative phosphorylation. In this connection, it has been reported that valinomycin produces marked ATP depletion in Ehrlich ascites tumor cells (Levinson, 1967) and the perfused guinea pig heart (Schneider & Sperelakis, 1974). If such is also the case in L cells, the blockade of oscillations on exposure to valinomycin would probably result from its action as a metabolic inhibitor. In fact, KCN and DNP blocked the spontaneous oscillations without affecting H.A. responses induced by electrical stimuli, as reported in the preceding paper (Okada *et al.*, 1977). These results suggest that the oscillations of membrane potentials are related to cellular biochemical activities which remain to be identified.

The observation of current-voltage curves could provide information on the characteristic of the K^+ current responsible for oscillations. In addition, effects of divalent cations on the oscillation should be studied to elucidate the regulating mechanism of this K^+ current. Studies along these lines are in progress and these results will be described in future publications.

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