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Oscillations of Cytoplasmic Concentrations of Ca^{2+} and K^+ in Fused L Cells

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Summary. Using Ca^{2+}- and K^+-selective microelectrodes, the cytosolic free Ca^{2+} and K^+ concentrations were measured in mouse fibroblastic L cells. When the extracellular Ca^{2+} concentration exceeded several micromoles, spontaneous oscillations of the intracellular free Ca^{2+} concentration were observed in the submicromolar ranges. During the Ca^{2+} oscillations, the membrane potential was found to oscillate concomitantly. The peak of cyclic increases in the free Ca^{2+} level coincided in time with the peak of periodic hyperpolarizations. Both oscillations were abolished by reducing the extracellular Ca^{2+} concentration down to 10^{-7} M or by applying a Ca^{2+} channel blocker, nifedipine (50 μM). In the presence of 0.5 mM quinine, an inhibitor of Ca^{2+}-activated K^+ channel, sizable Ca^{2+} oscillations still persisted, while the potential oscillations were markedly suppressed. Oscillations of the intracellular K^+ concentration between about 145 and 140 mM were often associated with the potential oscillations. The minimum phase of the K^+ concentration was always 5 to 6 sec behind the peak hyperpolarization. Thus, it is concluded that the oscillation of membrane potential results from oscillatory increases in the intracellular Ca^{2+} level, which, in turn, periodically stimulate Ca^{2+}-activated K^+ channels.

Key Words oscillation · Ca^{2+}-selective microelectrode · intracellular Ca^{2+} concentration · Ca^{2+}-activated K^+ channel · intracellular K^+ concentration · membrane potential · fibroblast

Introduction

L-strain mouse fibroblasts exhibit membrane potential oscillations composed of spontaneous repeated hyperpolarizations (Okada et al., 1977*a*; Nelson & Henkart, 1979). Previous studies have shown that the hyperpolarizing phase is caused by an increase in the K^+ conductance of the cell membrane (Nelson et al., 1972; Okada et al., 1977*b*; Roy & Okada, 1978). The electrical potential profile in L cells is also known to be affected by removal of extracellular Ca^{2+} (Okada et al., 1979; Ince et al., 1984), by the extracellular application of Ca^{2+} channel blockers (Okada et al., 1981, 1982) or Ca^{2+} ionophore (Okada et al., 1979) or by intracellular Ca^{2+} injection (Henkart & Nelson, 1979; Okada et al., 1979, 1982; Ince et al., 1984; Hosoi & Slayman, 1985). In addition, quinine and quinidine, known inhibitors of Ca^{2+}-activated K^+ channel, were shown to inhibit the oscillatory hyperpolarizing response (Okada et al., 1982). Therefore, it is likely that the hyperpolarizations are brought about by elevation of the cytoplasmic free Ca^{2+} level, which, in turn, activates Ca^{2+}-dependent K^+ conductance of the membrane. If this were the case, periodic increases in the free Ca^{2+} concentration would occur within the cells in association with the membrane potential oscillation. Furthermore, cyclic increases in the K^+ conductance might result in periodic decreases in the intracellular free K^+ concentration, inasmuch as the membrane permeability to Cl^- is very high in L cells (Lamb & MacKinnon, 1971; Okada et al., 1977*b*). However, no direct measurements of cytoplasmic Ca^{2+} and K^+ concentrations have been reported in L cells. We present here direct observations of oscillations of cytosolic Ca^{2+} and K^+ concentrations with Ca^{2+}- and K^+-selective microelectrodes. Based on these observations, it is concluded that the membrane potential oscillation results from the oscillation of intracellular free Ca^{2+} level, which, in turn, induces periodic stimulation of Ca^{2+}-activated K^+ conductance.

Some of these data have been published in a short report (Ueda et al., 1983).

Materials and Methods

L cells (a mouse fibroblast cell line) were cultured in Fischer medium supplemented with 10% bovine serum without adding any antibiotics. The monolayer of multinucleate giant L cells (of 10 to 20 μm thick and 50 to 200 μm in diameter, containing tens to hundreds of nuclei) produced by cell fusion with polyethylene glycol (Okada et al., 1984) was used.

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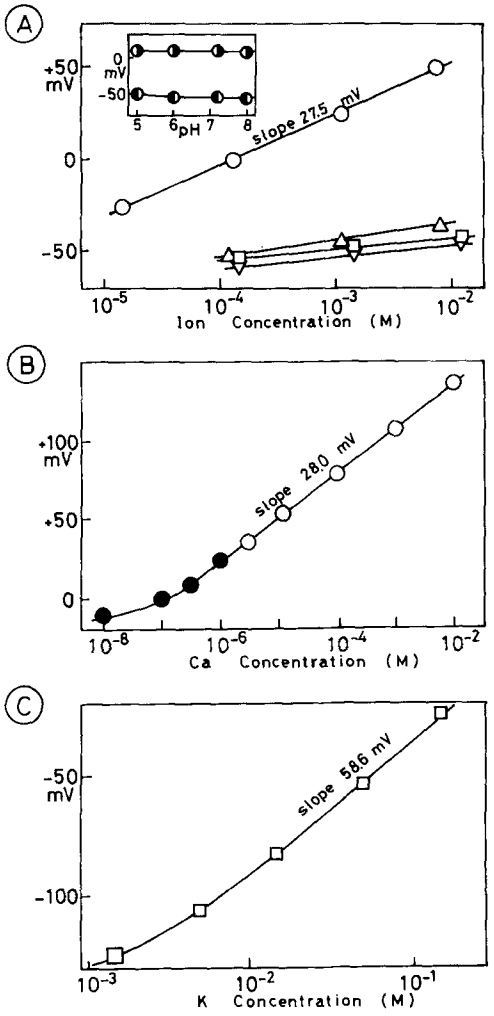

Fig. 1. Calibration of Ca^{2+}- and K^+-selective microelectrodes. (*A*) Ca electrode potentials in simple $CaCl_2$ (○), $MgCl_2$ (△), KCl (□) and NaCl (▽) solutions (pH ~6). Inset: Ca electrode potentials in 1 μM (◐) and 1 mM $CaCl_2$ (◑) solutions with different pH values. (*B*) Ca-electrode potentials in $CaCl_2$ solutions approximating the cytosol (130 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$ and 10 mM Tris-HCl, pH 7.20) in the presence (●) and absence (○) of 1 mM EGTA. (*C*) K electrode potentials in KCl-Na gluconate solutions (150 mM in total) (pH ~ 6). The selectivity coefficient against Na^+ was calculated to be 0.013 from this curve

The membrane potential (V_m) was recorded with intracellular electrodes (Okada et al., 1977*a*). The resistance of the microelectrodes (tip size ≤ 0.5 μm) filled with 3 M KCl or 0.5 M KCl was 20 to 100 MΩ, and the tip potential less than 5 mV.

Single-barreled Ca^{2+}-selective microelectrodes (Ca electrodes) were made with a neutral ligand sensor cocktail (Oehme et al., 1976) according to the methods of Rink and Tsien (1980) with a slight modification. The glass micropipettes (tip diameter, 1 to 1.5 μm) were made from Pyrex® capillaries (1 or 2 mm in diameter) and baked at 220°C for 30 min. Their surface was silanized by exposure to vapor of tri-*n*-butylchlorosilane at 220°C. The micropipettes were first backfilled with 0.1 M $CaCl_2$, and then filled by suction with Ca sensor resin up to 100 to 500 μm from the tip. The outer surface was coated with an electroconductive resinous paint (Dotite S-1, Fujikura Chemicals) or a silver paint (Silvest P-225, Tokuriki Chemicals) from the shoulder up to 30 to 100 μm of the tip. The resistance was 10 to 20 GΩ. The selectivity coefficients estimated at 1 mM by the separate solution method (Blinks et al., 1982) were 1.6×10^{-6}, 1.9×10^{-8} and 3.5×10^{-9} against Mg^{2+}, Na^+ and K^+, respectively (Fig. 1*A*). The electrodes were practically insensitive to pH in a range from 5 to 8 (Fig. 1*A*, Inset). Calibration of the Ca electrodes was carried out before and after each impalement in the solution containing the presumptive cationic composition of the cytoplasm (mM: 130 KCl, 10 NaCl, 1 $MgCl_2$ and 10 Tris-HCl, pH 7.20) with different $CaCl_2$ concentrations. The solutions containing 10^{-8} to 10^{-6} M Ca^{2+} were made from a Ca^{2+} buffer consisting of 1 mM ethylene glycol bis(β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA, Nakarai Chem. Co.). The free Ca^{2+} concentrations were calculated using the absolute values of stability constants of EGTA for binding of Ca^{2+}, Mg^{2+} and H^+ (Fabiato & Fabiato, 1979). All the Ca electrodes showed virtually ideal (Nernstian) responses between *p*Ca 2 and 6.5 (the mean slope 27.5 ± 0.4 mV, $n = 29$) and sub-Nernstian responses down to *p*Ca 8, as shown in Fig. 1*B*. Though polyvinyl chloride was not used for the sensor matrix, super-Nernstian responses and hysteresis (Tsien & Rink, 1980, 1981) were usually absent, probably because of the relatively large tip size of the Ca electrodes employed (Lanter et al., 1982). The electrodes exhibiting super- or sub-Nernstian responses between *p*Ca 3 and 6.5 were discarded. The response time for changes in Ca^{2+} concentration was measured in a series of calibration solutions. The Ca electrodes with response time of shorter than 1.5 sec (to 90% of the final value) were selected for the experiments. The response of the Ca electrode to changes in electrical potentials was tested within the cell by altering the V_m with a current pulse (5 nA) injected through the intracellular potential electrode (V_m electrode) using a bridge circuit. Some of the Ca electrodes were found to have a relatively low voltage sensitivity. This could not be attributed to the leakage around the impaled Ca^{2+} electrode, since identical potential changes were recorded from the two electrodes when a relatively blunt V_m electrode was impaled simultaneously with a sharp V_m electrode. The low voltage sensitivity of these Ca electrodes might be due to a significant shunt conductance across the glass wall (Okada & Inouye, 1976; Armstrong & Garcia-Diaz, 1980; Lewis & Willis, 1980) just outside the cell because of the high resistivity at the tip pore filled with the Ca sensor. When the voltage response of the Ca electrode was less than 90% of the V_m electrodes, the Ca electrode was discarded.

Single-barreled K^+-selective microelectrodes (K electrodes) were prepared with a K^+ sensor (Corning 477317) by an essentially identical method with that for Ca electrodes. However, coating with an electroconductive paint was not usually done, because the response time of K electrodes without painting was found to be as fast (<0.5 sec) as that of the electrodes with painting (*see* Fig. 4*A*,*B*). The K electrodes had a tip diameter of 0.5 to 1 μm, and their resistances were of the order of 10^9 Ω. The K electrodes showed Nernstian behavior to K^+ between 1 to 150 mM in a series of solutions containing only KCl (the mean slope 57.3 ± 7.7 mV, $n = 12$) and above 10 mM in a series of K^+ solutions containing KCl and Na-gluconate of 150 mM in total (the mean slope 59.0 ± 0.3 mV, $n = 4$) (Fig. 1*C*). The selectivity coefficients determined by the separate solution method were 1.8×10^{-2}, 4.4×10^{-3} against Na^{2+}, Mg^{2+} and Ca^{2+}, respectively, at 10 to 100 mM.

Intracellular Ca^{2+} and K^+ concentrations ($[Ca^{2+}]_i$ and $[K^+]_i$)

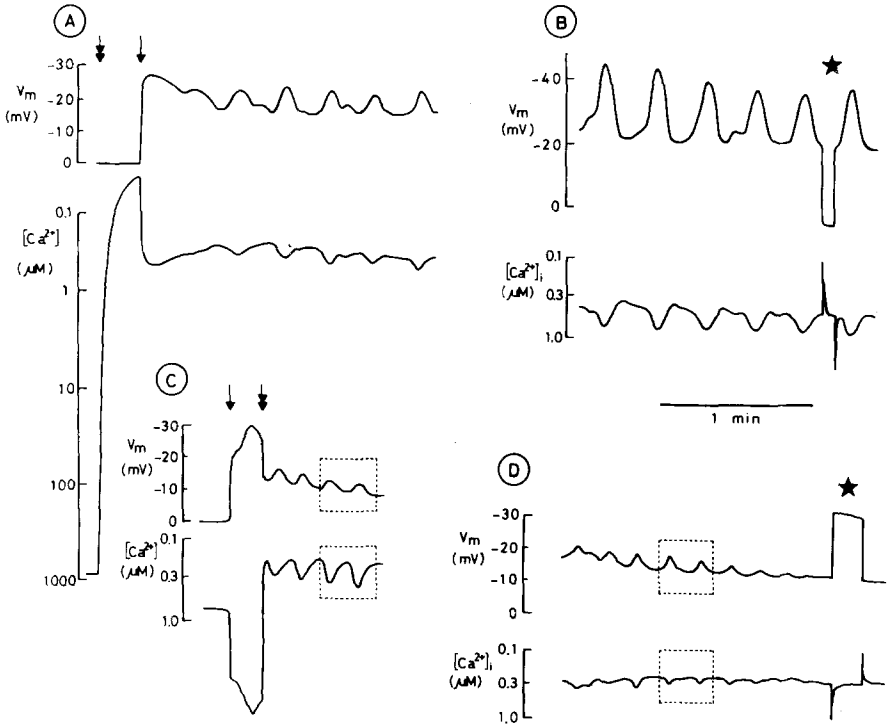

Fig. 2. Oscillations of intracellular free Ca^{2+} concentrations during membrane potential oscillations in giant L cells in the presence of 0.9 mM (*A,B*), 7 μM (*C*) or 5 μM extracellular Ca^{2+} (*D*). Each lower trace is the differential record between Ca and V_m electrodes representing $[Ca^{2+}]_i$ values (*A–D*) as well as extracellular free Ca^{2+} concentrations ($[Ca^{2+}]$: *A*, *C*). V_m or Ca electrodes were impaled at the time indicated by the arrows with single or double heads, respectively. Current pulses (±5 nA) were applied to the cell through the V_m electrodes with a bridge circuit at asterisks. The data designated by dotted squares are plotted in Fig. 5

were measured by subtracting the potential of the V_m electrode (recorded via WPI KS700) from that of the Ca or K electrode (via WPI FD223). These signals were monitored on a 3-channel pen recorder (Graphtec SR6221). All experiments were performed at room temperature (24 ± 2°C), instead of 37°C, to slow the frequency of membrane potential oscillations (Okada et al., 1981).

The control medium was a Tris-buffered saline (TBS) containing (mM): 143 NaCl, 4.2 KCl, 0.5 $MgCl_2$, 20 mannitol and 10 Tris-HCl (pH 7.2). This nominally Ca^{2+}-free TBS was found to contain 3 to 5 μM Ca^{2+} due to the contamination within the chemicals and distilled water employed. When necessary, 4 μM to 0.9 mM $CaCl_2$ or 0.1 mM EGTA was added to the nominally Ca^{2+}-free TBS.

Dimethylsulfoxide (DMSO) was used as a vehicle for nifedipine (a gift from Bayer Yakuhin Ltd., Osaka). Quinine (Nakarai Chem. Co.) was directly dissolved in TBS.

All data presented in the text and tables are expressed as the mean ± SE (*n*: number of the observations).

Results

Oscillation of $[Ca^{2+}]_i$

In almost all the L cells impaled with both V_m and Ca electrodes, oscillations of the membrane potential were observed in the TBS containing 0.9 mM Ca^{2+} (Fig. 2*A,B*), as reported previously (Okada et al., 1977*a*). In all the cells exhibiting potential oscillations, oscillatory changes in the free Ca^{2+} concentrations were simultaneously observed in the submicromolar or micromolar ranges. The $[Ca^{2+}]_i$ oscillations were nearly in phase with the V_m oscillations, and the $[Ca^{2+}]_i$ increase was always associated with the hyperpolarization. In the cells incubated in the TBS containing 3 to 10 μM Ca^{2+}, the synchronous oscillations of V_m and $[Ca^{2+}]_i$ were also observed, but both amplitudes gradually diminished (Fig. 2*C,D*). Since only those Ca electrodes which sensed faithfully the membrane potential deflection were selected for the experiments (*see* Materials and Methods), the response recorded with the Ca electrode could not be attributed to its poor voltage sensitivity or time resolution. In fact, when the membrane potential was deliberately altered by current pulse injections, the measured $[Ca^{2+}]_i$ was not affected (Fig. 2*B,D*, asterisks).

The $[Ca^{2+}]_i$ values at the depolarized (resting) state scattered from 0.2 to 1.6 μM within the cells incubated in 0.9 mM Ca^{2+} TBS, and the mean value (Table 1) was somewhat higher than those obtained in a variety of other cell species with the same type of Ca electrodes (e.g., *see* Table 6 in Blinks et al., 1982). Since the membrane potential was significantly reduced upon penetration with the Ca electrode (Fig. 2*C*), some of the data may have been artifactually affected by a leakage entry of extracellular Ca^{2+} ions around the impaled Ca electrodes. This possibility was further suggested by the fact that the $[Ca^{2+}]_i$ values observed at the resting state were always in the submicromolar range when the

Table 1. Intracellular Ca^{2+} concentration and membrane potential at the resting and spontaneously activated states in giant L cells

Extracellular Ca^{2+} concentration	Cell	$[Ca^{2+}]_i$ (μM)		V_m (mV)	
		resting	activated	resting	activated
0.9 mM	1	0.23	0.34	−16.6	−23.8
	2	0.26	0.40	−12.9	−23.2
	3	0.53	1.30	−14.8	−28.0
	4	0.55	0.87	−10.6	−19.1
	5	1.00	1.47	−17.0	−29.6
	6	1.13	1.47	−15.5	−20.0
	7	1.20	1.80	−14.4	−27.6
	8	1.63	3.13	−12.8	−23.0
	mean ± SE	0.82 ± 0.17	1.35 ± 0.30	−14.3 ± 0.7	−24.3 ± 1.3
3–10 μM	1	0.19	0.26	−16.5	−22.5
	2	0.20	0.37	−11.9	−15.4
	3	0.21	0.33	−13.1	−18.6
	4	0.21	0.43	−8.9	−13.0
	5	0.28	0.80	−10.5	−14.1
	6	0.29	0.73	−7.7	−10.6
	7	0.31	0.53	−15.4	−19.2
	8	0.33	0.73	−11.0	−19.6
	9	0.33	0.83	−16.6	−31.2
	10	0.40	0.57	−21.6	−28.6
	11	0.43	0.87	−13.6	−22.8
	12	0.60	0.83	−15.0	−25.0
	13	0.67	0.90	−21.8	−37.6
	14	0.70	1.80	−19.0	−37.2
	15	0.80	1.27	−18.2	−26.0
	16	0.83	1.27	−12.2	−22.4
	mean ± SE	0.42 ± 0.5	0.78 ± 0.10	−14.6 ± 1.0	−22.7 ± 1.9

Table 2. Changes in the intracellular K^+ concentration during membrane potential oscillations

Cells	Number of observations	$[K^+]_i$ (mM)		V_m (mV)	
		maximum	minimum	resting	activated
with $[K^+]_i$ change	10	145 ± 21	140 ± 21	−11.8 ± 1.5	−30.3 ± 3.6
without $[K^+]_i$ change	17	130 ± 7	—	−15.2 ± 1.0	−28.9 ± 1.5
total	27	136 ± 9	134 ± 9	−13.9 ± 0.9	−29.4 ± 1.6

cells were incubated in 3 to 10 μM Ca^{2+} TBS (Table 1).

The intracellular free Ca^{2+} always increased during the hyperpolarizing phase, and the $[Ca^{2+}]_i$ values at peak hyperpolarizations were around twice as large as those at the resting state, regardless of the extracellular Ca^{2+} level (Table 1).

A reduction in the extracellular Ca^{2+} concentration down to 10^{-8} to 10^{-7} M (using 0.1 mM EGTA) abolished the V_m oscillation (Fig. 3*A*). Under these conditions, the $[Ca^{2+}]_i$ oscillation was also abolished (Fig. 3*A*). It should be noted that $[Ca^{2+}]_i$ exceeded the extracellular Ca^{2+} concentration. The mean $[Ca^{2+}]_i$ of 4 cells incubated in 0.14 μM Ca^{2+} TBS was 0.25 ± 0.06 μM. A Ca^{2+} channel blocker, nifedipine, inhibited not only the V_m oscillation (Okada et al., 1982) but also the $[Ca^{2+}]_i$ oscillation (Fig. 3*B*). The $[Ca^{2+}]_i$ stayed at 0.2 to 0.3 μM without showing detectable fluctuations in the presence of nifedipine. These observations are in good agreement with the notion that an increase in the cytosolic free Ca^{2+} responsible for the hyperpolarizing response is mainly derived from the extracellular milieu via Ca^{2+} channels (Okada et al., 1979). However, the possibility that the Ca^{2+} origin is an intracellular Ca pool (Henkart & Nelson, 1979) cannot entirely be ruled out, if the pool is rapidly depleted and replenished from the extracellular solution via a nifedipine-sensitive route.

In the presence of 0.5 mM quinine, an inhibitor

of Ca^{2+}-activated K^+ channels, the V_m oscillation was markedly suppressed, while the $[Ca^{2+}]_i$ oscillation was consistently observed as in the absence of the drug (Fig. 3*C*). In the light of these observations, it is concluded that spontaneous V_m oscillations are due to the $[Ca^{2+}]_i$ oscillations which periodically stimulate quinine-sensitive, Ca^{2+}-activated K^+ channels.

Oscillation of $[K^+]_i$

It has been known that the membrane of L cell is appreciably permeable to Cl^- (Lamb & MacKinnon, 1971; Okada et al., 1977*b*). During the V_m oscillation, the intracellular Cl^- concentration was actually found to fluctuate in a cyclic manner in giant L cells with Cl^--selective microelectrodes (Ueda et al., 1981). Thus, it can be expected that the cyclic increases in the K^+ permeability may also produce detectable fluctuations in the intracellular K^+ concentration ($[K^+]_i$). In fact, small $[K^+]_i$ oscillations were observed in 10 out of 27 cells impaled simultaneously with a K electrode and a 3 M KCl-filled V_m electrode (Fig. 4*A*). The $[K^+]_i$ oscillation was about 100 to 120° out of phase with the V_m oscillation. The intracellular K^+ ions decreased by several millimoles about 5 to 6 sec after each peak hyperpolarization (Fig. 4*A*, Table 2). Again, the observed cyclic $[K^+]_i$ changes could not be attributed to the membrane potential changes as evidenced by the ability of K electrodes to respond to the passage of current pulses (Fig. 4*A*,*B*, asterisks). Such $[K^+]_i$ oscillations were not detected in 17 other cells with a relatively larger diameter (Fig. 4*B*). However, it should be noted that submillimolar changes in $[K^+]_i$ would be beyond the resolution of the present technique.

The steady $[K^+]_i$ value was about 140 mM in the cells incubated in TBS containing 4.2 mM K^+ and 0.9 mM Ca^{2+} (Table 2). Because the tip size of the K electrodes was smaller than that of Ca electrodes and because the K^+ gradient across the cell membrane was not so great, the leakage artifact would not be expected for the $[K^+]_i$ measurements. In fact, the impalement with a K electrode did not cause a significant reduction of the membrane potential. It must be argued that the possible K^+ leakage from 3 M KCl-filled microelectrodes (Fromm & Schultz, 1981; Blatt & Slayman, 1983; Stoner et al., 1984) affects the $[K^+]_i$ level measured even in the giant cells. However, with 0.5 M KCl-filled microelectrodes, from which much less leakage of K^+ occurs (Fromm & Schultz, 1981), essentially identical $[K^+]_i$ values (137 ± 9 mM, $n = 7$) were obtained in the steady state.

In the presence of nifedipine, by which the V_m oscillation was remarkably suppressed or abolished, no $[K^+]_i$ oscillations were observed (Fig. 4*C*). Reliable $[K^+]_i$ measurements could not be made in the presence of quinine, because the K electrode potentials always shifted by about 150 mV and spike-like noises were frequently induced by this drug.

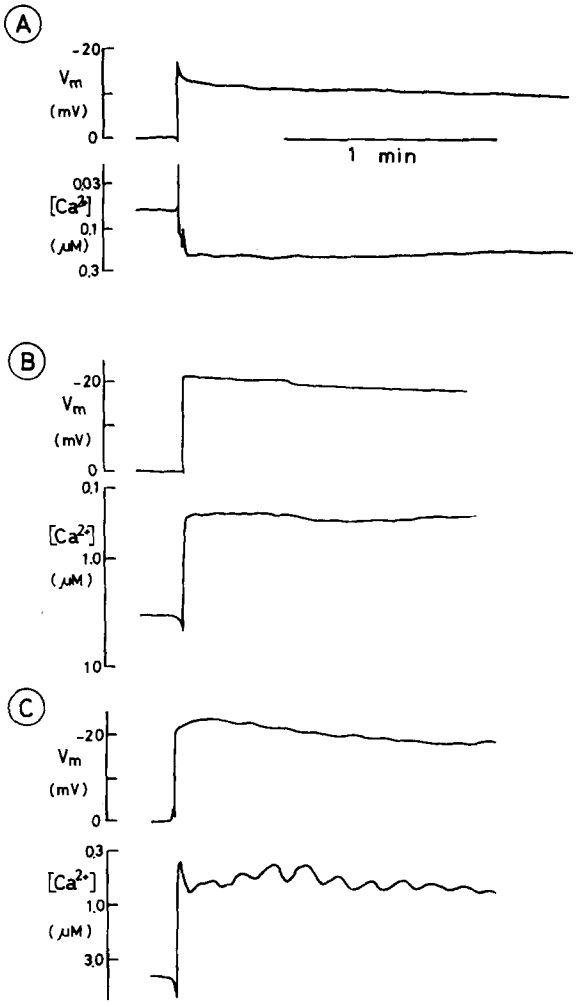

Fig. 3. Effects of extracellular EGTA (0.1 mM), nifedipine (50 μM) and quinine (0.5 mM) on the oscillations of $[Ca^{2+}]_i$ and V_m in giant L cells. V_m and Ca electrodes were simultaneously introduced into the cells 1 to 3 min after adding these drugs. In the three cells tested, qualitatively similar results were obtained

Discussion

The present study with Ca^{2+}-sensitive microelectrodes provides direct evidence for oscillations of the intracellular Ca^{2+} concentration responsible for the membrane potential oscillations in L cells. The use of relatively large-sized Ca electrodes (tip size of 1 to 1.5 μm) enabled one to attain their excellent

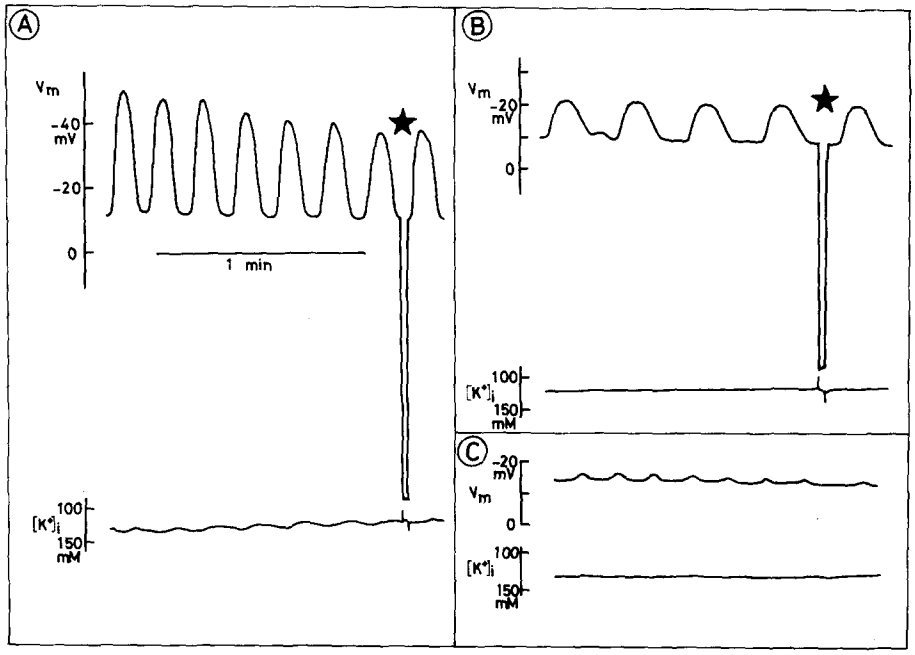

Fig. 4. Intracellular free K^+ concentrations during membrane potential oscillations in giant L cells with relatively small (70 to 100 μm: *A,C*) and large diameters (about 200 μm: *B*). Current pulses (+5 nA) were applied to the cell through V_m electrodes at asterisks

voltage sensitivity, fast response time and no super-Nernstian response, probably because their shunt conductance through the wall of glass micropipettes near the tip was negligible compared with the tip conductance filled with the liquid-ion exchanger (Lewis & Willis, 1980; Tsien & Rink, 1981). However, in principle, the larger the tip, the greater would be leakage around the electrode. In fact, the intracellular Ca^{2+} level measured in the bathing solution containing 0.9 mM Ca^{2+} was often over 1 μM. However, the $[Ca^{2+}]_i$ values of 0.2 to 0.3 μM were also obtained occasionally (Fig. 2*A,B*; Table 1). Furthermore, when the extracellular Ca^{2+} concentration was reduced down to 3 to 10 μM, in which the potential oscillations still persisted, the resting $[Ca^{2+}]_i$ was found to be always in the submicromolar ranges and often 0.2 to 0.3 μM (Fig. 2*C,D*; Table 1). Even under these conditions, the leakage artifact may have little affected some $[Ca^{2+}]_i$ measurements. Thus, it can be conceived that the intracellular free Ca^{2+} level is about 0.2 μM in the resting L cells. This is in line with values for $[Ca^{2+}]_i$ obtained in a variety of cell types by the Ca^{2+}-selective microelectrodes made with Simon's neutral carrier (*see* Blinks et al., 1982, for a review).

Figure 5 summarized the V_m values as a function of $[Ca^{2+}]_i$ observed in the solutions containing 3 to 10 μM Ca^{2+}. The relationship looks sigmoidal. In the submicromolar $[Ca^{2+}]_i$ ranges over 0.2 μM, the membrane is progressively hyperpolarized approximately parallel to the increase in $[Ca^{2+}]_i$ during an oscillation. The membrane potential reaches a plateau above 1.5 μM and below 0.2 μM. The half-maximum effect is observed at about 0.6 μM. A similar $[Ca^{2+}]_i$ dependency of Ca^{2+}-activated K^+ channels has been obtained by the single-channel recordings in cultured rat muscle (Barrett et al., 1982) or in red cell membrane (Grygorczyk & Schwarz, 1983), and by the measurements of ^{42}K or ^{86}Rb fluxes in red cell membrane (Simons, 1976; García-Sancho et al., 1982). However, it should be noted that the $[Ca^{2+}]_i$ value measured with Ca electrodes represents only that in a restricted region near the electrode tip which is presumably placed at a substantial distance from the active membrane. Since it is known that the Ca^{2+} concentration or Ca^{2+} buffering capacity is not uniform within the cytosol (Baker et al., 1971; Rose & Loewenstein, 1975; Loewenstein, 1976; Tillotson & Gorman, 1980; DiPolo et al., 1983; Gorman et al., 1984), the actual Ca dependency of the Ca^{2+}-activated K^+ channel at the active membrane would be somewhat different from that shown in Fig. 5.

Recently, it has been suggested that the membrane potential oscillation results from leakage of Ca^{2+} around the impaled microelectrode (Ince et al., 1984). However, seven lines of available evidence strongly argue against this possibility: 1) Regular potential oscillations can be observed at a higher incidence in giant cells than in small cells, although the latter should be susceptible to the leakage formation around the electrode (Okada et al., 1977*a*); 2) A number of records, in which the potential oscillation could be initiated at any phase, sug-

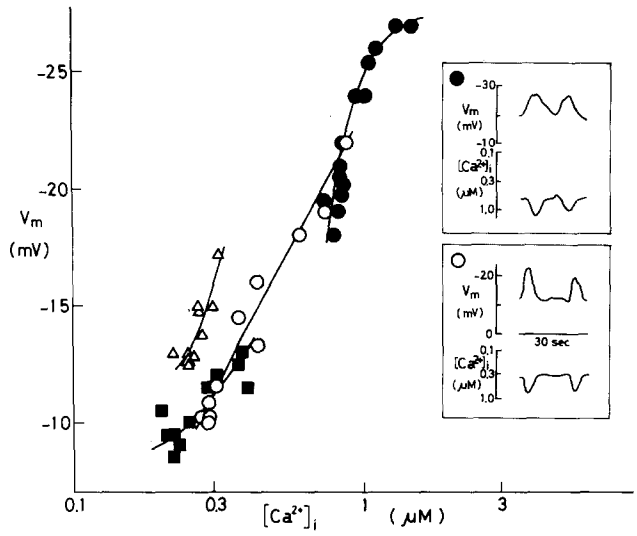

Fig. 5. Relationship between intracellular free Ca^{2+} concentrations and membrane potentials during oscillations of four giant L cells. Each symbol represents the data collected at 2-sec intervals from 2 cycles of the oscillations presented in Insets (●, ○), Fig. 2*C* (■) and Fig. 2*D* (△)

gest that the oscillation is endogenous (Okada et al., 1977*a*); 3) When a single hyperpolarizing response is elicited by mechanical stimulation upon the impalement with a microelectrode, it is superimposed on the intrinsic regular oscillation (Okada et al., 1981); 4) A variety of Ca^{2+} channel blockers inhibit the oscillations without blocking Ca^{2+}-activated K^+ channels (Okada et al., 1981, 1982); 5) Cytochalasin B or local anesthetics block the oscillations without inhibiting the K^+ channels (Tsuchiya et al., 1981); 6) Potential oscillations can be abolished in the presence of metabolic inhibitors, even when hyperpolarizing responses are still induced by electrical stimuli (Okada et al., 1977*a*; 1981); 7) The oscillations can be observed during whole-cell recordings with giga-sealed microelectrodes (Oiki et al., 1985). In addition, the present study showed that the intracellular free Ca^{2+} level increased little upon penetration with a second V_m electrode (Fig. 2*A*).

Similar $[Ca^{2+}]_i$ oscillations have been demonstrated by means of Ca electrodes in salivary gland epithelial cells stimulated by 5-HT (O'Doherty et al., 1980) or of optical Ca^{2+} indicators in *Physarum* (Ridgway & Durham, 1976; Kamiya et al., 1982), in molluscan neurones (Gorman et al., 1982) and in ferret papillary muscle (Orchard et al., 1983). Thus, the intracellular Ca^{2+} oscillation may be essential for some cellular functions. In mouse fibroblastic L cells, oscillatory hyperpolarizations are suggested to be related to phagocytosis (Okada et al., 1981), pinocytosis (Tsuchiya et al., 1981) and chemotaxis (Okada et al., 1983, 1984; Oiki et al., 1984). Potential oscillations were also observed (Okada et al., 1984) in human normal diploid fibroblasts under stimulation by exogenous ATP, which is thought to be one of the chemoattractants for fibroblasts (Okada et al., 1983). Taken together, the $[Ca^{2+}]_i$ oscillation is likely to be involved in Ca^{2+}-mediated, membrane-mobile cell activities in fibroblasts. Whether the resultant fluctuation of intracellular K^+ concentrations is merely a by-product of the K^+ conductance change to modulate the membrane potential or is by itself implicated in some physiological functions, for instance, regulation of the metabolic activity, still remains to be clarified.

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