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Ca^{2+}-Activated K^+ Conductance Causes Membrane Hyperpolarizations in a Monkey Kidney Cell Line (JTC-12)

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Summary. We have previously reported hyperpolarizing membrane potential changes in a monkey kidney cell line (JTC-12) which has characteristics resembling proximal tubular cells. These hyperpolarizations could be observed spontaneously or evoked by mechanically touching adjacent cells. In this report, we have shown further evidence that these hyperpolarizations are elicited by an increase in membrane conductance to K^+ which is caused by an increase in cytosolic Ca^{2+} concentration. In addition, we have found another type of hyperpolarization which is evoked by applying flow of extracellular fluid to the cell. Intracellular injection of Ca^{2+} and Sr^{2+} evoked hyperpolarizations, while intracellular injection of Mn^{2+} and Ba^{2+} did not. Intracellular injection of EGTA suppressed both spontaneous and mechanically evoked hyperpolarizations. In Ca^{2+}-free medium, both spontaneous and flow-evoked hyperpolarizations were not observed, while mechanical stimuli consistently evoked hyperpolarization. In Na^+-free medium, the incidence of cells showing the spontaneous or flow-evoked hyperpolarization increased, and the amplitude and the duration of the mechanically evoked hyperpolarization became greater. Quinidine inhibited all types of hyperpolarization. These data suggest that hyperpolarizations in JTC-12 cells are due to an increase in Ca^{2+}-activated K^+ conductance.

Key Words hyperpolarization · Ca^{2+}-activated K^+ conductance · JTC-12 cells · quinidine · fluid flow · proximal tubule

Introduction

JTC-12 cell is a cell line derived from monkey kidney [18], which possesses properties characteristic of the proximal tubular cells including existence of microvilli in apical membranes [29], sodium-dependent phosphate transport [28], adenylate cyclase activity sensitive to parathyroid hormone and prostaglandin E_1 [16], and 25-hydroxyvitamin D_3-24-hydroxylase activity induced by 1,25-dihydroxy-vitamin D_3 [21]. We have reported spontaneous and mechanically induced hyperpolarizing membrane potential changes in JTC-12 cells, which are caused by an increase in K^+ conductance [4]. Similar spontaneous membrane hyperpolarizations have been reported in several kinds of cells such as macrophages [7], fibroblastic L cells [24–26] and mammalian eggs [13, 14, 22, 23]. In these cells, hyperpolarizing membrane potential changes have been shown to be caused by an increase in Ca^{2+}-activated K^+ conductance. However, it is not known whether an increase in K^+ conductance during hyperpolarization in JTC-12 cells is due to a rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). In the present study, we extended our previous observations and examined the role of Ca^{2+} in hyperpolarization in JTC-12 cells. In addition, we have found that application of fluid flow to the cell also evoked membrane hyperpolarization in JTC-12 cells.

Materials and Methods

Cell Culture

JTC-12 cells were cultured at 37°C under 5% CO_2 humidified air in Eagle's minimum essential medium (Nissui, Japan) containing 10% (vol/vol) heat-inactivated fetal calf serum and 60 μg/ml kanamycin. Confluent monolayer cells were detached from culture dishes by incubation in Ca^{2+}, Mg^{2+}-free phosphate-buffered saline containing 0.02% (wt/vol) EDTA for 5 min. They were seeded on 35-mm culture dishes and subjected to electrophysiological study after 1 to 3 days, when cells were in their logarithmic growth phase. Medium was changed every other day and the day before an experiment.

Electrophysiological Analysis

A glass microelectrode filled with 3 M KCl was used to impale cells for intracellular membrane potential record. The tip resistance of the electrodes ranged from 30 to 50 MΩ. Current was passed through the intracellular electrode by means of a bridge circuit. The input resistance was calculated from the ratio of the membrane potential change to the applied current. The duration of the applied current was over 300 msec, which was much longer than the time constant of JTC-12 cell membrane (less than

Table 1. Composition of the extracellular media (mM)

	Na^+	K^+	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}	Choline$^+$	Li^+	La^{3+}	EGTA	Cl^-
Standard	128.75	6	1	2.5	0	0	0	0	0	0	141.75
Na^+-free	0	6	1	2.5	0	0	128.75	0	0	0	141.75
Ca^{2+}-free, 0.5 mM La^{3+}	132.5	6	1	0	0	0	0	0	0.5	0	142.0
Ca^{2+}-free, 2 mM EGTA Na^+-free	0	6	1	0	0	0	132.5	0	0	2	140.5
2.5 mM Sr^{2+}, Na^+-free	0	6	1	0	2.5	0	128.75	0	0	0	141.75
2.5 mM Ba^{2+}, Na^+-free	0	6	1	0	0	2.5	128.75	0	0	0	141.75
5/6 Li^+	21.67	6	1	2.5	0	0	0	107.08	0	0	141.75

The pH of the Na^+ containing media were adjusted to 7.3 by 20 mM HEPES (Na^+-salt). The pH of the Na^+-deficient media was adjusted to 7.3 by 20 mM HEPES (TMA-salt). All media contained 4 mg/ml bovine serum albumin (BSA) and 1 mg/ml glucose.

20 msec). Relatively large cells (diameter over 30 μm) were chosen in the present experiments because of technical reasons. Mechanical stimulation of adjacent cells (not the impaled cell) was conducted by finely touching neighboring cells with another glass electrode, which evoked hyperpolarization of the impaled cell, as reported previously [4]. The details of the electrophysiological methods were essentially the same as reported in a previous study [14].

For intracellular Ca^{2+} injection, the second electrode containing 0.5 M $CaCl_2$ was advanced into the recording cell. Ca^{2+} was iontophoretically injected by applying inward current of varying amplitude (0.4–3.0 nA) and duration (250 to 2000 msec). Intracellular injection of Sr^{2+}, Ba^{2+}, and Mn^{2+} was similarly performed. For intracellular EGTA injection, the second microelectrode was filled with 0.2 M EGTA (K^+-salt) and outward current was applied.

In experiments in which the effect of quinidine was examined, the tip of a glass capillary (tip diameter of a few microns) containing 5×10^{-4} M quinidine dissolved in the same solution with extracellular solution was positioned 30 to 40 μm away from the recording cell and quinidine was delivered to the proximity of the cell by applying pressure of 0.2 to 0.3 kg/cm^2 to the capillary. This pressure application method was the same as previously reported [31].

Whole cell variation of the patch-electrode voltage clamp was carried out in a similar manner as described by Hamill et al. [11]. Patch electrodes were filled with a solution containing 151.3 mM KCl, 1 mM $MgCl_2$, and 0.5 mM EGTA. The pH was adjusted to 7.4 with 20 mM HEPES (K^+-salt). The resistance of the patch electrode ranged from 8 to 10 MΩ. The seal resistance reached over 10 GΩ after application of negative pressure of less than 50 cm H_2O. After formation of giga-seal, negative pressure over 100 cm H_2O was applied in order to obtain whole-cell clamp condition, which was assumed by a sudden increase in the capacitative surge and the membrane noise. A liquid junction potential between the standard extracellular medium (*see below*) and the internal solution was −4 mV; this value was added to the measured potential.

In flow-application experiments, a glass microcapillary was pulled by a two-step puller, making a tip diameter of about 10 μm. These capillaries were filled with extracellular solution. The tip was positioned 150–250 μm apart from the recording cell. By applying pressure of about 10 cm H_2O for 10 sec to the capillary, fluid flow around the recording cell was generated. In separate experiments, the velocity of extracellular fluid flow around the recording cell was estimated in the solution suspending human red blood cells. By measuring the velocity of movement of red blood cells under microscopy, flow velocity was calculated to be 170 ± 40 μm/sec (n = 44).

All studies were carried out at temperature of 30–32°C. The ionic compositions of the external media are summarized in Table 1. All media contained 4 mg/ml bovine serum albumin (BSA) and 1 mg/ml glucose. The pH was adjusted to 7.3 by 20 mM HEPES. Data were expressed as the mean ± SD and analyzed by χ^2 or unpaired Student's t tests as appropriate.

Results

Reversal Potential of Hyperpolarization

The resting membrane potential of JTC-12 cells was −17.3 ± 5.2 mV and the input resistance was 80.2 ± 41.3 MΩ (n = 73) in the standard solution, which are similar to the values reported previously (−15.3 ± 7.4 mV and 78.0 ± 51.7 MΩ, respectively) [4]. Since the resting potential was not significantly changed in 20 mM K^+, Na^+ free, or Cl^- free solutions (*data not shown*), nonselective channel or leakage current may be determining the resting potential in JTC-12 cells. Resting potential of JTC-12 is lower than those reported for perfused proximal tubular cells (ca. −70 mV) [2, 6, 8] or other cultured tubular cell lines (ca. −50 mV) [3, 17]. The reason for such difference is presently unknown. However, as discussed previously [4], calculated membrane resistance of JTC-12 cells (2100 Ω cm^2) is much higher than those reported for luminal (260 Ω cm^2) or basolateral (90 Ω cm^2) membranes of proximal tubular cells [6], which might suggest that relatively poor expression of K^+ conductance in resting state may be the cause of low membrane potential in JTC-12 cells. However, the possibility cannot be excluded that the difference in the degree of cell-cell coupling may underlie these differences in membrane resistances. As reported previously, spontaneous and mechanically evoked hyperpolarizing membrane potential change was observed in JTC-12 cells [4]. These hyperpolarizations were accompanied by an increase in membrane conductance and the reversal potential was −72.7 mV, an observation which suggests that the hyperpolariza-

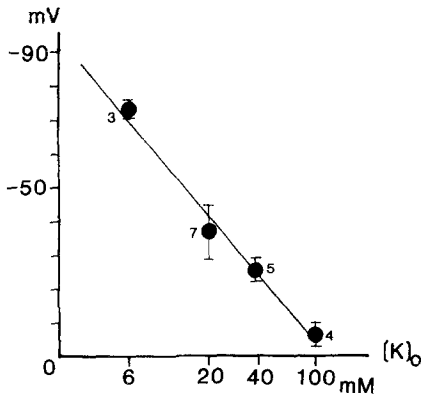

Fig. 1. Reversal potentials in various extracellular K^+ concentrations. Current-voltage relationships were obtained both at the resting state and at the peak of the hyperpolarization, by applying varying amplitude of inward and outward currents. Reversal potential was estimated as the the intercept of these two current-voltage relationships, both of which were linear between −110 to +20 mV. Extracellular K^+ concentration was changed by replacing Na^+ with K^+. Results are from mechanically evoked hyperpolarizations. Filled circles represent the mean value, and each bar indicates SD. Number of experiments is shown beside the circle. Line was drawn to be fitted by the least squares method. The slope was −56 mV/decade. Similar results were also obtained from spontaneous hyperpolarizations

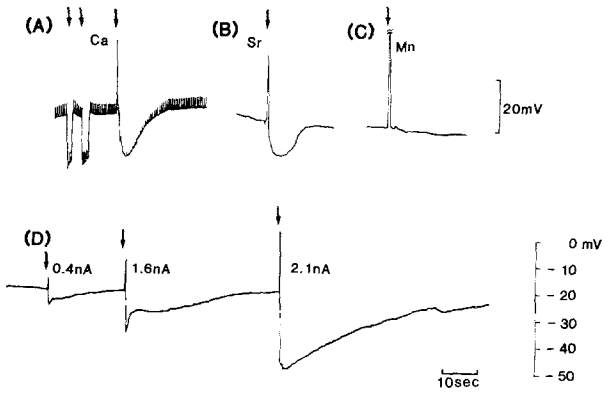

Fig. 2. Membrane potentials and the intracellular injection of divalent cations in the Ca^{2+}-free medium containing 0.5 mM La^{3+}. (*A*) Ca^{2+} injection. Outward currents of −0.4 nA and 400 msec were applied every 500 msec. Two outward currents of 1.8 nA (1,200 msec, 1,800 msec) showed almost no change in the membrane potential, while inward current (1.8 nA, 500 msec) evoked hyperpolarization with an increase in membrane conductance. The resting potential was −10 mV. (*B*) Sr^{2+} injection. Inward current pulse of 2 nA, 500 msec, was applied. The resting potential was −28 mV. (*C*) Mn^{2+} injection. The inward current pulse of 3 nA, 1,000 msec, was applied. The resting potential was −18 mV. (*D*) Dependence of the hyperpolarization in response to Ca^{2+} injection on the amplitude of the applied inward current. The duration of each applied current was 500 msec. The resting potential was −20 mV. Each arrow indicates the timing at which current pulse was applied

tions were due to an increase in membrane conductance to K^+. In order to further clarify the role of K^+ conductance, reversal potentials were evaluated in varying extracellular K^+ concentrations. As shown in Fig. 1, reversal potential changed by 56 mV with a tenfold change in extracellular K^+ concentration. The result indicates these hyperpolarizations are indeed due to increased membrane conductance to K^+.

Intracellular Injection of Ca^{2+} and Other Divalent Cations

In order to examine the role of intracellular Ca^{2+} in producing hyperpolarization, Ca^{2+} was iontophoretically injected into JTC-12 cells. Figure 2*A* depicts a tracing of a Ca^{2+} injection study. The extracellular medium was Ca^{2+}-free and contained 0.5 mM La^{3+}, in which Ca^{2+} influx from extracellular medium should be negligible. An outward current pulse, −0.4 nA and 400 msec duration, was applied every 500 msec to monitor input resistance. The outward current pulses elicited no hyperpolarizing phenomenon. In contrast, the inward current pulse (1.8 nA, 500 msec) which introduces Ca^{2+} into the cell caused membrane hyperpolarization with decreased input resistance, a response similar to spontaneous and mechanically evoked hyperpolarizations [4]. As can be seen in Fig. 2*A*, membrane conductance changed little when membrane potential was hyperpolarized by outward current pulse, an observation suggesting that voltage-dependent conductance, if any, contributes little to the observed increase in membrane conductance during the hyperpolarizing phenomena. The hyperpolarization by Ca^{2+} injection was reproduced in nine additional cells examined. The amplitude and the duration of Ca^{2+}-induced hyperpolarization was dependent on the amplitude and the duration of the applied current, i.e., the amount of Ca^{2+} injected (Fig. 2*D*). These results indicate that the hyperpolarization was caused by an increase in $[Ca^{2+}]_i$. Intracellular injection of Sr^{2+} evoked hyperpolarizing membrane potential changes similar to those with Ca^{2+} injection (Fig. 2*B*), while intracellular injections of Ba^{2+} and Mn^{2+} had no effect (Fig. 2*C*; a record of Mn^{2+} injection). These data strongly suggest that the hyperpolarization in JTC-12 cells is caused by Ca^{2+}-activated K^+ conductance. That the hyperpolarization produced by mechanical stimulation was larger in amplitude and longer in duration than the spontaneous hyperpolarizations [4] suggests that an increase in $[Ca^{2+}]_i$ in the mechanically evoked hyperpolarization was larger than that in the spontaneous hyperpolarization.

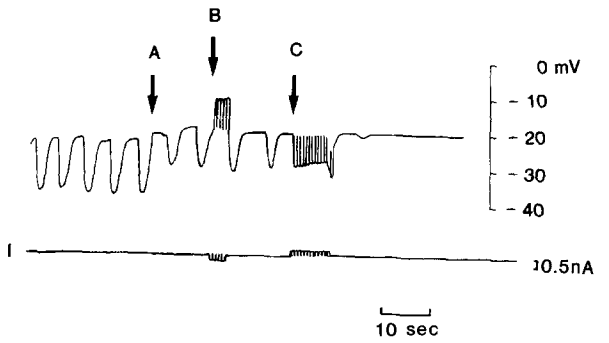

Fig. 3. Membrane potentials and intracellular injection of EGTA in the Na^+-free medium. The impalement of the second microelectrode filled with 0.2 M EGTA (K^+-salt) is indicated by arrow *A*. The application of inward current (arrow *B*) showed no effect on hyperpolarization, while outward current (arrow *C*) suppressed spontaneous hyperpolarization. The resting potential was −20 mV

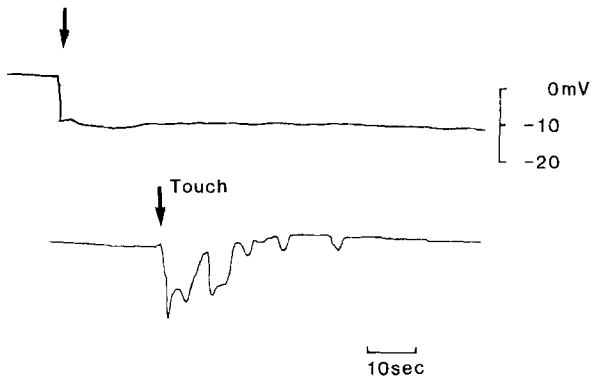

Fig. 4. Membrane potential recording in the Ca^{2+}-free medium containing 2 mM EGTA. The arrow in the upper trace indicates the timing of electrode impalement. Lower trace shows mechanically evoked hyperpolarization in this Ca^{2+}-free medium. Mechanical stimulation by touching the adjacent cell is indicated by an arrow (*Touch*)

Intracellular Injection of EGTA

In order to examine the effect of lowering $[Ca^{2+}]_i$, intracellular injection of EGTA was performed. Figure 3 shows the recording of a cell which demonstrates periodic spontaneous hyperpolarizations. Impalement of the second electrode depolarized membrane potential by a few mV with concomitant reduction of the amplitude of hyperpolarizations (arrow *A*), presumably due to an increased leakage conductance. Application of the inward current (arrow *B*) had no effect on spontaneous hyperpolarization. However, the outward current which introduced EGTA into the cell completely abolished spontaneous hyperpolarization (arrow *C*). Similar results were obtained in five additional cells (maximum applied outward current was 0.5 nA, 7.5 sec). In separate experiments, spontaneous hyperpolarizations were not abolished either by an inward or an outward current pulse, when the second electrode contained 3 M KCl ($n = 3$). Mechanically evoked hyperpolarization was also inhibited by EGTA injection ($n = 10$), although the inhibition was partial in most cells probably because a larger increase in $[Ca^{2+}]_i$ was not completely buffered by EGTA injected. These data suggest that reduction of $[Ca^{2+}]_i$ concentration inhibits hyperpolarizations and further support the notion that these hyperpolarizations are caused by an increase in $[Ca^{2+}]_i$, thus stimulating Ca^{2+}-activated K^+ conductance.

Effect of Extracellular Ca^{2+} on Hyperpolarization

To further assess the role of extracellular Ca^{2+} on these hyperpolarizations, membrane potential changes were examined in Ca^{2+}-free medium containing 2 mM EGTA. Figure 4 exemplifies recordings obtained in the Ca^{2+}-free medium. Persistent spontaneous hyperpolarization was not observed in this medium (Fig. 4, upper trace) ($n = 9$), although a few successive hyperpolarizations may appear in a few cells immediately after the impalement of the electrode. In contrast, mechanical stimulation consistently evoked a single or several successive hyperpolarizations (Fig. 4, lower trace). These results indicate that the spontaneous hyperpolarization, but not the mechanically evoked hyperpolarization, requires the presence of extracellular Ca^{2+}. In addition, these results indicate that the mechanically evoked hyperpolarization was not caused by an influx of Ca^{2+} into the recording cell around the microelectrode impalement site. Although a rise in $[Ca^{2+}]_i$ was essential for both types of hyperpolarizations, the influx of Ca^{2+} from extracellular solution was necessary for the generation of spontaneous hyperpolarization. Both spontaneous and mechanically evoked hyperpolarizations were observed in 2.5 mM Sr^{2+} medium, but not in 2.5 mM Ba^{2+} medium. Therefore, extracellular Sr^{2+} could substitute for Ca^{2+}, consistent with the results of our intracellular divalent-cation injection experiments.

Role of Na^+ on Hyperpolarization

In the previous study [4], we showed that the number of the cells showing spontaneous hyperpolarization was increased in Na^+-free medium. We examined this issue further. Table 2 compares the hyperpolarization in the standard and Na^+-free media. The incidence of the cells showing the sponta-

Table 2. Characteristics of hyperpolarizations

	Media		
	Standard	Na^+-free	5/6 Li^+
% of cells showing SH[a]	18.8% (19/101)	60.9%[d] (25/41)	52.0%[d] (13/25)
Amplitude of SH (mV)	7.2 ± 6.1[b] (n = 19)	8.2 ± 5.5 (n = 25)	11.3 ± 5.5[c] (n = 13)
Duration of SH (sec)	4.1 ± 1.2 (n = 19)	4.8 ± 2.7 (n = 25)	7.9 ± 1.5[d] (n = 5)
Amplitude of MEH[a] (mV)	18.9 ± 7.2 (n = 47)	23.2 ± 10.1[c] (n = 19)	25.0 ± 10.4[d] (n = 20)
Duration of MEH (sec)	22.4 ± 9.6 (n = 47)	44.1 ± 20.7[d] (n = 19)	49.4 ± 18.7[d] (n = 20)

[a] SH: spontaneous hyperpolarization; MEH: mechanically evoked hyperpolarization.
[b] Mean ± SD.
[c] Significant $P < 0.05$ *vs.* standard.
[d] Significant $P < 0.01$ *vs.* standard.

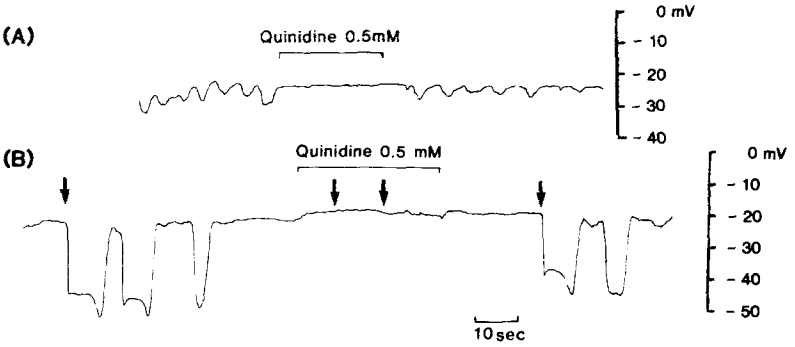

Fig. 5. The effects of quinidine on hyperpolarization in the Na^+-free medium. (A) Suppression of spontaneous hyperpolarization by pressure application of 0.5 mM quinidine as indicated by a horizontal bar. The resting potential was −26 mV. (*B*) Inhibition of mechanically evoked hyperpolarization by quinidine. Mechanical stimulation is indicated by arrows. This cell showed three successive hyperpolarizations by a single mechanical stimulus. The resting potential, −20 mV

neous hyperpolarization was 60.9% (25/41) in Na^+-free medium, 52.0% (13/25) in 5/6 Li^+ (21 mM Na^+) medium, and 18.8% (19/101) in standard medium. Both the amplitude and the duration of the mechanically evoked hyperpolarization were greater in Na^+-free medium than in standard medium. These findings may suggest that the increase in $[Ca^{2+}]_i$ was actively buffered, in part, through the Na^+-Ca^{2+} exchange across plasma membranes.

Effects of Pharmacological Agents

Quinidine and tetraethylammonium (TEA) are known to inhibit Ca^{2+}-activated K^+ conductance. As shown in Fig. 5*A*, spontaneous hyperpolarizations were reversibly suppressed by quinidine. When 0.5 mM quinidine was present in the Na^+-free medium, the spontaneous hyperpolarization was observed only in 1 of 13 cells and its amplitude was only 4 mV, values much less than those observed in the quinidine-free control condition (*see* Table 2). Quinidine also inhibited the mechanically evoked hyperpolarization (Fig. 5*B*); in the presence of quinidine, the mechanically evoked hyperpolarization was observed only in 6 of 13 cells and its amplitude was greatly reduced (6.2 ± 2.9 mV, *see* Table 2).

TEA (50 mM) in the external medium did not inhibit spontaneous and mechanically evoked hyperpolarizations. We have also examined the effects of Ca^{2+} channel blocker, verapamil. However, the presence of 10^{-5} M verapamil in the external medium did not affect spontaneous and mechanically evoked hyperpolarizations. In addition, colchicine (10^{-6} M) and cytochalasin B (2×10^{-5} M) had no effect on spontaneous or mechanically evoked hyperpolarizations, suggesting that these membrane hyperpolarizations were not related to either colchicine or cytochalasin B sensitive cytoskeletal systems.

Whole-Cell Clamp Recording

In order to rule out the possibility that an increase in $[Ca^{2+}]_i$ is caused by a leakage influx of extracellular Ca^{2+} around the impaled electrode [15], we used the whole-cell variation of patch-electrode voltage-clamp technique with tight seal condition to minimize the leakage current. Spontaneous increase of outward or inward current was observed at holding potential of −4 and −104 mV, respectively (Fig. 6). These spontaneous hyperpolarizations could be observed only in a few minutes after the establishment of whole-cell recording, probably because diffusion

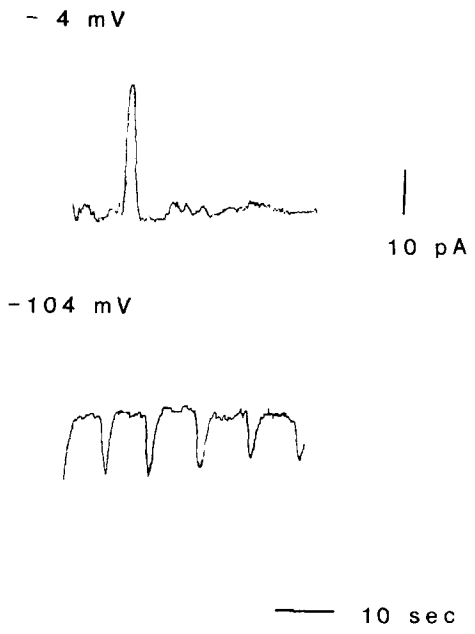

Fig. 6. Spontaneous hyperpolarizations recorded by whole-cell clamp method. The tracings show the current monitoring at the holding potential of −4 mV (top), and −104 mV (bottom). The upward deflection indicates outward current

of patch-pipette solution containing EGTA attenuated the increase in $[Ca^{2+}]_i$. The reversal potential was −71 mV, a value similar to that obtained by the microelectrode technique. The frequency of the spontaneous hyperpolarization at holding potential of −4 mV was smaller than that at −104 mV, probably due to the decreased driving force for Ca^{2+} at less negative holding potential. These results indicate that the spontaneous hyperpolarization was not caused by a leakage of extracellular Ca^{2+}.

Flow-Induced Hyperpolarization

As shown in Fig. 7, application of fluid flow to impaled cell caused hyperpolarizing responses. Successive applications of fluid flow also elicited similar hyperpolarizing responses. Increasing the duration of flow application resulted in greater amplitude and duration of flow-evoked hyperpolarization (Fig. 7*B*). The frequency of cells showing flow-evoked hyperpolarizations was 29.4% (10/34) in the standard medium, and the amplitude and duration of the hyperpolarization evoked by application of flow for 10 sec was 7.7 ± 5.7 mV and 19.0 ± 16.2 sec, respectively. Input resistance was decreased during the hyperpolarization, and the reversal potential was −80.0 ± 4.2 mV (n = 3) in the standard solution. These responses were completely inhibited by 10^{-4} M quinidine (Table 3). In Ca^{2+}-free solution containing 2 mM EGTA, the flow-elicited hyperpolarization could not be observed. As in the case of spontaneous and mechanically induced hyperpolarizations, the incidence, amplitude and duration of this flow-elicited hyperpolarization increased in Na^+-free solution (Table 3). Cytochalasin B (2×10^{-5} M) and colchicine (10^{-6} M) had no effect on flow-elicited hyperpolarization. The flow-elicited hyperpolarization was also observed under whole-cell clamp condition, indicating that it was not caused by leakage of Ca^{2+} at the impalement site. These data indicate that extracellular fluid flow causes hyperpolarizing membrane potential change probably through increased Ca^{2+}-activated K^+ conductance.

Table 3. Flow-elicited hyperpolarizations

	Media	
	Standard	Na^+-free
% of cells showing FEH[a]	29.4% (10/34)	57.9%[c] (22/38)
Amplitude of FEH (mV)	7.5 ± 5.7[b] (n = 10)	14.3 ± 8.9[c] (n = 22)
Duration of FEH (sec)	19.0 ± 16.2 (n = 7)	30.3 ± 12.4 (n = 20)

[a] FEH: flow-elicited hyperpolarization.
[b] Mean ± SD.
[c] Significant $P < 0.05$ *vs.* standard.

Although all the experiments reported above were done using JTC-12 cells in logarithmic growth state, all spontaneous, mechanically evoked and flow-elicited hyperpolarizations were observed also in the cells in confluency.

Discussion

The present study has extended our previous observations [4] and revealed that JTC-12 cells showed membrane hyperpolarizations which occur spontaneously or are evoked either by touching adjacent cells or by applying fluid flow. These hyperpolarizations were due to an increase in Ca^{2+}-activated K^+ conductance demonstrated by the intercellular injection of Ca^{2+} and EGTA. This conclusion was further supported by the finding that these hyperpolarizations were abolished by quinidine, an agent which inhibits Ca^{2+}-activated K^+ conductance. A recent study by Kolb and associated [19] have demonstrated by using patch-clamp technique the presence of Ca^{2+}-activated K^+ channels of large unit conductance of about 200 pS in the apical membrane of JTC-12 cells. Such K^+ channels may ac-

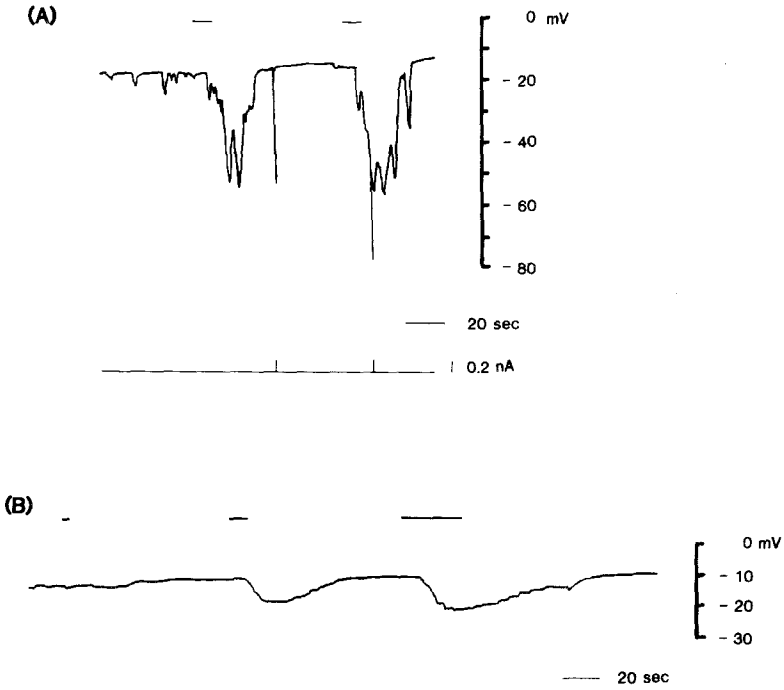

Fig. 7. Flow-elicited hyperpolarization. (*A*) Extracellular fluid flow was applied to a recording cell for 10 sec as indicated by horizontal bars above the tracing. With a time lag of about 6 sec, hyperpolarizing response was reproducibly observed. At two points, 0.2 nA hyperpolarizing pulses were applied, showing decreased input resistance during hyperpolarization. The study was performed in the Na^+-free solution. The resting membrane potential was −18 mV. (*B*) The flow was applied for various durations. From left to right, flow was applied for 3, 10 and 30 sec, respectively, as indicated by the horizontal bars above the trace. Increasing the duration of fluid flow application caused the amplitude and duration of the hyperpolarization to become larger

count for the K^+ conductance observed in our previous [4] and present studies.

Spontaneous hyperpolarizations in JTC-12 cells were abolished by extracellular Ca^{2+} deprivation as in fibroblasts and hamster eggs, in which continuous Ca^{2+} influx through Ca^{2+} channels is required to maintain hyperpolarization [13, 25, 26]. The presence of spontaneous hyperpolarization in 2.5 mM Sr^{2+} medium and the results of intracellular Sr^{2+} injection suggest that Sr^{2+} permeates through plasma membranes and can substitute Ca^{2+} to increase Ca^{2+}-activated K^+ conductance. Mechanical stimulation evoked hyperpolarization by an increase in $[Ca^{2+}]_i$, which occurs even in the absence of extracellular Ca^{2+}. This contrasts the properties of spontaneous and flow-elicited hyperpolarizations which required the presence of extracellular Ca^{2+}.

It has been suggested that hyperpolarizations in fibroblasts and macrophages may be caused by the leakage of Ca^{2+} introduced at the impalement of the electrode and that hyperpolarizations do not occur before the impalement of the electrode [15]. This possibility is unlikely in JTC-12 cells because: (i) spontaneous hyperpolarizations were observed by a whole-cell clamp recording method in which tight seal condition makes leakage current negligible; (ii) mechanically elicited hyperpolarizations were observed in Ca^{2+}-free medium containing 2 mM EGTA; (iii) after mechanically elicited hyperpolarization has ceased, input resistance recovered to the level prior to the stimulation; and (iv) in the presence of quinidine to block Ca^{2+}-activated K^+ conductance, the change in input resistance in response to mechanical stimulation was absent.

It has been shown that cell damage increases $[Ca^{2+}]_i$, which then may seal gap junctions with neighboring cells [27]. Thus, it is possible that the mechanically evoked hyperpolarization was caused by cell damage. However, this possibility is unlikely since mechanical stimulation consistently caused hyperpolarization not in the touched cell itself but in the adjacent cells.

In the present study, 5×10^{-2} M TEA showed almost no effects on hyperpolarization while quinidine suppressed hyperpolarization. Although TEA is widely used as an antagonist of K^+ channels, the sensitivity of Ca^{2+}-activated K^+ channels to TEA varies in cell types. Thus, it appears that Ca^{2+}-activated K^+ channels in JTC-12 cells belong to the group of Ca^{2+}-activated K^+ channels insensitive to TEA, as those seen in *Tritonia* [30], *Arcidoris* and *Anisodoris* [1, 5] and *Helix* [12, 20].

The order of effectiveness of divalent cations to activate Ca^{2+}-activated K^+ conductance has been reported to be $Ca^{2+} > Cd^{2+} > Hg^{2+} > Sr^{2+} > Mn^{2+} > Fe^{2+}$, while Ba^{2+} is usually ineffective [9]. JTC-12 cells showed hyperpolarization in response to intracellular injection of Ca^{2+} or Sr^{2+}, but not to Mn^{2+} or Ba^{2+}, observations consistent with these results. The absence of spontaneous and mechanically evoked hyperpolarization in 2.5 mM Ba^{2+} might be due to the blockade of Ca^{2+}-activated K^+ channels by Ba^{2+}, as reported in rat pituitary cells [10].

Flow-elicited hyperpolarization resembles spontaneous hyperpolarization in that the incidence and the amplitude were increased in Na^+-free medium and that both required the presence of extracellular Ca^{2+}. The precise mechanism by which extracellular fluid flow elicits hyperpolarization in JTC-12 cells is unknown. However, the possibility of increased leakage current around the impalement site is unlikely, because flow-elicited hyperpolarization could be observed under whole-cell clamp recording, in which leakage current would be negligible. Other possibilities, such as change in local concentration of electrolytes or substrates, or existence of specific mechanism sensing extracellular fluid flow, await further investigation. Physiological significance of these hyperpolarizations are unknown. However, flow-elicited hyperpolarization might be of interest in this regard. If present in normal tubular cells, it could afford to them a sensing machinery responding to extracellular fluid flow, enabling them to adapt to changing flow rate of renal tubule.

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