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MEMBRANE POTENTIAL FLUCTUATION IN PARAMECIUM

Toshikazu MAJIMA
Department of Biophysical Engineering, Faculty of Engineering Science, Osaka University,
Toyonaka, Osaka, 560, Japan

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The fluctuation of the membrane potential in *Paramecium caudatum* cells was measured. The fluctuation was very large and composed of slow and periodic components and fast and random ones. The power of fast fluctuation depended on the environmental salt condition, particularly on the ratio of the potassium ion concentration to the square root of the calcium ion concentration, $[K^+]_0/[Ca^{2+}]_0^{1/2}$, in the medium. The power greatly increased with the decrease of this ratio. The power density spectrum of fluctuation was of the $1/(1+(f/f_c)^2)$ type when the total power was large. There, the fluctuation arose mainly from the local current of potassium ions. Some cooperation among potassium channels to increase the fluctuation was suggested. The spectrum became of the $1/f$ type when the total power was small.

1. Introduction

Many investigators have reported on the subthreshold fluctuation of the membrane potential or current in nerves and other excitable cells [1–4]. The potential fluctuation contains useful information about the underlying molecular mechanism and the main problem studied and discussed has been the kinetics of ion channels in the membrane.

Recently, Moolenaar, de Goede and Verveen [5] measured the fluctuation of the membrane potential and current in *Paramecium* cells. They obtained power density spectra of a type of $1/f$ (f: frequency) and suggested participation of potassium ions in the fluctuating current.

The purpose of this paper is to describe our results of analyses of the spontaneous fluctuation of the spontaneous fluctuation of the membrane potential in *Paramecium* cells. The time correlation function and the power density spectrum at various environmental conditions are shown. The effects of calcium and potassium ions are examined.

2. Materials and methods

Paramecium caudatum supplied by Dr. Shimizu of Osaka Univ. was cultured in a hay infusion with *Aerobactor aerogenes* at 25°C. The cells were concentrated by filtration and washed with an equilibration solution containing 0.5 mM KCl, 1.0 mM $CaCl_2$ and 1.0 mM Tris-HCl buffer of pH 7.2. They were incubated in the same solution for 30 min at 25°C before the experiment.

Glass capillary microelectrodes filled with 0.1 M KCl were inserted into a *Paramecium* cell fixed on a slide glass, according to the method of Naitoh [6]. An agar bridge containing 3 M KCl and 1% agar was used as a reference electrode in the external medium. The potential difference was recorded on an FM magnetic tape recorder. The medium was replaced by solutions containing 1.0 mM Tris-HCl of pH 7.2 and various concentrations of KCl and $CaCl_2$. The temperature of the medium was maintained at 25°C.

To detect a slow change of the resting potential, the autocorrelation function $C(\tau)$ was calculated by the equation

$$C(\tau) = \overline{(V(t) - \bar{V})(V(t+\tau) - \bar{V})}\,, \qquad (1)$$

where $V(t)$ is the membrane potential (the voltage) at time t and τ is the time difference. The bar means the average with respect to time t. Calculations were carried out using the data of 600 points at 500 ms intervals for 5 min.

The power density spectrum (PDS) of the fluctuating potential was obtained from the record by a computer in a frequency range of 0.4 Hz to 40 Hz at intervals of 0.2 Hz. Parts of the record of the length of about 20 s were used after sharp spikelike depolarizing and hyperpolarizing fluctuation was removed. Five to ten spectra from different parts of the record from the same cell in the same condition were averaged.

The total fluctuation power was defined by

$$I = \sum_i I(f_i)\Delta f \,, \tag{2}$$

where $I(f_i)$ is the power density in the interval Δf which was taken to be 0.2 Hz. The summation was performed in the range of 0.4 Hz to a maximum frequency where the density almost vanished.

3. Results

3.1. Slow and fast components of potential fluctuation

The intracellular membrane potential of *Paramecium caudatum* fluctuates as shown in fig. 1. The average potential was about —30 mV under the physiological condition and the amplitude of the fluctuation was in the range of 0.5 mV to 5 mV. Sharp spikelike depolarization and hyperpolarization were included. In general, the fluctuation consisted of two components; a slow and periodic one, and a fast and random one.

As shown in fig. 2, the autocorrelation function $C(\tau)$ decreased gradually with τ, having negative values at 30 — 50 s and positive values at 60 — 100 s. It did not tend to zero but showed oscillations. The period was between 60 — 100 s and the amplitude was within 2.5 mV.

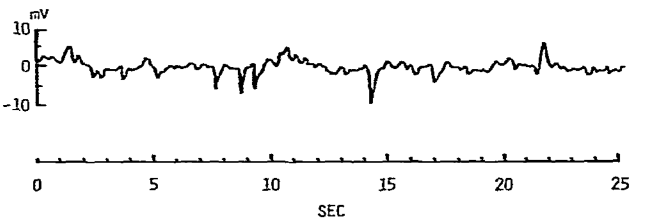

Fig. 1. Record of the fluctuating potential in a *Paramecium* cell in the presence of 0.5 mM KCl and 1.0 mM $CaCl_2$ at 25°C.

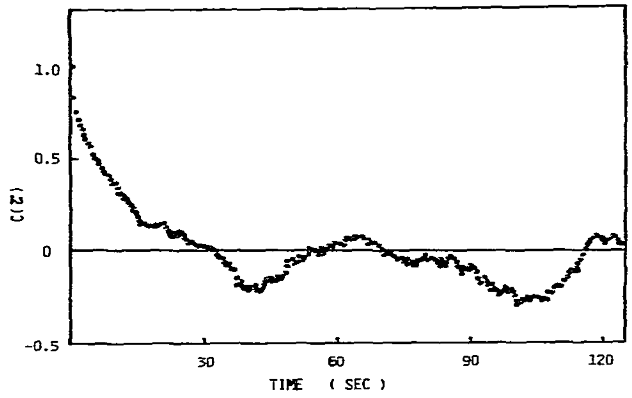

Fig. 2. Autocorrelation function $C(\tau)$ showing a slow periodi change of the resting potential.

The fast component was analysed after omitting the slow oscillation by a high pass filter with a cut-off frequency of 0.02 Hz. The histogram obtained was nearly gaussian. The total intensity and the PDS of the fluctuation greatly depended on the environment conditions. Calcium and potassium ions are known to be important for *Paramecium* cells to produce the membrane potential [7]. Therefore, the effects of these cations on the fast component of the fluctuatio have been investigated by varying the concentrations of KCl and $CaCl_2$ in the medium.

3.2. Effects of K^+ and Ca^{2+} on the total fluctuation power

When the KCl concentration was increased at a constant concentration of $CaCl_2$ (the K series), the total power of the potential fluctuation decreased. When the $CaCl_2$ concentration was increased at a constant concentration of KCl (the Ca series), the power increased. As shown in table 1, at 1.0 mM $CaCl_2$, the power at 0.5 mM KCl was about eighty times larger than that at 8.0 mM KCl. At 1.0 mM KCl, the power at 4.0 mM $CaCl_2$ was about one hundred times larger than that at 0.016 mM $CaCl_2$. These effects of KCl and $CaCl_2$ were reversible. The absolute value of the fluctuation power varied from cell to cell as far as examined. However, the relative magnitude of the power at different concentrations of KCl and $CaCl_2$ was similar in different cells.

In the above two series of experiments, the total

Table 1
Fluctuation power and resting potential at various values of the ratio $[K^+]_o/[Ca^{2+}]_o^{1/2}$ in the K series and the Ca series

$[K^+]_o/[Ca^{2+}]^{1/2}$	0.5	1.0	2.0	4.0	8.0
KCl mM ($CaCl_2$ 1 mM)	0.5	1.0	2.0	4.0	8.0
Normalized fluctuation power	1*	0.27	0.17	0.017	0.013
Resting potential (mV)	−36	−33	−28	−23	−14
$CaCl_2$ mM (KCl 1 mM)	4.0	1.0	0.25	0.063	0.016
Normalized fluctuation power	1*	0.38	0.30	0.042	0.011
Resting potential (mV)	−18	−32	−45	−51	−56
Ratio of the power in the two series	1	0.7	0.6	0.4	1.2

fluctuation power was found to have the same order of magnitude at the same value of the ratio of the K^+ concentration to the square root of the Ca^{2+} concentration, $[K^+]/[Ca^{2+}]^{1/2}$. To confirm this relation, the potential fluctuation was measured changing the concentrations of KCl and $CaCl_2$ keeping the ratio $[K^+]/[Ca^{2+}]^{1/2}$ equal to 0.5. As shown in table 2, the total fluctuation power was nearly constant as far as $[K^+]/[Ca^{2+}]^{1/2}$ was kept constant.

3.3. Power density spectra of the potential fluctuation

The PDS obtained from the same cell in the K series are shown in fig. 3 and the PDS in the Ca series are shown in fig. 4. The spectrum from the same cell at the same condition was reproducible and the profile of the spectrum from different cells in the same condi-

Table 2
Fluctuation power and resting potential at a constant value of $[K^+]_o/[Ca^{2+}]_o^{1/2}$

$[K^+]_o/[Ca^{2+}]_o^{1/2}$	0.5	0.5	0.5	0.5
KCl (mM)	0.25	0.5	1.0	2.0
$CaCl_2$ (mM)	0.25	1.0	4.0	16
Normalized fluctuation power	0.75	1*	1.1	0.9
Resting potential (mV)	−49	−35	−20	−8

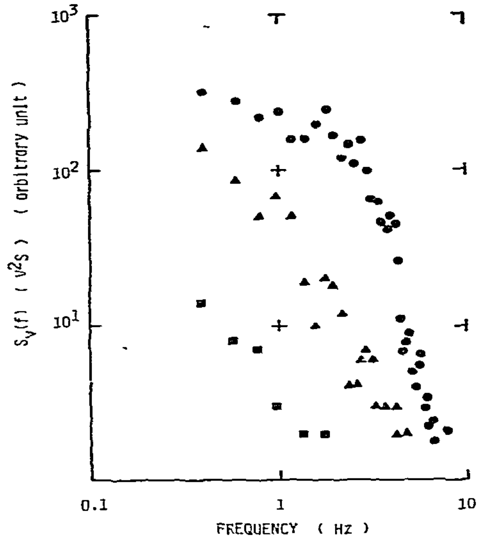

Fig. 3. Power density spectra of the fluctuating potential in the presence of 1.0 mM $CaCl_2$ and 0.5 mM KCl (●), 2.0 mM KCl (▲) or 8.0 mM KCl (■).

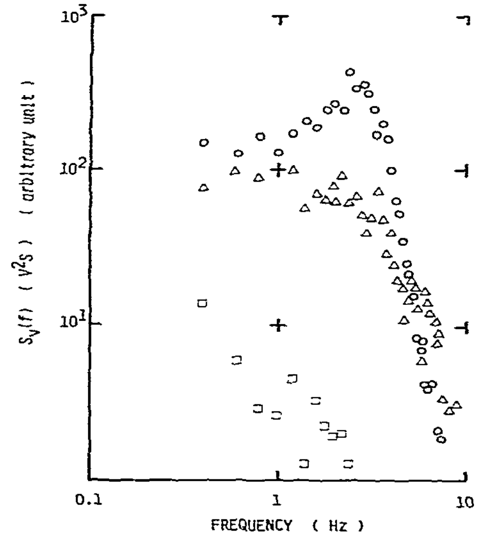

Fig. 4. Power density spectra of the fluctuating potential in the presence of 1.0 mM KCl and 4.0 mM $CaCl_2$ (○), 0.25 mM $CaCl_2$ (△) or 0.016 mM $CaCl_2$ (□).

tion was quite similar.

When the total fluctuation power was large, the PDS had a profile nearly of the $1/(1+(f/f_c)^2)$ type. Below 1 Hz, it had a plateau or a small slope and above a few Hz, the density decreased with increasing frequency in proportion to $1/f^{\alpha}$ where α was about 2 or a little larger. The corner frequency f_c was apparently in the range of 1 to a few Hz. Sometimes a peak appeared near f_c in PDS.

With the decrease of the total fluctuation power, the corner frequency shifted to the low frequency side, becoming undefinable and the slope of PDS at high frequencies decreased. Finally, the PDS became of the $1/f$ type over the whole range of frequency. These results suggested that the PDS is composed of two components, the $1/f$ type and the $1/(1+(f/f_c)^2)$ type. The latter component decreased with the decrease of the total fluctuation power.

Fig. 5 shows the PDS when the concentrations of KCl and $CaCl_2$ were varied keeping the ratio $[K^+]/[Ca^{2+}]^{1/2}$ at 0.5. The profile of PDS was mostly maintained, although the slope became a little steeper and f_c slightly shifted to the high frequency side with increasing concentrations of KCl and $CaCl_2$.

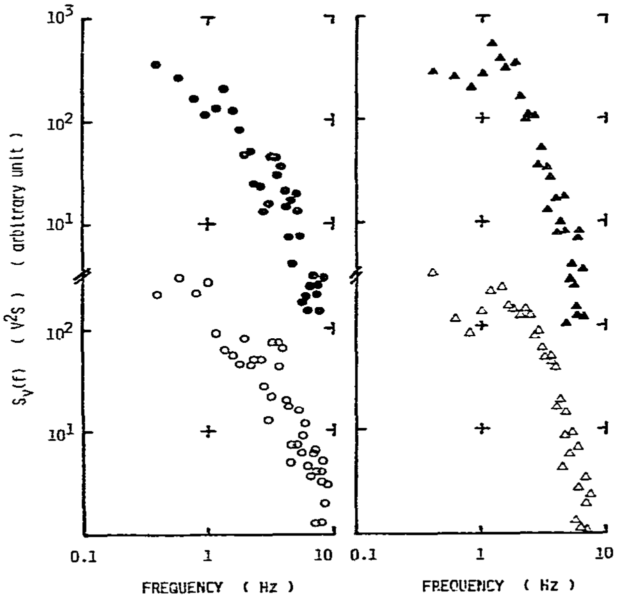

Fig. 5. Power density spectra of the fluctuating potential at a constant ratio $[K^+]_o/[Ca^{2+}]_o^{1/2} = 0.5$. 0.25 mM KCl and 0.25 mM $CaCl_2$ (●), 0.5 mM KCl and 1.0 mM $CaCl_2$ (○), 1.0 mM KCl and 4.0 mM $CaCl_2$ (▲) or 2.0 mM KCl and 16 mM $CaCl_2$ (△).

3.4. Effects of K^+ and Ca^{2+} on the resting potential

As already reported in several papers, the resting potential (the average potential) depolarized with increasing concentration of KCl at a constant concentration of $CaCl_2$. It also depolarized with increasing concentration of $CaCl_2$ at a constant concentration of KCl (table 1). Fig. 6 and table 2 show the change of the resting potential when the concentrations of KCl and $CaCl_2$ were varied keeping the ratio $[K^+]/[Ca^{2+}]^{1/}$ equal to 0.5. The resting potential depolarized in proportion to the logarithm of the concentration of KCl or $CaCl_2$, by about 45 mV per ten times increase of the KCl concentration and by 22.5 mV per ten times increase of the $CaCl_2$ concentration. Thus, if $[K^+]/[Ca^{2+}]^{1/2}$ was kept constant, the fluctuation remaine constant, whereas the resting potential showed a large change with the change of $[K^+]$ and $[Ca^{2+}]$.

3.5. Effects of tetraethylammonium ion

Friedman and Eckert showed that tetraethylammo-

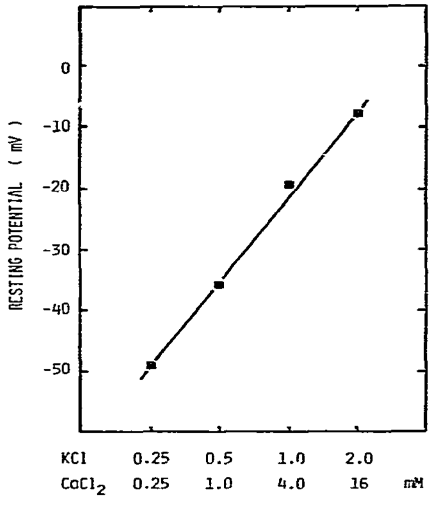

Fig. 6. Relation between the resting potential and the concentration of KCl or $CaCl_2$ at a constant ratio $[K^+]_o/[Ca^{2+}]_o^{1/2} = 0.5$.

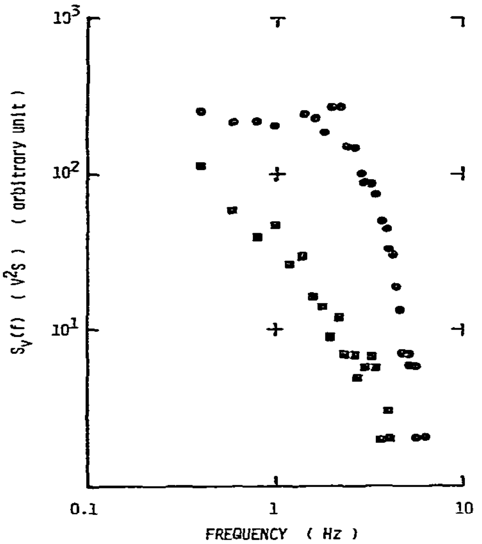

Fig. 7. Effect of TEA^+ on the power density spectra in 0.5 mM KCl and 1.0 mM $CaCl_2$. In the presence of 1.0 mM TEACl (■) and in the absence of TEACl (●).

nium ion (TEA^+) blocked the potassium conductance in *Paramecium* [8]. The external application of 1.0 mM TEA-Cl to a medium containing 0.5 mM KCl and 1.0 mM $CaCl_2$ reduced the total power of potential fluctuation to about 1/60. The PDS changed its profile from the $1/(1 + (f/f_c)^2)$ type to the $1/f$ type, as shown in fig. 7. The resting potential depolarized only a little, from —36 mV to —34 mV.

3.6. Power density spectrum of current fluctuation

The PDS described above were obtained from the potential fluctuation under the condition that the total current was kept (nearly) zero. The PDS of the current fluctuation can be derived from that of potential fluctuation by using data on the membrane impedance previously reported [9]. The membrane resistance of *Paramecium* was found to be in the range of 2.2 to 6.7 kΩ cm^2, depending on the ionic condition and the membrane capacitance was in the range of 3.9 to 9.3 μF/cm^2. The calculated value of the time constant of the membrane impedance was in the range of 25 to 30 ms, which agreed with the result of direct measurements of time constant by Saji [10]. This value corresponded to the frequency of 5 to 6 Hz, which was larger than the corner frequency f_c in the PDS of potential fluctuation. Therefore, after correction by the impedance spectrum, the PDS of the current fluctuation had the same characteristics as that of the potential fluctuation. An example is shown in fig. 8.

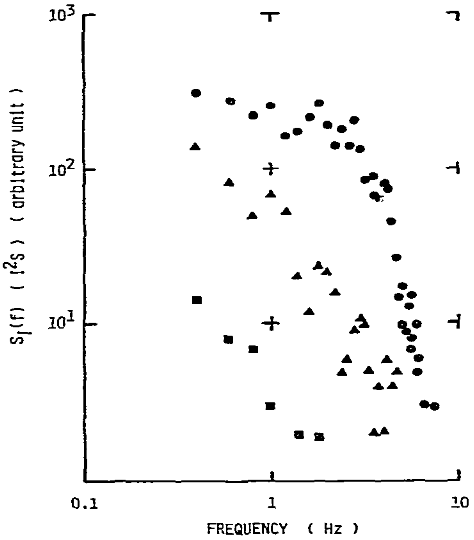

Fig. 8. Calculated power density spectra of the current fluctuation. 0.5 mM KCl (●), 2.0 mM KCl (▲) or 8.0 mM KCl (■); where the membrane resistance was assumed to be 37 MΩ, 40 MΩ, or 32 MΩ, respectively and the membrane capacitance to be 860 pF.

4. Discussion

4.1. Resting potential, local current and the ratio $[K^+]/[Ca^{2+}]^{1/2}$

Potassium ions and calcium ions are considered to be the main contributors to the membrane current in *Paramecium*. The potassium current I_K and the calcium current I_{Ca} across the membrane are assumed to be given by

$$I_K = g_K(E_m - E_K)\,, \qquad I_{Ca} = g_{Ca}(E_m - E_{Ca})\,, \qquad (3)$$

where g_K and g_{Ca} are the membrane conductance, and E_K and E_{Ca} are the equilibrium potential defined by

$$\begin{aligned} E_K &= (RT/F)\ln([K^+]_o/[K^+]_i)\,,\\ E_{Ca} &= (RT/2F)\ln([Ca^{2+}]_o/[Ca^{2+}]_i)\,, \end{aligned} \tag{4}$$

where R is the gas constant, T the absolute temperature and F the Faraday constant. The factor RT/F is 26 mV at 25°C. The suffixes i and o mean the inside and the outside of the cell. Under the condition that the total current is zero, that is,

$$I_K + I_{Ca} = 0\,, \tag{5}$$

the membrane potential E_m is given by

$$E_m = [g_K/(g_K + g_{Ca})]E_K + [g_{Ca}/(g_K + g_{Ca})]E_{Ca}\,, \tag{6}$$

which is rewritten by using (4) as

$$\begin{aligned} E_m = &-(RT/F)[g_{Ca}/(g_K + g_{Ca})]\ln([K^+]_o/[Ca^{2+}]_o^{1/2})\\ &+ (RT/F)\ln([K^+]_i/[K^+]_o)\\ &+ (RT/F)[g_{Ca}/(g_K + g_{Ca})]\ln([K^+]_i/[Ca^{2+}]_i^{1/2})\,. \end{aligned} \tag{7}$$

This equation can explain the result of fig. 6. The motive force for each ion is given as

$$\begin{aligned} E_m - E_K &= [g_{Ca}/(g_K + g_{Ca})](E_{Ca} - E_K)\,,\\ E_m - E_{Ca} &= -[g_K/(g_K + g_{Ca})](E_{Ca} - E_K)\,, \end{aligned} \tag{8}$$

where

$$\begin{aligned} E_{Ca} - E_K = &-(RT/F)\ln([K^+]_o/[Ca^{2+}]_o^{1/2})\\ &+ (RT/F)\ln([K^+]_i/[Ca^{2+}]_i^{1/2})\,. \end{aligned} \tag{9}$$

When the internal concentrations $[K^+]_i$ and $[Ca^{2+}]_i$ are constant, the difference $E_{Ca} - E_K$ changes in proportion to the logarithm of the ratio $[K^+]_o/[Ca^{2+}]_o^{1/2}$ Therefore, the motive force for each ion also depends on this ratio. The increase of fluctuation with the decrease of $[K^+]_o/[Ca^{2+}]_o^{1/2}$ is due to the increase of the motive force.

To estimate the value of the motive force, we assumed the following values for the internal concentrations of K^+ and Ca^{2+}; that is, 18 mM for $[K^+]_i$ according to the result of Oretel et al. [11] and 10^{-7} M for $[Ca^{2+}]_i$ because the critical concentration of Ca^{2+} for ciliary reversal was reported to be about 10^{-6} M by Naitoh and Kaneko [12]. The calculated values of $E_m - E_K$ and $E_m - E_{Ca}$ in the K series are listed in table 3. The value of the ratio of conductance g_K/g_{Ca} can be calculated using the observed values of E_m from (8). The total conductance $g_K + g_{Ca}$ at the same conditions has been reported previously [13]. Thus,

Table 3
Data in the K series

$[K^+]_o/[Ca^{2+}]_o^{1/2}$	0.5	1	2	4	8	16
E_m (mV)	−36	−33	−28	−23	−14	−1
E_K (mV)	−92	−74	−57	−39	−21	−3
$E_m - E_K$ (mV)	56	41	29	16	7	2
E_{Ca} (mV)	207	207	207	207	207	207
$E_m - E_{Ca}$ (mV)	−243	−240	−235	−230	−221	−208
$g_K + g_{Ca}$ (10^{-8} $\mho$)	2.70	2.38	2.13	2.22	3.13	4.17
g_K (10^{-8} $\mho$)	2.19	2.03	1.90	2.08	3.03	4.13
g_{Ca} (10^{-8} $\mho$)	0.51	0.35	0.23	0.14	0.10	0.04
$I_K = I_{Ca}$ (nA)	1.23	0.83	0.55	0.33	0.21	0.08
observed power	1*	0.27	0.17	0.017	0.013	(0.004)
calculated						
$(g_K/(g_K + g_{Ca}))^2$ $\times (E_m - E_K)^2$	1*	0.59	0.32	0.11	0.022	0.0019
$\overline{\delta g_K^2}/g_K^2 + \overline{\delta g_{Ca}^2}/g_{Ca}^2$	1*	0.46	0.53	0.15	0.59	1.89

the conductances g_K and g_{Ca} can be separately obtained, as shown in table 3. The value of the current I_K or I_{Ca} calculated is also included in table 3.

4.2. Total power and power density spectrum

Fig. 9 shows the relation between the fluctuation power and the motive force at a logarithmic scale. Above 15 mV of $E_m - E_K$, the power I increased approximately in proportion to $(E_m - E_K)^{3.15}$ and below 15 mV, the power increase with the increase of $E_m - E_K$ was much smaller. Moolenaar, de Goede and Verveen measured the current fluctuation changing the clamping voltage, at 4.0 mM KCl and 1.0 mM $CaCl_2$. The relation between the power at 1 Hz and the motive force $E_m - E_K$ obtained by them is very similar to ours.

Let us consider the fluctuation of local currents I_K and I_{Ca} given by (3); that is,

$$\begin{aligned} \delta I_K &= \delta g_K(E_m - E_K) + g_K(\delta E_m - \delta E_K)\,, \\ \delta I_{Ca} &= \delta g_{Ca}(E_m - E_{Ca}) + g_{Ca}(\delta E_m - \delta E_{Ca})\,, \end{aligned} \tag{10}$$

where δ means variation due to fluctuation. In the present experimental condition, we may put approximately

$$\delta I_K + \delta I_{Ca} = 0\,, \tag{11}$$

because the time constant of the membrane impedance

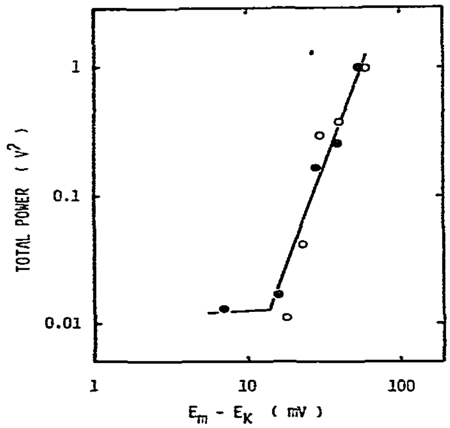

Fig. 9. Relation between the normalized power of the potential fluctuation and the motive force of potassium ions. From the K series (●) and the Ca series (○).

was very much smaller than that of the fluctuation. Then, if the fluctuations of conductances and equilibrium potentials were all independent, the power of the potential fluctuation is given by

$$\overline{\delta E_m^2} = \frac{1}{(g_K + g_{Ca})^2}\,[\overline{\delta g_K^2}(E_m - E_K)^2 + \overline{\delta g_{Ca}^2}(E_m - E_{Ca})^2 + g_K^2\,\overline{\delta E_K^2} + g_{Ca}^2\,\overline{\delta E_{Ca}^2}]\,. \tag{12}$$

The fluctuation of the equilibrium potential is due to the fluctuation of the local ion concentration. Usually, the concentration fluctuation has very short relaxation times, so that its contribution to the observed δE_m may be neglected. Then

$$\overline{\delta E_m^2} = \left(\frac{\overline{\delta g_K^2}}{g_K^2} + \frac{\overline{\delta g_{Ca}^2}}{g_{Ca}^2}\right)\frac{g_K^2}{(g_K + g_{Ca})^2}\,(E_m - E_K)^2\,. \tag{13}$$

The observed fluctuation power increased with $(E_m - E_K)$ but not exactly in proportion to its second power. If we apply (13) and compare with experimental data, the value of the factor $(\overline{\delta g_K^2}/g_K^2 + \overline{\delta g_{Ca}^2}/g_{Ca}^2)$ becomes dependent on the motive force or the ion concentration, as shown in table 3.

The conductance fluctuation is probably due to the fluctuation of the number of opened channels. By the addition of TEA^+, the profile of PDS was changed from the $1/(1 + (f/f_c)^2)$ type to the $1/f$ type and the total power was very much decreased. It is likely, therefore, that the large increase of the potential fluctuation at large values of $E_m - E_K$ is mainly due to the increase of the fluctuation of the potassium current and to this fluctuation, the open–close kinetics of channels is applicable. On the other hand, the calcium current seems not to have much contribution to the fluctuation at large values of $E_m - E_K$. The fluctuation at small values of $E_m - E_K$, however, might come from the calcium current and other origins.

If the open–close kinetics is applied, the ratio $\overline{\delta g_K^2}/g_K^2$ is approximately inversely proportional to the number of K^+ channels. In a *Paramecium* cell, the amplitude of the potential fluctuation was found to be about 3.2 mV under the condition that the motive force $E_m - E_K$ was about 56 mV. The number of channels estimated from these values was of the order of 1000. The number of cilia on a *Paramecium* cell was calculated to be 5000 [14]. Therefore, the number of channels per cilium is in the order of unity.

The magnitude of the fluctuation in *Paramecium* is very large as compared with other excitable membranes. Fishman et al. [4] employed the sucrose gap method and analysed the fluctuation in a small area of a squid axon membrane. The area was 10^{-4} to 10^{-5} cm^2, which was the same order of magnitude as the surface area of a *Paramecium* cell including the membrane of cilia. The amplitude of the potential fluctuation in the axon was several tens μV at a resting potential of −60 mV. The estimated value of the channel density in the axon was about 500/μm^2 for the Na$^+$ channel and about 50/μm^2 for the delayed K$^+$ channel [15–18]. The above value of the number of channels in *Paramecium* corresponds to a density of 0.02 – 0.03/μm^2. The static conductance of the membrane was not very different between the *Paramecium* cell and the squid axon. Therefore, the unit conductance of the channel in *Paramecium* must be very large. In other words, a certain number of single channels in *Paramecium* may work cooperatively as a unit for the fluctuation. Such a cooperativity among channels may be effective to increase the fluctuation in proportion to the high power of the motive force.

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