VOLTAGE SENSITIVE Ca-CHANNELS AND THE TRANSIENT INWARD CURRENT IN PARAMECium TETRAURELIA

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(Received 11 April 1978)

SUMMARY

Transient inward currents across the membrane of P. tetraurelia are recorded upon step depolarizations with a voltage clamp in solutions where Ca$^{2+}$ is the only added inorganic cation. It is shown that the current is normally carried by Ca$^{2+}$ through the Ca-channels which activate and inactivate in time.

The transient inward current is dependent on both the size of the depolarizing step and the holding level before the step. Maximum inward current ($I_{\text{max}}$) occurs when the membrane is first held at the resting level ($-30$ mV), then stepped to $0$ mV in a solution containing $0.91$ mM-Ca$^{2+}$. The $I_{\text{max}}$ is smaller when the membrane is first held at depolarized level. This is due to the depolarization-sensitive inactivation of the Ca-channels. The $I_{\text{max}}$ is also smaller when the membrane is first held at a hyperpolarized level. This may be explained by the activation of hyperpolarization-sensitive K-channels known to exist in the Paramecium membrane.

$I_{\text{max}}$ increases with concentration of Ca$^{2+}$ up to $0.9$ mM. Further increase in the Ca$^{2+}$ concentration does not affect $I_{\text{max}}$. This apparent saturation at $0.9$ mM-Ca$^{2+}$ may reflect a rate-limiting step of Ca$^{2+}$ permeation. The increase in Ca$^{2+}$ concentration shifts the $V-I_{\text{peak}}$ curve in the direction of less sensitivity. This result is best explained as the effect of bound Ca$^{2+}$ on the surface potential of the Paramecium membrane.

These results provide the first detailed description of the properties of the action current through the Ca-channel in Paramecium. They also define the conditions under which future voltage-clamp studies of wild-type and mutant membranes of P. tetraurelia should be performed, i.e. to maximize the resolution of the Ca-channel activity, the membrane should be held at or near the resting potential and there should be over $0.9$ mM-Ca$^{2+}$ in the test solutions. The behaviour of the Paramecium Ca-channel and small $I_{\text{max}}$ in the presence of K$^+$ are discussed.

INTRODUCTION

Paramecium has been used for a multidisciplinary study of membrane excitation. One approach makes use of membranes made defective by single-gene mutations (Kung, 1971; Kung & Eckert, 1972; Satow & Kung, 1976a, b, c; Schein, Bennett &
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Kate, 1976; Takahashi & Naitoh, 1978). In spite of the large volume of literature on the physiology of *Paramecium* (for review see Eckert, Naitoh & Machemer, 1976 on *P. caudatum* and Satow, 1978a on *P. tetraurelia*) and the larger number of mutants available (Kung et al. 1975; Takahashi & Naitoh, 1978), the analysis of the ion channels in the *Paramecium* membrane remains difficult. The difficulty in part results from the fact that the membrane potential of *Paramecium* was not controlled in most previous experiments which recorded spontaneous discharges or events evoked by injected currents or mechanical stimulation. In this study of normal and mutant membranes, the membrane currents were recorded under a voltage clamp. A slow voltage clamp was used by Machemer & Eckert (1975) in their studies of ciliary beat in *P. caudatum*. The preliminary results of the study of membrane currents in *P. caudatum* using a fast voltage clamp were reported by Naitoh (1974) and Naitoh & Eckert (1974). Oertel, Schein & Kung (1977) studied the membrane currents of *P. tetraurelia* and used a mutant devoid of functional Ca-channels as well as Mg$^2+$ substitution of Ca$^2+$ to separate the transient inward current from the outward current. However, the characteristics of the Ca current ($I_{Ca}$) have not been fully explored. The transient inward currents of the *Paramecium* membrane induced by step depolarization using a voltage clamp are described in this paper. Further analysis of membrane currents in wild type and mutants (Satow & Kung, in preparation) is based on the findings reported here.

**METHODS**

**Stock and culture**

Stock 51s, a wild-type *P. tetraurelia* (previously species 4 of *P. aurelia*) was used. Cells were cultured in the Cerophyl medium bacterized with *Enterobacter aerogenes* 20 h before use (Sonneborn, 1970). Only robust cells in log-phase growth were used at room temperature (22 ± 1 °C).

**Solutions**

The ionic compositions of the various test solutions are listed in Table 1. The free Ca concentration was calculated from the dissociation constant of calcium citrate (Martell & Smith, 1977).

**Recordings**

Two microelectrodes filled with 3 to 3·5 M-KOAc mixed with 10 to 20% KCl were used, and the electrode resistances measured in the Ca-K(A) solution, were between 8 and 18 MΩ. Details on the clamp circuit were given in Oertel, Schein & Kung (1978). A block diagram of the voltage clamp circuit, similar to that of Oertel et al. was presented in Fig. 1. Membrane currents were measured by a current-voltage converter inserted between the indifferent electrode and ground. The voltage stabilized at the command level about 1 msec after a step change in the command. The membrane was usually held at the resting potential ($V_h$) before stepping to a new level ($V$). The voltage was measured from the reference level established by placing the voltage-measuring electrode in the Ca-K (A) solution. The membrane currents ($I$) corresponding to step changes in voltage were recorded.
To examine if the interior of the cell is isopotential, the membrane potential \((V_{\text{mon}})\) at a point 50 to 100 \(\mu\)m from the recording electrode was monitored by a third electrode. Potential changes recorded through the two recording electrodes were indistinguishable. This finding verifies the conclusion of Eckert & Naitoh (1970) that the interior of the *Paramecium* is isopotential. The third electrode was not used in the experiments below. The current at the holding potential was considered zero-current, and the peak inward currents \((I_{\text{peak}})\) were measured and plotted in Figs. 3–6. The leakage currents, estimated to be less than 1 nA (see Results and Fig. 2) were not added to the measurements of the inward currents in these plots.

The cell was first transferred from the culture to the test solution about 10 min before recording. Recordings were made within 10 min without perfusion of the test solution. The cell membrane began to deteriorate after 10 min due to the low-resistance electrodes. We analyse and present only the results from cells having reasonable membrane potential and membrane resistance in each test solution. Figs. 6, 7 and 8 each include data from 5 to 10 cells.
RESULTS

Transient inward membrane currents and their voltage sensitivities

Membrane potential is held at the resting level (-30 mV) before the sudden step depolarizations. An inward current can be observed with depolarization beyond -25 mV. The maximum inward current ($I_{\text{max}}$) is observed at about 0 mV.

In experiments where the Paramecia are bathed with a solution having Ca$^{2+}$ as the sole cation besides Tris (the Ca-solution), the inward transient can be observed clearly (Fig. 2, second column from left). In these experiments the membrane is held at -30 mV (the resting level in the Ca-solution) before the voltage steps are applied. The inward current rises to a peak ($I_{\text{peak}}$) in 3 msec and subsides afterward. The time course of such transients and the activation and inactivation of the Ca-channels have been reported by Oertel et al. (1977) and is not analysed in this paper.

To study the voltage dependency of this inward current, the peak current ($I_{\text{peak}}$) is plotted against the membrane potential after the step ($V$) (Fig. 3, left). The maximum $I_{\text{peak}}$, which we call the $I_{\text{max}}$, is 6.7 nA (6.8 ± 0.7 nA; mean ± s.d. $n = 7$) at a $V$ ($V_{\text{max}}$) of 1 mV (1.4 ± 2.3 mV) in this experiment. The $I_{\text{peak}}$ becomes smaller when $V$ is above 0 mV.
A steady-state outward current is observed after the inward transient, as in other excitable membranes (Fig. 2). The outward current is less than 2 nA up to a V of 0 mV. Above 0 mV, the outward current becomes much bigger due to the depolarization-activated K+ current. This finding agrees with that of Oertel et al. (1977) that major K+ activation does not occur at V of 0 mV, even though their experiments were performed with Paramecia bathed in a different solution. Thus, the I_{peak} up to and including I_{max} represent mostly the Ca^{2+} action current minus a small outward current mainly due to the leakage conductance. For this and other reasons (see Discussion), we use the I_{peak} up to I_{max} as an approximate indicator of the Ca-channel activity.

In an attempt to reduce the leakage current, TEA+, a compound which is known to affect membrane potential in Paramecium (Satow & Kung, 1976a) was added to the bath. The experiment with the Ca-TEA solution is shown in Fig. 2. The inward transients can again be seen. The I_{max} is 6.1 nA (at a V of −8 mV from a holding potential of −37.5 mV). V−I_{peak} relation in the Ca-TEA solution (Fig. 3, centre) is very similar to that in Ca-solution (I_{max} 6.2 ± 0.7 nA; V_{max}−4.0 ± 5.2 mV, n = 4). Thus, the external addition of TEA+ has no dramatic effects on the transient inward current. The effect of internal application of TEA+ is not examined, since the internal TEA+ suppresses both K-conductance and the Ca-spike (Satow & Kung, 1976b).

Most behavioural and physiological experiments with Paramecium are performed in solutions containing Ca^{2+} and K+ (Kamada & Kinosita, 1940; Naitoh & Eckert, 1968a, b; Satow & Kung, 1976a, b, c; Oertel et al. 1977). However, the inward transient has not previously been examined in detail under a voltage clamp in this kind of solution. Fig. 2 (right) shows such a transient. When the membrane was first held at −36 mV and then step depolarized to −7.8 mV the peak inward current was 3.7 nA. The inward currents from cells bathed in the Ca-K solution were considerably smaller than those in the Ca- or the Ca-TEA solution. Fig. 3 (right) shows the V−I_{peak} relation in Ca-K (A) solution (I_{max} 4.4 ± 1.2 nA; V_{max}−2.2 ± 5.6 mV; n = 14). However, the voltage sensitivity of the inward current in this solution was very similar to those in other solutions despite the smaller I_{peak} at all step voltages (see Discussion).
Fig. 4. $V-I_{\text{peak}}$ relations (A) and the inactivation curve (B) in the Ca-TEA solution. The order of this set of experiments is as follows: $-24 \text{ mV}$ (●), 2nd, $-14.5 \text{ mV}$ (x), 3rd, $-35 \text{ mV}$ (○), 4th, $-54 \text{ mV}$ (♯), 5th, $-44 \text{ mV}$ (∆) and 6th, $-19.5 \text{ mV}$ (□). The maximum inward current ($I_{\text{max}}$) at each holding potential ($V_h$) were plotted on the graph (right). The resting potential was $-25 \text{ mV}$. Note that $I_{\text{max}}$ is smaller when the membrane is held at depolarization and hyperpolarization.

**Inactivation by prior depolarization or hyperpolarization**

As reported in other systems with Ca action potentials (Geduldig & Gruener, 1970; Iwasaki, Satow & Kuroda, 1973; Standen, 1975; Mounier & Vassort, 1975), the inward transient is reduced by conditioning depolarization and hyperpolarization. For future research with *Paramecium*, it is important to know the holding potential at which the inward transient can be best observed.

The $V-I_{\text{peak}}$ relations from cells kept at various holding potentials before the step depolarizations is shown in Fig. 4A. This experiment was performed in the Ca-TEA solution when the cell has a resting potential of $-25 \text{ mV}$ (experiments with the Ca-solution gave very similar results). The inward transient can be triggered by step depolarizations of the membrane which was previously held at a large range of potential levels. However, the $V-I_{\text{peak}}$ relation shifted slightly when the membrane was first held at a very hyperpolarized ($-54 \text{ mV}$) or depolarized ($-14 \text{ mV}$) level. The reason is not known. The $I_{\text{peak}}$ was also considerably smaller under these conditions. The maximum $I_{\text{peak}}$ ($I_{\text{max}}$) is plotted against the holding potential ($V_h$) in Fig. 4B. The highest $I_{\text{max}}$ ($I_{\text{max}}^*$) is detected when the membrane is held between $-22$ and $-32 \text{ mV}$, very close to the resting level.

Similar experiments in the Ca-K(A) solution gave equivalent results (Fig. 5A). However, the inward currents were smaller, and were further reduced by strong hyperpolarizing ($-45 \text{ mV}$) and depolarizing ($-15 \text{ mV}$) holding potentials. The optimal holding potential giving the $I_{\text{max}}^*$ was again near the resting level ($-33 \text{ mV}$) (Fig. 5B).

**Effects of external Ca$^{2+}$ concentration**

The experiments shown in Figs. 1–5 used test solutions of 0.91 mM-Ca$^{2+}$. In view of the Ca$^{2+}$ effects on other membrane functions (Frankenhaeuser & Hodgkin, 1957; Hagiwara & Naka, 1964; Okamoto, Takahashi & Yoshii, 1976) and its complex
Fig. 5. $V-I_{\text{peak}}$ relations (A) and the inactivation curve (B) of cells in the Ca-K(A) solution. The experiment was done in the order of $-34.5$ mV (x), $-45$ mV (O), $-15$ mV (#), and $-21$ mV (•) of the holding potentials. The resting potential was $-33$ mV. The character of the inactivation curve is very similar to that in Ca-TEA solution in Fig. 4.

Fig. 6. $V-I_{\text{peak}}$ relations of cells in various external Ca$^{2+}$ concentrations (0.38, 0.91, 2.69 and 5.60 mM) at fixed K$^+$ concentration (4 mM). The holding potentials were $-37.5$, $-34.5$, $-18.5$, and $-9$ mV in [Ca$^{2+}$]$_0$ 0.38, 0.91, 2.69 and 5.60 mM, respectively. $I_{\text{max}}$ in 0.38 mM-Ca$^{2+}$ is smaller and the voltage sensitivities of the Ca channels shift along the voltage axis.

effects on the resting and excited membranes of Paramecium (Eckert et al. 1976; Satow, 1978 a), we have systematically investigated the relations between the inward transient and the external concentration of Ca$^{2+}$, [Ca$^{2+}$]$_0$.

The [Ca$^{2+}$]$_0$ in the Ca-K solutions was changed, as shown in Table 1, while the K$^+$ concentration was kept constant. To measure the inward transient, the membranes were held at the resting potential in each of the solutions. The $V-I_{\text{peak}}$ relations in various Ca-K solutions are shown in Fig. 6. The maximum inward currents were 2.8 nA, 4 nA, 3.8 nA, and 4 nA in [Ca$^{2+}$]$_0$ 0.38 mM, 0.91 mM, 2.69 mM, and 5.60 mM, respectively. The voltages where the maximum currents were induced were $-15$ mV, $-35$ mV, $+18.5$ mV and $+27$ mV in [Ca$^{2+}$]$_0$ 0.38 mM, 0.91 mM, 2.69 mM and 5.60 mM, respectively. The mean values and standard deviations of the $I_{\text{max}}$ are plotted against [Ca$^{2+}$]$_0$ (Fig. 7). $I_{\text{max}}$ and the voltage ($V_{\text{max}}$), at which the $I_{\text{max}}$ were observed in various [Ca$^{2+}$]$_0$, are listed in Table 2. The results show that the voltage sensitivity of the Ca channels and $I_{\text{max}}$ changes with [Ca$^{2+}$]$_0$, and appears to saturate above [Ca$^{2+}$]$_0$ of 0.91 mM. The effects of [Ca$^{2+}$]$_0$ on the inward current in the Ca- or
Fig. 7. The maximal inward current ($I_{\text{max}}$) of cells in various $[\text{Ca}^{2+}]_0$ (mean ± s.d.). The holding potentials were near the resting potentials in each test solution.

Table 2. The maximum inward current and the voltage sensitivity at the various external Ca concentrations

<table>
<thead>
<tr>
<th>$[\text{Ca}^{2+}]_0$ (mM)</th>
<th>$I_{\text{max}}$ (nA)</th>
<th>$V_{\text{axx}}$ (mV)</th>
<th>$V_{\text{rest}}$ (mV)</th>
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<tr>
<td>0.38</td>
<td>2.4 ± 0.3</td>
<td>-12.2 ± 1.2</td>
<td>-30.7 ± 3.0</td>
</tr>
<tr>
<td>0.91</td>
<td>4.4 ± 1.2</td>
<td>-2.2 ± 0.5</td>
<td>-34.0 ± 1.5</td>
</tr>
<tr>
<td>2.69</td>
<td>4.1 ± 1.1</td>
<td>+20.4 ± 3.5</td>
<td>-16.1 ± 2.5</td>
</tr>
<tr>
<td>5.60</td>
<td>4.7 ± 0.4</td>
<td>+29.1 ± 2.5</td>
<td>-11.0 ± 2.0</td>
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KCl 4 mM was contained in all solutions (see Table 1).

$I_{\text{max}}$, the maximum inward current when the membrane was held at the resting potential.

$V_{\text{axx}}$, the voltage level where $I_{\text{max}}$ was seen.

$V_{\text{rest}}$, the resting membrane potential in test solution.

$n$, number of cells.

Ca-TEA solution were similar to those in Ca-K solution. The resting potentials of cells in various Ca concentrations also changed (Table 2) as expected (Naitoh & Eckert, 1968a; Satow & Kung, 1976a).

The inactivation curves in various $[\text{Ca}^{2+}]_0$ are shown in Fig. 8. Data from several cells in each $[\text{Ca}^{2+}]_0$ are pooled and the ratio of $I_{\text{max}}/I_{\text{max}}^*$ in each cell is plotted against the holding potential. The curves clearly shift in the positive direction along the potential axis when $[\text{Ca}^{2+}]_0$ is increased.
The transient inward current studied in this paper is largely carried by calcium ions. The evidence for this is as follows. (1) Behavioural, electrophysiological and genetic evidence all indicate that the action potential in *Paramecium* is Ca-dependent (Naitoh & Eckert, 1968a, b; Eckert, 1972; Kung & Eckert, 1972; Satow & Kung, 1976a, b, c; Schein et al. 1976; Satow, 1978a). (2) A mutant defective in its Ca-channels does not exhibit the inward transient upon step depolarization under voltage clamp (Oertel et al. 1977). (3) The inward transient is seen when Ca²⁺ is the only external cation besides Tris (Figs. 1, 2). (4) Replacing most of the Ca²⁺ with Mg²⁺ eliminates the inward transient (Oertel et al. 1977).

In theory, the total inward current is the algebraic sum of the inward (Ca-), the outward (K-), and the leakage currents. In practice, most of the currents (e.g. Fig. 2), examined from steps below and up to \( V_{\text{max}} \), have only a small outward component in *Paramecium*, for the following reasons. (1) These currents are measured at depolarizations below that needed for major K-activation (Fig. 2, Oertel et al. 1977; Naitoh & Eckert, 1974; Satow & Kung, unpublished). (2) When it occurs, K-activation, whether voltage-induced or Ca-mediated, must be delayed (Naitoh & Eckert, 1974). Therefore, when the peak of inward current appears (within 3 msec), there should be little activated K⁺ current. (3) Mutants with reduced Ca-conductance (Satow & Kung, 1976c; Schein et al. 1976) show reduced transient inward current whereas a mutant with a large increase in K-conductance (Satow & Kung, 1976b) shows an almost unimpaired peak inward current (Satow & Kung, in preparation). Thus, if the inward transient is obtained by step depolarization to a level below the voltage-dependent K-activation level, it closely describes the behaviour of the Ca-channels of the *Paramecium* membrane. The small leakage current, estimated to be less than 1 nA is not considered in our analysis.
The Ca-channels of Paramecium are clearly sensitive to depolarization. Fig. 3 shows that as the step-depolarization increases, the inward current becomes larger. This observation substantiates many studies of the Ca-action potential (Naitoh & Eckert, 1968a, b; Satow & Kung, 1976b, c). Very large step-depolarizations (e.g. from \( V_h \) to over 0 mV in the Ca-solution) suppress the net inward current. This is probably due to the K-activation, as shown by the quasi-steady-state outward current seen after the inward transient (Fig. 2; Oertel et al. 1977) although the reduction of the Ca current as \( V \) approaches \( E^+ \) may also be a factor. Thus, in the \( V-I_{peak} \) plots, the curves up to and including the maximum describe the behaviour of the Ca-channels.

A major finding in our experiments is that the inward transient is highest when the membrane is initially held at the resting potential before step depolarization. This effect was seen using a variety of solutions which induced different resting potentials (Figs. 4, 5). The reduction of the inward current by depolarization before the steps is consistent with the observation that the Ca-channels of Paramecium inactivate. Naitoh, Eckert & Friedman (1972) showed a refractory period in the Ca-action potential in P. caudatum and Oertel et al. (1977) showed that a 5 msec step depolarization completely inactivates the Ca-channel for up to 20 msec in P. tetraurelia. Thus, the calcium channel of Paramecium shows time and voltage dependent inactivations similar to those of the sodium channel in the squid axon (Hodgkin & Huxley, 1952a, b).

The reduction of the inward current by prior hyperpolarization (Figs. 4, 5) is similar to those observed in other excitable membranes which exhibit Ca-currents (Geduldig & Gruener, 1970; Iwasaki et al. 1973; Standen, 1975; Mounier & Vassort, 1975). In these membranes, the effects of membrane hyperpolarization (i.e. suppression of the inward Ca-current or reducing the rate of rise of the action potential) are abolished after inhibition of K-conductance by TEA+. In P. tetraurelia, externally applied TEA+ affects the resting membrane potential but does not change the membrane conductance (Satow & Kung, 1976a). Internally applied TEA+ suppresses both the K- and Ca-conductances (Satow & Kung, 1976b) as in Helix neurones (Meech & Standen, 1975; Heyer & Lux, 1976). As shown in Fig. 4, externally applied TEA+ does not relieve the suppression of inward current by the membrane hyperpolarization. Because of its effect on the Ca-conductance, TEA+ cannot be applied internally to solve this problem. Although the external TEA+ experiment fails to show the involvement of hyperpolarization-activated K+ channels in the suppression of the inward transient, such channels do exist in the Paramecium membrane. The activity of these channels results in anomalous rectification (Naitoh & Eckert, 1968a; Satow, 1978a), and the up-side-down action potential (K-spike) (Satow & Kung, 1977). Voltage-clamp studies show that K-channels are activated by the membrane hyperpolarization (Oertel et al. 1978).

It is well known that external Ca\(^{2+}\) decreases the excitability of the membrane. This has been explained by the 'stabilizing effect' of Ca\(^{2+}\) through its binding to the channel or in its vicinity (Frankenhaeuser & Hodgkin, 1957), or, more recently, the effect on the electrostatic surface potential (McLaughlin, Szabo, and Eisenman, 1971; McLaughlin, 1976). The Paramecium membrane is no exception. Fig. 6 shows that increasing external Ca\(^{2+}\) 'stabilizes' the membrane, i.e. increase the \( V \) at which...
the activation of the inward transient begins and at which it is maximal. It is possible that these changes reflect not a change in the properties of the Ca-channel but a decrease in the effective surface concentration of Ca$^{2+}$ (Ohmori & Yoshii, 1977). This 'stabilizing' effect exists regardless of the holding potential level before the step depolarizations (Fig. 8). Ca$^{2+}$ also 'stabilizes' the channels responsible for the delayed rectification. The V level which activates the depolarization-sensitive K$^+$ conductance also increases with [Ca$^{2+}$]$_0$ (data not shown). The resting potential of *Paramecium* decreases (depolarizes) with increasing external Ca$^{2+}$ concentration (Table 2). The shift of $V-J_{\text{peak}}$ relations in various external Ca$^{2+}$ in Fig. 6 parallels the change of the level of the resting membrane. We believe that the change in resting potential with [Ca$^{2+}$]$_0$ is due largely to the indirect effects on the major permeability by the changes in surface-charge pattern and not to the Nernst effect on a fixed permeability of Ca$^{2+}$ (Naitoh & Eckert, 1974). The Gouy-Chapman equation for the diffuse double layer predicts that the surface charge is large in the low-ionic strength solutions used in experiments with *Paramecium* (McLaughlin, 1976).

It is interesting that the maximal inward current is constant at the range of external Ca$^{2+}$ of 0.9 to 5.6 mM (Fig. 7, Table 2). Below 0.9 mM, however, the inward current decreases. This cannot be explained simply by the 'stabilizing effect' of Ca$^{2+}$, since the membrane should be less stabilized and more excitable at a lower concentration of external Ca$^{2+}$. It has been postulated that one of the steps of Ca$^{2+}$ permeation through the Ca-channel is a saturable binding step (Hagiwara, 1973). If this is the case in the *Paramecium* Ca-channel, 0.9 mM-Ca$^{2+}$ appears to be the concentration at which the rate-limiting binding step is saturated.

Figs. 2, 4, 5 show that the addition of K$^+$ suppresses the Ca$^{2+}$ inward current. It is possible that added K$^+$ alters the distribution of surface charges (McLaughlin, 1976), which could greatly change the electric field actually detected by the Ca-channel. On the other hand, it is also possible that some of the Ca$^{2+}$-binding sites in the Ca-channel become occupied by K$^+$.

Preliminary results show that the addition of Ba$^{2+}$, decreases the inward current and slows down its activation and inactivation (Satow, 1978b). Externally added Na$^+$ does not inhibit or enhance the maximal inward current but does slow down its inactivation. Internally applied EGTA$^{2-}$ appears to affect both activation and inactivation.

Some of the electrophysiological techniques for analysing the membrane currents cannot be used with *Paramecium*. The cell does not survive Ca$^{2+}$-free solutions. Divalent cations reduce the inward transient. There are no strong and specific inhibitors for the individual ion channels of the *Paramecium* membrane. However, there is a large collection of membrane mutants with identified defective ion channels (Kung & Eckert, 1972; Satow & Kung, 1976a, b, c; Schein *et al.* 1976; Takahashi & Naitoh, 1978). Studies of these mutants should help us to describe further the behaviour of Ca-channels in *P. tetraurelia*. Voltage-clamp studies of these mutants under the optimal conditions described by this paper are in progress.

We thank Drs D. Oertel and S. Schein for criticisms. This work was supported by NSF grant BNS 75-10433 and NSF grant BNS 77-20440.
REFERENCES


**I_{Ca} of Paramecium**


