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# VOLTAGE-CLAMP AND CURRENT-CLAMP STUDIES ON THE ACTION POTENTIAL IN *NITELLA TRANSLUCENS*

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SUMMARY

1. The primary aim of the work was to establish the relative importance of $Na^+$, $K^+$, $Ca^{2+}$ and $Cl^-$, as the membrane charge-carriers during the action potential.

2. The results suggest that neither $Na^+$ nor $Ca^{2+}$ carries a significant part of the initial leakage current. It is argued that this current arises as a result of a large increase in the membrane conductance to $Cl^-$, but the cation-exchange properties of the cell wall do not allow a definite conclusion to be made from the experimental results.

3. The results indicate that $K^+$ is also involved in the action potential and it is suggested that this is consistent with the hypothesis that there is an increase in the membrane conductance to both $Cl^-$ and $K^+$ during activity.

4. It is suggested that the bulk of the electrical activity takes place at the plasmalemma.

5. In general, the results are in good agreement with those obtained from similar work performed on other species of the Characeae.

## INTRODUCTION

The action potential in the giant internodal cells of the Characeae has been the subject of persistent investigation in recent years. These investigations have involved the measurement of the electrical properties of the cell membranes[1–7] and also the ionic fluxes across these membranes[8–11]. Much difficulty has been experienced in establishing the identity of the ions which carry the membrane currents during the action potential. The most definitive evidence comes from the ion flux measurements where it has been demonstrated that there is a large increase in the chloride efflux during activity, presumably as a result of an increased membrane conductance to chloride. Thus the chloride ion is directly involved in the action potential. The identities of other ions which may also be involved in the electrical activity have not been established.

The difficulties encountered with the Characeae are largely due to the fact that there are three barriers to ionic diffusion between the large central vacuole of the

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cell and the environmental solution. These are the tonoplast, the plasmalemma, and the cell wall, and it is experimentally somewhat difficult to resolve the individual responses of these three components to external influences. Nevertheless, certain general features of the system have now been established. In the first place, it appears that the main part of the cell's resting potential is located between the cytoplasm and the bathing medium[5,12–15]. Secondly, the major proportion of the resting electrical resistance is associated with the plasmalemma[5,16–19]. During the action potential large transient changes occur in both the electrical potential and the electrical resistance. Only in the case of *Chara australis*[5] has it been observed that both tonoplast and plasmalemma are involved in these changes, *i.e.* that both membranes are excitable. Even so, the bulk of the electrical changes take place at the plasmalemma.

In these present experiments we have studied the electrical responses of the cells of *Nitella translucens*, bathed in various ionic media, to both constant-voltage and constant-current pulses with the object of identifying the charge-carriers of the membrane currents. We have not attempted to distinguish between the tonoplast and the plasmalemma in these experiments but have simply applied the pulses between the vacuole and the bathing medium. Experiments carried out under constant-voltage conditions are conventionally termed "voltage-clamp" experiments; in a like manner, we shall use the term "current clamp" for our constant-current experiments.

## MATERIALS AND METHODS

Strands of *Nitella translucens*, which had been collected from a freshwater lake, were stored in large tanks in a sheltered place, out of doors. The cells were brought into the laboratory a week before the experiments were begun and kept in an artificial pond water of the following composition: NaCl, 1.0 mM; KCl, 0.1 mM; $CaCl_2$, 1.0 mM; this solution will be referred to as Ca-APW. Cells presoaked in solutions containing $Ca^{2+}$ concentrations substantially lower than 1.0 mM were observed to give rather sluggish action potentials or were completely inexcitable. The cells selected for the experiments were those which showed rapid protoplasmic streaming. The lengths of the cells were usually about 8 cm, though short cells of 1–1.5 cm were also used when available; the diameters of all the cells were close to 1 mm.

The experimental chamber, in which the cells were mounted during the experiments, was approximately 20 cm long, 1 cm wide and 2 cm deep. It was divided into two compartments by means of a removable perspex partition. The partition had a 1.1-mm hole drilled through it and a cell could be drawn through this hole so that only a short section of the cell, not exceeding 1.5 cm in length, protruded into one of the compartments. To prevent electrical leakages between the environmental solutions in the two compartments, the partition was lined with white petroleum jelly beforehand. Solutions were circulated through each compartment separately and during all the experiments the solutions in the two compartments at any given time were identical.

Ca-APW was used as the standard reference solution in the experimental chamber. It could be replaced by any of what will be referred to as High Cl, Low Ca, Medium Na–K, and High K–Low Na solutions. On completion of a clamp experiment using a particular bathing solution, the cell was allowed to re-equilibrate in Ca-APW before proceeding to a further solution change. The ionic composition of the solutions is given in Table I and they were chosen with the aim of examining the effects of

$Na^+$, $K^+$, $Ca^{2+}$, and $Cl^-$ on the membrane responses to constant-voltage and constant-current pulses.

TABLE I

THE IONIC COMPOSITION OF THE EXTERNAL BATHING MEDIA

| *Medium* | *Composition* | | |
|---|---|---|---|
| | *KCl* (*mM*) | *NaCl* (*mM*) | $CaCl_2$ (*mM*) |
| Ca-APW | 0.1 | 1.0 | 1.0 |
| High Cl | 0.1 | 10.0 | 1.0 |
| Low Ca | 0.1 | 1.0 | 0.1 |
| Medium Na–K | 0.5 | 0.6 | 1.0 |
| High K–Low Na | 1.0 | 0.1 | 1.0 |

The internal current electrode was a micro-Ag/AgCl wire electrode inserted transversely into the cell at its midpoint; the method for making these electrodes has been described elsewhere[20]. The tip diameters of the electrodes used in the experiments ranged from 10 to 30 $\mu$; electrodes with finer tips do not have the necessary mechanical strength to penetrate the tough cell wall. About 0.8 mm of the electrode tip was left uninsulated and this was the depth to which the electrode tip was thrust into the cell. The electrical resistance of a typical electrode was about 60 k$\Omega$. The external current electrode was a 1.5-cm length of platinum wire. It was positioned in the experimental chamber so as to lie parallel to the length of the cell under study and 1 mm distant from it.

The membrane potential was measured using a conventional 3 M KCl-filled glass micro-electrode inserted transversely into the cell, and a calomel half-cell in the bathing medium; the internal voltage electrode was positioned about 1 mm from the internal current electrode. The tip diameters of the micro-electrode were never in excess of 2 $\mu$. Each electrode was tested for tip potentials and those having tip potentials in Ca-APW of more than 5 mV were discarded. The electrical resistance of these micro-electrodes was between 1 and 5 megohms. The tip of the calomel reference electrode was located not more than a few mm from the point of insertion of the micro-electrode. This was done in order to eliminate any spurious ohmic potentials in the bathing medium; this was an especially important consideration in the present work where the bathing media were all very dilute salt solutions and therefore of high resistivity (about 5 k$\Omega$·cm).

The cells of *Nitella translucens* have the electrical properties of a short, leaky, coaxial cable[21]. This means that if an electric current is injected into a cell at a point, then there will be an attenuation of both the membrane current density and voltage response with distance from the point of current injection. The attenuation is characterised by the space constant, $\lambda$, of the cell. The space constant of the cell is expressed as:

$$\lambda = [r_m/(r_i + r_o)]^{\frac{1}{2}}$$

where $r_m$ is the resistance of unit length of the cell membrane (k$\Omega$·cm), and $r_i$ and $r_o$ are, respectively, the resistances per unit length of the internal and external solutions

with respect to the cell membrane ($k\Omega \cdot cm^{-1}$); $\lambda$ has the dimensions of length. The resting value of $\lambda$ for *Nitella translucens* lies between 2.5 and 3.0 cm (refs. 20, 21). In the present experiments it was observed that the membrane resistance decreased during the action potential to about a fifth of its resting value; $r_m$ will decrease by the same amount under these conditions because it is directly related to the membrane resistance. Hence $\lambda$ will decrease to about one half of its resting value, *i.e.* to between 1.3 and 1.5 cm. Clearly, in order that voltage- and current-clamp studies shall be meaningful the attenuation effects must be minimised over the clamped area of the membrane. This presents an obvious difficulty in experiments in which the membrane current is injected at a single point as was the case in the present work. However, it is possible to overcome this to a large extent by clamping sections of cells whose half-lengths are appreciably shorter than the space constant of the cell in its excited state. For this reason, only cell sections with half-lengths not exceeding 0.8 cm were clamped. The insulation of a short length of cell from the rest of the cell was accomplished by using the two-compartment chamber already described. In a few experiments, short cells of total length between 1.0 and 1.5 cm were used and in these experiments the perspex partition was omitted from the chamber. It is preferable to use short cells in these experiments but since suitable cells of large diameter were not in very plentiful supply, it was necessary to use short sections of long cells in the majority of the experiments. No systematic difference was detected in the membrane parameters obtained for the two types of cell.

A schematic diagram of the system used in the voltage-clamp experiments is shown in Fig. 1. The membrane potentials were recorded by the electrometer pre-amplifier *A*. This preamplifier had differential inputs of resistance $10^{14}$ ohms and the time constant of the response to an input voltage step function from a 10 megohm source was less than 100 $\mu$sec. Such a response time is clearly more than adequate in the present experiments in which the source resistance, *i.e.* that of the micro-electrode, was between 1 and 5 megohms and the time constants of the transient membrane potentials were never less than 20 msec. The output from this preamplifier was fed

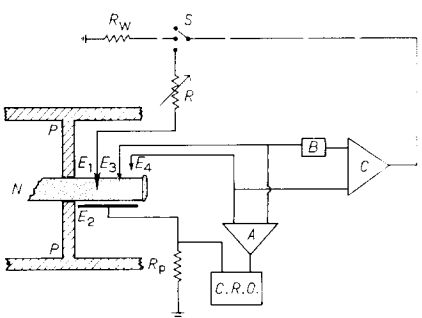



Fig. 1. A schematic diagram of the experimental arrangement for voltage-clamping. *N* denotes the Nitella cell. *PP* represents the partition in the two-compartment bath which isolates a short length of one end of the Nitella cell. $E_1$ is the Ag/AgCl electrode through which current enters the cell and is inserted into the cell or cell section at its mid point; $E_2$ is the external current electrode (platinum); $E_3$ is a conventional 3 M KCl-filled glass micro-electrode and is positioned about 1 mm from $E_1$; $E_4$ is a standard calomel reference electrode. *R* is a resistance box with a range of resistances from 1 megohm to zero; $R_W$ is a standard 1-megohm resistance and $R_P$ is a standard 10-k$\Omega$ resistance. *S* is a two-way switch. *A* and *C* are Keithley high-input impedance electrometer amplifiers (Model 603). *B* is a potentiometer providing potentials up to 200 mV. *C.R.O.* denotes the recording oscilloscope (Tektronix, Model 502).

into the oscilloscope. $B$ was a voltage source which could be switched stepwise from zero up to 200 mV and was essentially a potentiometer driven by a battery; its impedance was close to 100 k$\Omega$. The output voltage of amplifier $A$ was subtracted from the voltage generated by $B$ and the difference fed into the differential control amplifier $C$; $A$ and $C$ had identical specifications. The signal fed into $C$ was amplified 1000 times and inverted, the output current being passed through the variable control resistance $R$ and the membrane, thus completing the feedback loop. The membrane current was measured by feeding the potential difference across $R_p$ directly into the oscilloscope; the value of $R_p$ was 10 k$\Omega$ and the input impedance of the oscilloscope was 1 megohm. The oscilloscope traces were photographed as required.

The operation of the system was as follows: the membrane potential recorded by $A$ was backed off to zero using $B$ and the output from $C$ was fed to earth through $R_w$ by proper positioning of the switch $S$. $R_w$ was equal to the maximum value of $R$, *i.e.* about 1 megohm. $C$ was then adjusted until its output current was less than 0.1 $\mu$A. This output from $C$ was then fed into the membrane through $R$ using the switch $S$. The gain of the control amplifier $C$ was maintained at 1000. Finally, reducing the value of $R$ to about 300 k$\Omega$ brought the membrane under complete voltage control. By stepwise switching of $B$ the membrane potential could be clamped at any desired level. The membrane potential was not clamped during the complete duration of an experiment and normally the feedback loop was only employed for a few seconds at a time, this being long enough for a voltage pulse capable of controlling the membrane during activity to be produced. All the pulses were manually switched; automatic switching is unnecessary with the Characeae because of the long duration of the action potential.

The current-clamp experiments were performed separately from the voltage-clamp experiments. The electrode system and the equipment for measuring and recording the membrane potential and current was the same as for the voltage-clamp experiments. Constant-current pulses were passed into the cell through the Ag/AgCl current electrode. The pulses were obtained from a manually switched battery power supply with a 10-megohm series impedance; the maximum battery voltage was 250 V. The rise time of the current pulses was always about 1 msec.

It must be emphasised that both the internal Ag/AgCl electrode and the glass micro-electrode were inserted into the cell at least halfway across its diameter and their tips were therefore presumably located in the cell vacuole. Thus the applied voltage or current pulses were applied between the vacuole and the bathing medium. In other words, no discrimination was made between the electrical characteristics of the tonoplast and the plasmalemma.

Experiments were carried out on four batches of cells; voltage-clamp experiments were carried out on the first and second batches while current-clamp experiments were performed on the third and fourth batches. The external solutions used with the first batch (25 cells) and the third batch (10 cells) were Ca-APW, High Cl and Low Ca; with the second batch (20 cells) and the fourth batch (10 cells), the external solutions were Ca-APW, Medium Na–K and High K–Low Na. The application of voltage or current pulses was always initiated about an hour after a solution change was made in order to allow for the re-establishment of the steady state. Furthermore, because of the relatively long refractory period of the membrane, voltage and current pulses could only usefully be applied every 10 min or so. Thus in order to avoid overlong

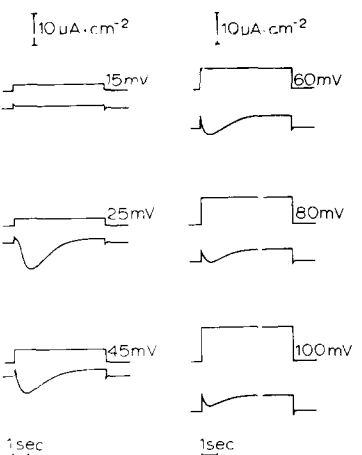
experiments with any given cell, the experimental system was operated on a batch production basis.

## RESULTS

### *Ca-APW as the external medium*

Examples of the current responses of a cell to a series of depolarising clamp voltages are shown in Fig. 2. The resting potential for this particular cell was —60 mV and the magnitude of the action potential was 80 mV. The responses closely resemble those obtained for *Nitella axillaris*[7]. At the beginning and end of each current response, membrane capacitance charging and discharging effects are discernible. These transients confirm that the membrane capacitance is close to 1.0 $\mu F \cdot cm^{-2}$ (see ref. 21). The excitation threshold potential for an unclamped Nitella cell is about 20 mV above the resting potential and for depolarisations below this level the membrane current is outward and small. Above the threshold level a large transient inward current appears, followed by a steady outward current. The steady outward current increases smoothly with increasing membrane depolarisation while the transient inward component is reduced; the transient inward current becomes a transient outward current when the membrane is depolarised by more than 80 mV, *i.e.* by a value in excess of the magnitude of the action potential. The membrane behaves as a simple resistance–capacitance network when it is hyperpolarised by less than about 40 mV; at higher levels of hyperpolarisation, delayed rectification and occasional negative resistance effects are observed[22].

The conventional current–voltage characteristic curves for the excitable membrane were obtained from the traces in Fig. 2 by plotting the peak transient, the steady outward, and the resting-state current density, as functions of the membrane potential (see Fig. 3). The resting-state and peak transient curves are tentatively



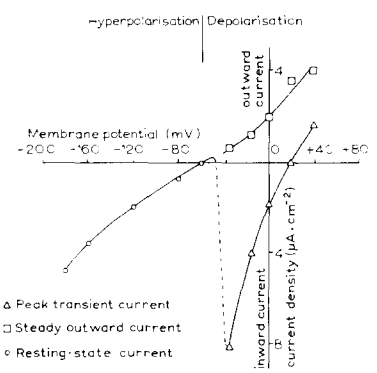




Fig. 2. The oscilloscope traces of typical membrane current responses to a series of depolarising voltage pulses.

Fig. 3. The current–voltage characteristic curves for a typical Nitella cell bathed in Ca-APW obtained from a voltage-clamp experiment. The dotted line shows the region of negative differential membrane resistance.

connected so that the resulting curve exhibits voltage-controllable negative differential resistance. This negative resistance effect is responsible for the instability of the membrane near the threshold potential and the initial potential-switching phenomena associated with the action potential.

Certain membrane parameters can be deduced from the graphs of the type shown in Fig. 3. These are: the resting membrane potential at zero current ($E_m$); the resting membrane resistance ($R_m$) which is taken as the slope of the resting-state curve at zero current; the excited-state membrane potential ($E_e$) which is the potential at which the peak transient current is zero; and finally, the excited-state membrane resistance ($R_e$) which is obtained from the slope of the peak transient curve at zero current. The mean values obtained for $E_m$, $E_e$, $R_m$ and $R_e$ for 45 cells bathed in Ca-APW are given in Table II. These results are the combined results for two batches of 25 and 20 cells, the separate results for the two batches being given in Tables IV and VI.

TABLE II

THE MEMBRANE PARAMETERS OBTAINED FROM VOLTAGE-CLAMP EXPERIMENTS ON 45 CELLS BATHED IN Ca-APW SOLUTION

$E_m$ and $E_e$ are respectively the resting and the excited-state membrane potentials at zero current (all membrane potentials are expressed as the potential of the inside of the cell with respect to the bathing medium). The difference ($E_m - E_e$) gives the magnitude of the action potential. $R_m$ and $R_e$ are respectively the resting and excited-state membrane resistances at zero current. The quoted errors for these parameters are standard errors.

| $E_m$ (mV) | $E_e$ (mV) | $E_m - E_e$ (mV) | $R_m$ ($k\Omega \cdot cm^2$) | $R_e$ ($k\Omega \cdot cm^2$) |
|---|---|---|---|---|
| $-63 \pm 2$ | $+7 \pm 2$ | $70 \pm 2$ | $27 \pm 3$ | $5.0 \pm 0.4$ |

The traces of a series of typical potential responses to a series of stepwise-increasing, depolarising (outward) current pulses are shown in Fig. 4. The voltage response is that of a resistance–capacitance network for subthreshold pulses. When the applied pulse is supra-threshold, the familiar action potential profile appears, with the ohmic potential due to the applied current superimposed. The membrane capacitance has little effect on this superimposed potential because the membrane time constant, which is about 50 msec in *Nitella translucens*[21], is very much shorter than the duration of the peak transient potential. With increasing applied current, the peak transient potential and the subsequent steady-state potential both increase steadily. The results for hyperpolarising pulses confirm those obtained by voltage-clamping in that the membrane responses for hyperpolarisations below about 40 mV are those of a resistance–capacitance network, while for greater hyperpolarisations the responses exhibit delayed rectification and occasional negative resistance effects[22]. It will be noted from the traces in Fig. 4 that the membrane potential does not return immediately to its normal resting value when the current pulse is terminated. This is a common observation, particularly with large currents. The effect is not, however, long lasting and the normal resting potential is usually re-established within 5 min.

From the data that can be obtained from the responses shown in Fig. 4, the peak transient, the steady-state, and the resting-state potential can be plotted as functions of the membrane current density. The resulting curves are shown in Fig. 5.

As might be expected, they resemble those obtained from the voltage-clamp experiments except for the fact that the variation of the peak transient potential with inward current is unobtainable by the current-clamp method. The voltage-clamp curves show the variation of the peak transient, steady outward, and resting-state current density with membrane potential; the current-clamp curves show the respective variations of the peak transient, steady-state, and resting-state potential with membrane current density.

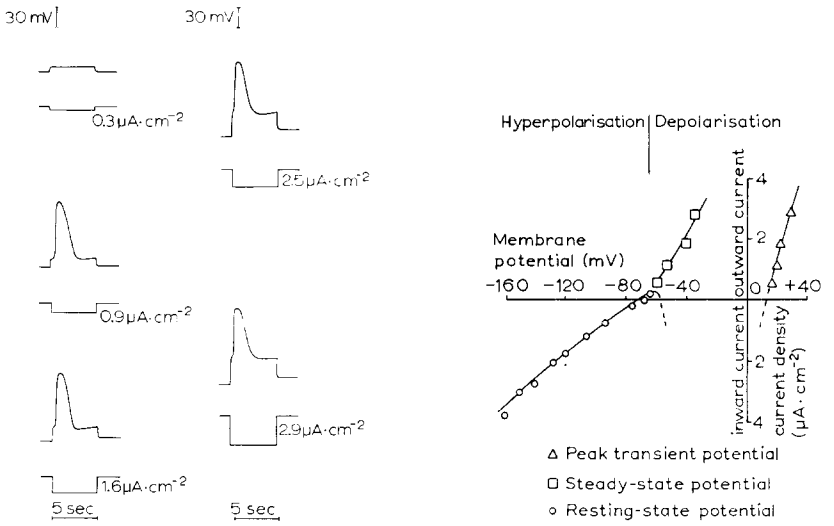



Fig. 4. The oscilloscope traces of typical potential responses to a series of depolarising current pulses.

Fig. 5. The current–voltage characteristic curves for a typical Nitella cell bathed in Ca-APW obtained from a current-clamp experiment.

The membrane parameters $E_m$ and $R_m$ are easily deduced from graphs of the type shown in Fig. 5 and by projecting the peak transient curve to cut the current axis the parameters $E_e$ and $R_e$ may be deduced. The mean values of the parameters obtained for 20 cells bathed in Ca-APW are given in Table III. These results are the combined results for two batches of 10 cells each, the separate results being shown in Tables V and VII.

There appears to be good agreement between the values of the membrane parameters obtained by the voltage-clamp method and those obtained by the current-clamp method. The small variations that do exist are most likely due to the use of

TABLE III

THE MEMBRANE PARAMETERS OBTAINED FROM CURRENT-CLAMP EXPERIMENTS ON 20 CELLS BATHED IN Ca-APW SOLUTION

| $E_m$ (mV) | $E_e$ (mV) | $E_m - E_e$ (mV) | $R_m$ ($k\Omega \cdot cm^2$) | $R_e$ ($k\Omega \cdot cm^2$) |
|---|---|---|---|---|
| $-69 \pm 2$ | $+4 \pm 4$ | $73 \pm 4$ | $22 \pm 3$ | $6.0 \pm 0.7$ |

different cell batches, which were collected in different seasons, for the two kinds of experiments.

*Ca-APW, High Cl and Low Ca as the external solutions*

Sets of current–voltage characteristics are shown in Fig. 6 for a typical Nitella cell for which the bathing media were in turn Ca-APW, High Cl and Low Ca. The cell was studied under voltage-clamp conditions and the current responses from which the characteristics were plotted were not significantly different in the three solutions. There is also very little difference in the shapes of the curves for the three solutions. In fact the only significant observation that can be made is that in changing from Ca-APW to Low Ca solution, both curves are shifted to the left along the voltage axis by about 30 mV, *i.e.* both $E_m$ and $E_e$ become more negative by practically the same amount when the external $Ca^{2+}$ concentration is decreased ten-fold.

TABLE IV

THE MEMBRANE PARAMETERS OBTAINED FROM VOLTAGE-CLAMP EXPERIMENTS ON 25 CELLS BATHED IN Ca-APW, LOW Ca AND HIGH Cl SOLUTIONS

| *External medium* | $E_m$ (*mV*) | $E_e$ (*mV*) | $E_m - E_e$ (*mV*) | $R_m$ ($k\Omega \cdot cm^2$) | $R_e$ ($k\Omega \cdot cm^2$) |
|---|---|---|---|---|---|
| Ca-APW | − 64 ± 3 | + 7 ± 2 | 71 ± 2 | 24 ± 4 | 4.6 ± 0.6 |
| High Cl | − 68 ± 3 | + 4 ± 3 | 72 ± 3 | 23 ± 4 | 4.4 ± 0.5 |
| Low Ca | − 85 ± 4 | −15 ± 5 | 70 ± 5 | 24 ± 4 | 6.1 ± 0.8 |

The mean values of the membrane parameters obtained for 25 cells are shown in Table IV. It can be seen that the replacement of the Ca-APW solution by High Cl has a negligible effect on the membrane parameters. This particular solution change involves a ten-fold change in the $Na^+$ concentration and a four-fold change in the $Cl^-$ concentration but, as will be shown in the DISCUSSION, any observed effect on the action potential should be associated with the increased $Cl^-$ concentration. The results in Table IV also show that the average changes in both $E_m$ and $E_e$ when the reference solution is replaced by Low Ca are almost equal. This fact is very difficult to reconcile with the fact that there is a very significant difference between $R_m$ and $R_e$ which undoubtedly reflects a change in the resistance of the membrane. Since the cell wall is known to have the properties of a cation-exchange resin, then it seems reasonable to suggest that these potential changes take place in the cell wall and are not associated with the membrane. Table IV also shows that the values of $R_m$ and $R_e$ are little affected by any of the changes of bathing media; this is confirmed by the current-clamp experiments.

With cells under current-clamp conditions, the membrane potential responses, both to applied inward and outward current pulses, were substantially the same in all three solutions and the shapes of the characteristics curves are therefore very similar. A typical set of current–voltage curves is shown in Fig. 7. It is again significant that in changing from Ca-APW to Low Ca solution the values of $E_m$ and $E_e$ became more negative by almost the same amount; the average changes for 10 cells were respectively 19 mV and 16 mV. This observation confirms that made in the voltage-clamp

experiments. The mean membrane parameters deduced from the results for 10 cells are given in Table V.

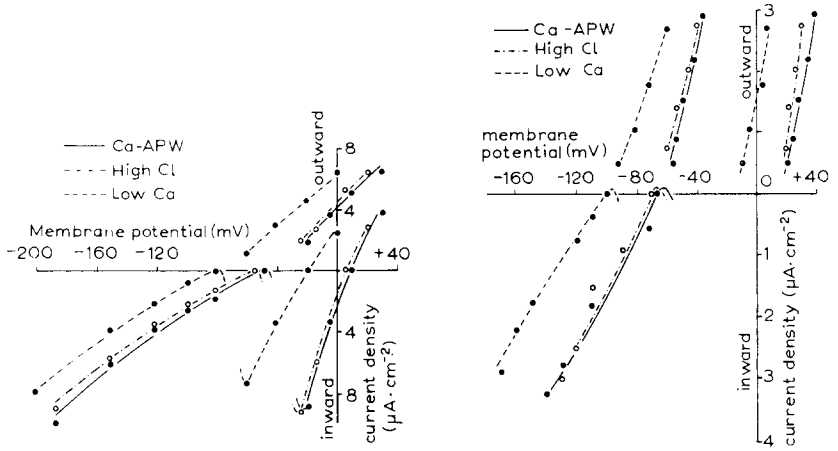



Fig. 6. The current–voltage curves for a cell bathed in Ca-APW, High Cl and Low Ca solutions obtained from voltage-clamp experiments.

Fig. 7. The current–voltage curves for a cell bathed in Ca-APW, High Cl and Low Ca solutions obtained from current-clamp experiments.

TABLE V

THE MEMBRANE PARAMETERS OBTAINED FROM CURRENT-CLAMP EXPERIMENTS ON 10 CELLS BATHED IN Ca-APW, LOW Ca AND HIGH Cl SOLUTIONS

| *External medium* | $E_m$ (*mV*) | $E_e$ (*mV*) | $E_m - E_e$ (*mV*) | $R_m$ ($k\Omega \cdot cm^2$) | $R_e$ ($k\Omega \cdot cm^2$) |
|---|---|---|---|---|---|
| Ca-APW | $-68 \pm 3$ | $-2 \pm 6$ | $66 \pm 6$ | $28 \pm 5$ | $7.2 \pm 0.9$ |
| High Cl | $-68 \pm 3$ | $-3 \pm 6$ | $65 \pm 5$ | $25 \pm 2$ | $7.4 \pm 1.1$ |
| Low Ca | $-87 \pm 4$ | $-18 \pm 4$ | $69 \pm 4$ | $28 \pm 4$ | $9.7 \pm 0.8$ |

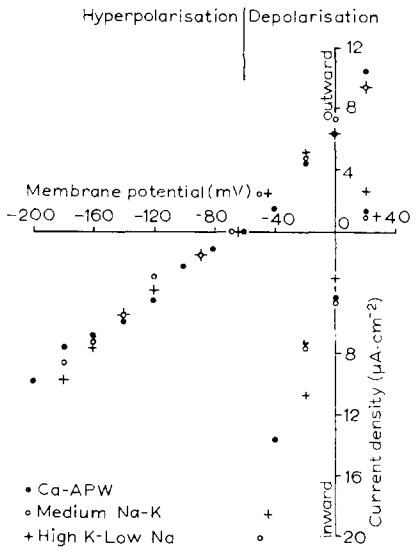
*Ca-APW, Medium Na–K, and High K–Low Na as the external solutions*

Neither the current responses for cells under voltage-clamp conditions nor the resulting membrane current–voltage characteristics were found to be very different in detail in any of these solutions. Fig. 8 shows the relation between membrane current density and potential obtained from a typical cell immersed in turn in the three solutions. In the particular example it is clearly not possible to distinguish between the individual curves for the three solutions and consequently no attempt has been made to draw them. The mean values of the membrane parameters derived from experiments on 20 cells are given in Table VI. The effect on $E_e$ of increasing the $K^+/Na^+$ concentration ratio is small and the errors are such as to indicate that it is not a significant trend. There is, however, a marginally significant increase in the size of the action potential as the concentration ratio increases. Neither $R_m$ nor $R_e$ are much affected by the solution changes and this is confirmed by the current-clamp experiments.

TABLE VI

THE MEMBRANE PARAMETERS OBTAINED FROM VOLTAGE-CLAMP EXPERIMENTS ON 20 CELLS BATHED IN Ca-APW, MEDIUM Na–K, AND HIGH K–LOW Na SOLUTIONS

| *External medium* | $E_m$ (*mV*) | $E_e$ (*mV*) | $E_m - E_e$ (*mV*) | $R_m$ ($k\Omega \cdot cm^2$) | $R_e$ ($k\Omega \cdot cm^2$) |
|---|---|---|---|---|---|
| Ca-APW | −62 ± 3 | + 6 ± 2 | 68 ± 4 | 30 ± 4 | 5.4 ± 0.6 |
| Medium Na–K | −67 ± 4 | + 8 ± 3 | 75 ± 5 | 27 ± 4 | 5.7 ± 0.8 |
| High K–Low Na | −67 ± 4 | + 10 ± 4 | 77 ± 6 | 25 ± 4 | 6.5 ± 0.9 |



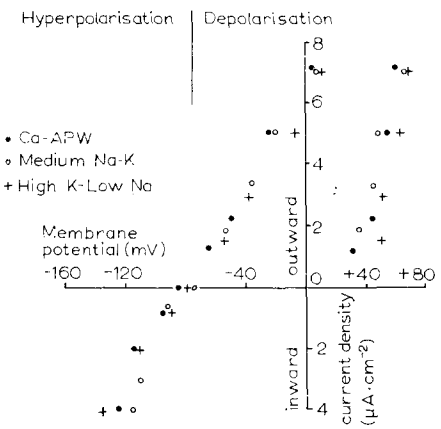




Fig. 8. The relation between membrane current and membrane potential obtained from a voltage-clamp experiment on a cell bathed in Ca-APW, in Medium Na–K and in High K–Low Na solutions.

Fig. 9. The relation between membrane current and membrane potential obtained from a current-clamp experiment on a cell bathed in Ca-APW, in Medium Na–K and in High K–Low Na solutions.

With the current clamp, the potential responses for cells bathed in the three solutions are very similar. Fig. 9 shows a typical example of the relation between the membrane current density and potential for the three solutions. It is clearly very difficult to distinguish between the individual curves for the resting membrane and

TABLE VII

THE MEMBRANE PARAMETERS OBTAINED FROM CURRENT-CLAMP EXPERIMENTS ON 10 CELLS BATHED IN Ca-APW, MEDIUM Na–K, HIGH K–LOW Na SOLUTIONS

| *External medium* | $E_m$ (*mV*) | $E_e$ (*mV*) | $E_m - E_e$ (*mV*) | $R_m$ ($k\Omega \cdot cm^2$) | $R_e$ ($k\Omega \cdot cm^2$) |
|---|---|---|---|---|---|
| Ca-APW | −71 ± 2 | + 11 ± 4 | 82 ± 5 | 16 ± 2 | 4.2 ± 0.9 |
| Medium Na–K | −70 ± 4 | + 14 ± 3 | 84 ± 4 | 17 ± 2 | 4.4 ± 0.6 |
| High K–Low Na | −73 ± 5 | + 21 ± 5 | 94 − 6 | 17 ± 2 | 3.8 ± 0.4 |

therefore no attempt has been made to draw them. However, the peak transient curve for the High K–Low Na solution could clearly have been distinguished from those for the other two solutions. This observation reflects a general trend for all 10 cells. The mean values of the membrane parameters for the 10 cells are given in Table VII. It will be noted that the solution changes have a larger and more significant effect on $E_e$ than was the case with the voltage-clamp experiments. There is again an increase in the magnitude of the action potential with increasing $K^+/Na^+$ concentration ratio.

## DISCUSSION

The experiments described herein have demonstrated substantial agreement between the results obtained by the voltage-clamp and current-clamp techniques for cells of *Nitella translucens* bathed in different ionic media. Any small variations in the values of the membrane parameters obtained by the two methods can be reasonably attributed to seasonal variations between batches of cells. In addition, the value for the resting membrane resistance, $R_m$, for cells bathed in Low Ca solution compares favourably with previously reported values[20,21]. The observed four- to five-fold decline in $R_m$ during the activity of the cell is to be expected on the basis of similar observations with other species of the Characeae[5,7]. In the present experiments, the overall mean value for $E_m$ for cells in Low Ca solution is 86 mV, which is about 15 % lower than the values obtained previously[13–15,20]. This is probably attributable to the fact that in the previous work the cells were cultivated in Low Ca for long periods before the experiments, whereas in the present work the cells were immersed in Ca-APW prior to the experiments and during the experiments were allowed to equilibrate in Low Ca for only 1 h before $E_m$ was measured. It is interesting to note that the average change in $E_m$ caused by a 10-fold change in the external $Ca^{2+}$ concentration was 21 mV under clamp conditions; this value compares very well with that obtained under conditions in which no current flowed through the membrane[15].

The primary objective of the present work was the identification of the ions which carry the membrane currents during the action potential. It is reasonable to assume that the action potential arises as the result of an increase in the membrane conductance to one or more of the ions in the bathing media, *i.e.* $Na^+$, $K^+$, $Ca^{2+}$ and $Cl^-$. It has been shown[13–15] that the electrochemical potential gradients for $Na^+$ and $Ca^{2+}$ are directed from the cytoplasm towards the bathing medium while those of $K^+$ and $Cl^-$ are oppositely directed. The present work has demonstrated that the electrical potential of the inside of the cell is more positive during activity than it is at rest. This could clearly be accomplished by an increased membrane conductance to any of the three ions, $Na^+$, $Ca^{2+}$ or $Cl^-$; the particular ion would then flow down its electrochemical potential gradient and drive the internal potential towards a positive value. It has already been suggested in a previous section that the changes in $E_m$ and $E_e$, which are observed when the external $Ca^{2+}$ concentration is changed, are most easily interpreted as being due to changes in the cell wall potential. In other words, $Ca^{2+}$ is not involved directly in the action potential, although it may be noted that the presence of $Ca^{2+}$ in the bathing medium is necessary for the initiation of an action potential. We are thus left with the possibility that the initial leakage current is due to an increase in the membrane conductance to $Na^+$ or $Cl^-$.

If the action potential were due solely to an increase in the membrane conductance to $Na^+$, then the membrane potential at the peak of the action potential ($E_e$) would be equal to the $Na^+$ equilibrium potential between the inside of the cell and the bathing medium, as given by the Nernst equation, and the membrane would behave as a reversible $Na^+$ electrode under these conditions. But the results shown in Tables II and III show that $E_e$ has a small positive value in solutions containing 1 mM $Na^+$. Thus, if $E_e$ were equal to the $Na^+$ equilibrium potential, then the internal $Na^+$ concentration of the cells would be less than 1 mM. Chemical analyses of the cell contents[13,15,18], indicate that this is not the case. It is therefore suggested that an increased membrane conductance to $Na^+$ is not an important contributory cause of the action potential and that there was fair justification in the assumption that in replacing Ca-APW by High Cl solution the increase in the $Na^+$ concentration was unimportant as far as the active membrane was concerned. This leaves, as the alternative, $Cl^-$ as one of the important current-carrying ions during the action potential. Certainly the positive values of $E_e$ could be explained on the basis of a $Cl^-$ action potential because the $Cl^-$ equilibrium potential is about + 100 mV (see refs. 13, 20). Unfortunately, the present work does not provide direct positive evidence in favour of this hypothesis since $E_e$ is apparently insensitive to changes in the $Cl^-$ concentration. Thus far, the definitive experiment for testing the Cl hypothesis in *Nitella translucens* by measuring the $Cl^-$ efflux during activity has not been made and the only favourable evidence[20] is, of necessity, indirect. In other species of the Characeae the observations of a greatly increased $Cl^-$ efflux during activity[8–11] strongly favour the Cl hypothesis and it seems reasonable to assume, at least until evidence to the contrary is forthcoming, that a similar situation exists in *Nitella translucens*. The lack of any observed effect of $Cl^-$ on $E_e$ in the present work can only be attributed to the cation-exchange properties of the cell wall which give rise to an electrical masking effect on any potential changes. Similar difficulties have been encountered with *Nitella flexilis*[7].

The fact that the peak of the action potential falls short of the $Cl^-$ equilibrium potential indicates that the membrane conductance to some other ion also increases during activity. The present work has shown that there is a tendency, though rather limited in the voltage-clamp experiments, for $E_e$ to assume a more positive value when the external $K^+$ concentration is increased; this observation is consistent with an increase in the $K^+$ conductance of the membrane during the activity of the cell.

Thus a satisfactory picture can be built up in which the action potential arises as a result of an increase in the membrane conductance to $Cl^-$ and also to $K^+$. Our experiments have not provided conclusive support for this hypothesis but at least the results are not in contradiction to it. Undoubtedly the presence of the cell wall, with its particular cation-exchange properties, makes for difficulties not only from the experimental point of view but also in the interpretation of the experimental results. It is a matter of some urgency that these particular difficulties should be overcome.

There remains one point to be discussed and this concerns our somewhat unspecific usage of the term "membrane". This lack of specificity of definition arises because, in the experimental work, the voltage and current pulses were applied between the central vacuole of the cell and the external bathing medium and the recorded responses were therefore those of a complex system made up of the cell wall, the plasmalemma, and the tonoplast, all in series. Thus no distinction was made between the electrical properties of the component parts of the system. This is not to

say that no conclusions can be drawn about these properties. In the first place, the cell wall is unlikely to be an important factor as far as the electrical resistance is concerned and it will certainly not be involved in the action potential. Furthermore, direct electrical measurements[19] suggest that the resistance of the plasmalemma is about ten times greater than that of the tonoplast; measurements of the $Na^+$ and $K^+$ fluxes across the two membranes[18] support these observations. Thus the observed four- to five-fold decrease in the cell's electrical resistance during activity must be mainly associated with the plasmalemma and this membrane is therefore excitable. We can say nothing at present about the excitability of the tonoplast. In *Chara australis*, both membranes have been observed to take part in the electrical activity but with the major part of this activity taking place at the plasmalemma. It may therefore be concluded that in referring to "the membrane" in the present paper, we are alluding principally to the plasmalemma.

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