THE ELECTRIC IMPEDANCE OF THE SQUID AXON MEMBRANE MEASURED BETWEEN INTERNAL AND EXTERNAL ELECTRODES

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The impedance of various cells and tissues has been measured extensively and analyzed in detail by Cole. The impedance locus, i.e., Cole-Cole plot, so far obtained is generally a circular arc, the center of which lies mostly below the resistance axis. Impedance locus of this type is expectable when the membrane impedance has a constant phase angle for the change of frequency. Such a property is qualitatively similar to that of the polarization impedance of metal electrodes in solutions.

A large decrease of the membrane impedance during excitation is caused mainly by a large increase of the membrane conductance. A possibility of decrease of capacity less than two per cent in the squid axon membrane was suggested by Cole and Curtis. These results were referred to by Hodgkin and Huxley in many aspects of analysis of their voltage clamp data. They assumed that the membrane capacity remained constant during excitation.

Almost all impedance measurements have been carried out between a pair of external parallel plate electrodes. Thus, it has been a problem to eliminate the effect of tangential current flow over the cell surface. Taylor measured the impedance of the squid axon membrane directly between an internal axial wire electrode and an external electrode, both being the platinized platinum. In the frequency range from 10 kHz to 70 kHz they found no appreciable change in capacity even with a long continued polarization or deterioration down to a resting potential of -20 mv.

In this report capacity and conductance of the squid axon membrane are measured between a pair of internal and external electrodes in a wide frequency range from 50 Hz to 1 MHz. The impedance locus thus obtained is not necessarily a circular arc, but rather can be expressed with an impedance having at least two time constants. An equivalent circuit for the axon mem-

Received for publication Feb. 27, 1970
brane having two time constants is introduced. The capacity which is extrapolated to the low frequency extreme decreases with the increase of the external potassium concentration.

MATERIAL AND METHOD

The giant axon of the Japan Sea squid, Doryteuthis bleekeri Keferstein, was used throughout the experiments. This species of squid is available at Ine Fishermen's Cooperative Association at the northern part of Kyoto Prefecture. The axon was dissected out for about 5 cm long and was carefully cleaned by removing a major portion surrounding connective tissues and small nerve fibers. The diameter of cleaned axon was between 0.4 and 0.7 mm.

The measuring chamber is shown in Fig. 1. An enamel-coated platinum wire of 100 microns in diameter was used as an internal axial electrode. The tip of the wire was freed from the enamel for 5 mm in length and was then platinized. Another platinized platinum wire of 500 microns in diameter was used as the external electrode. Both ends of measuring region of the axon were sealed with vaseline. Generally, impedances of these two electrodes were negligibly small at low frequency comparing with the impedance of the axon membrane. However, it was needed to subtract these electrode impedances vectorially from the measured value at high frequency measurements.

The conventional A.C. Wheatstone bridge method as shown in Fig. 2 was used for measuring membrane impedance. Internal and external electrodes were connected to an unknown arm of the bridge. A series capacity $C_o$ was needed to cut off the D.C. current flowing through the axon membrane. This capacity was about 20 $\mu$F, which was large enough not to disturb the measured values of parallel capacity $C_p$ and parallel conductance $G_p$ (Fig. 4a). The output of the bridge was amplified with a tuned amplifier (Yokogawa Hewlett-Packard 4403A) for the frequency range between 50 Hz and 20 kHz, and with a hand-made one for higher frequency measurements. The output was monitored with an oscilloscope (Matsushita, VP-543A).
RESULTS

Frequency Dependence of $C_p$ and $G_p$.

Values of parallel capacity $C_p$ and parallel conductance $G_p$ as defined in Fig. 4a were measured over a wide frequency range between 50 Hz and 1 MHz. The values of $C_p$ and $G_p$ themselves were different to some extent among different axons. However, the error of measurements on each axon was about 1 per cent. Generally, $C_p$ remained constant in the low frequency range, while it decreased in the high frequency range with the increase of frequency (Fig. 3). On the other hand, $G_p$ remained constant both at high and low frequency extremes. The apparent dispersion frequency of $C_p$ in Fig. 3 is about 10 kHz.

Impedance Locus of the Axon Membrane.

$C_p$ and $G_p$ in Fig. 4a can be transformed into a series circuit, i.e., resistance $R_s$ in series to a reactance $X_s$, as shown in Fig. 4b. Thus, the impedance of the axon membrane $Z$ is given by the following equation.

$$Z = R_s + jX_s$$  \hspace{1cm} (1)

where

$$R_s = R_p/(1 + \omega^2 C_p^2 R_p^2)$$  \hspace{1cm} (2)

$$X_s = -\omega C_p R_p^2 / (1 + \omega^2 C_p^2 R_p^2)$$  \hspace{1cm} (3)

In a complex impedance plane (the reactance is plotted against the resistance,
FIG. 3. A typical example of frequency dependences of parallel capacity $C_p$ and parallel conductance $G_p$ of the squid axon membrane.

FIG. 4. $a$: Definition of parallel capacity, $C_p$, and parallel resistance, $R_p (=1/G_p)$. $b$: Circuit of $a$ is transformed into $R_s$ in series to a reactance $X_s$.

the parameter being the frequency) the impedance locus of the axon membrane was almost a semi-circle in a frequency range lower than 1 kHz, the center of which lied on the real axis (FIG. 5). It is worth noting in this Cole-Cole plot that a clear deviation from a complete semi-circle was observed in every case of our experiments at frequencies higher than 1 kHz (see also FIG. 7b).

Changes of $C_p$ and $G_p$ During Excitation.

About thirty years ago COLE and CURTIS showed a large impedance change during excitation of the squid axon membrane with a pair of external electrode system. They concluded that such a large change was mainly caused by the large increase of the membrane conductance, while the change of the membrane capacity was negligibly small. Almost the same result was obtained with the transmembrane impedance measurements (FIG. 6). The bridge was
FIG. 5. Impedance locus of the squid axon membrane. Circles are measured values. Full line is a semi-circle which is the locus for an impedance having a single time constant. Dotted curve is the locus calculated for a model having two time constants such as shown in Fig. 9.

FIG. 6. Changes in the transmembrane impedance during excitation. a: Bridge is balanced at the resting state. b: Bridge is balanced at the peak of excitation. Frequency is 20 kHz.

balanced at the resting state in one case (Fig. 6a), while it was balanced at the peak of the action potential in another case (Fig. 6b). The decrease of $C_p$ was about 4 per cent at 5 kHz, 2 per cent at 10 kHz and 1 per cent at 20 kHz respectively. In other words, the decrease of $C_p$ was smaller, the larger the frequency. These values are of the same order to that obtained by Cole et al. Measurements of $C_p$ and $G_p$ during excitation at frequencies lower than
Fig. 7. a: Frequency dependence of parallel capacity and parallel conductance of the axon membrane for different external K+ concentration. b: The result in a is expressed in a complex plane (Cole-Cole plot).
5 kHz were not tried with the ordinary bridge method, since the duration of the action potential is about 1 msec.

**Effect of External Potassium Ion Concentration on \( C_p \) and \( G_p \).**

External K\(^+\) concentration was changed maintaining Na\(^+\) and Ca\(^{2+}\) concentrations constant. The external solution was also maintained isotonic at 1.2 molar with glycerol, and pH was adjusted to 8.0 with 10 mM tris-HCl buffer.

\( C_p \) and \( G_p \) for various external K\(^+\) concentrations are plotted in Fig. 7a against frequency. Also, these results are expressed as Cole-Cole plot in Fig. 7b by using equations (1), (2) and (3). The impedance loci in various external K\(^+\) concentrations were almost semi-circle in the low frequency range, while these deviated clearly from the circle in the high frequency range. It is clear from curves in this figure that changes in \( C_p \) and \( G_p \) with respect to the change in the external K\(^+\) concentration were large at low frequencies, while these were not detectable at high frequencies. Anyway, \( C_p \) at frequencies lower than 400 Hz remained almost constant for each K\(^+\) concentration. Thus, \( C_p \) at 400 Hz in Fig. 7a was plotted against the external K\(^+\) concentration in Fig. 8. There were some differences in the absolute values of \( C_p \) and \( G_p \) among 4 axons tested. However, it is evident that \( C_p \) and \( G_p \) remained practically constant at K\(^+\) concentrations less than 20 mM. \( C_p \) decreased when the external K\(^+\) concentration was larger than about 30 mM, while it reached again

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**Fig. 8.** Parallel capacity \( C_p \) and parallel conductance \( G_p \) at 400 Hz of 4 axons are plotted against change of external K\(^+\) concentration.
a constant value for the concentration larger than about 100 mM. In other words, $C_p$ has two limit values for the change in external $K^+$ concentration. On the other hand, $G_p$ increased with the increase in the $K^+$ concentration larger than 20 mM, while it remained constant for the external $K^+$ concentrations less than about 20 mM. The amplitude of the action potential decreased with the increase of the external $K^+$ concentration and went out practically at about 30 mM or larger. The axon membrane lost its excitability, when $C_p$ showed a tendency to decrease at about 30 mM of $K^+$. However, the excitability recovered gradually, when the external solution was replaced with the original low $K^+$ solution.

On the other hand, changes in external $Na^+$ concentration from 400 mM to 250 mM had no appreciable effect on $C_p$ and $G_p$. In this experiment the external $K^+$ and $Ca^{++}$ concentrations were kept constant. Changes of the external $Ca^{++}$ concentration from 10 mM to 60 mM had also no appreciable effect on $C_p$ and $G_p$.

**DISCUSSION**

Between internal and external electrodes there are several phases such as external solution, connective tissues, Schwann cell and axoplasm. If the sum of the resistances, $r_s$, of connective tissues, Schwann cell and else were comparable to or larger than the impedance of the axon membrane itself, the measured parallel capacity, $C_p$, would never be equal to the capacity of the axon membrane. Fortunately, however, the parallel conductance, $G_p$, at low frequency extreme was generally less than 1 per cent of that at high frequency extreme (FIGS. 3 and 7). $r_s$ calculated from our results was about 5 ohm $\cdot$ cm$^2$ on the average, which is of the same order as 7 ohm $\cdot$ cm$^2$ of HODGKIN and HUXLEY's result$^{3}$ and 3 ohm $\cdot$ cm$^2$ of COLE et $al.$.$^{2}$ Moreover, at the low frequency region the parallel capacity $C_p$ remained practically constant notwithstanding the increase in $G_p$ with the increase in the external $K^+$ concentration beyond 80 mM. These results indicate that $C_p$ at low frequency limit or practically at 400 Hz can be safely regarded as the capacity of the axon membrane.

As mentioned earlier the impedance locus would be a perfect semi-circle, if the membrane impedance were to be expressed with a single time constant circuit such as shown in FIG. 4a. Clear deviation from a semi-circle such as shown in FIG. 5 or in FIG. 7b, therefore, cannot be explained with this type of circuit. The impedance locus obtained by COLE and his college$^{3}$ in the axon membrane or some other cells was generally a circular arc. However, the center of this circular arc lied generally below the resistance axis. He explained such a property of the membrane as having a frequency-dependent impedance with a constant phase angle. However, the physical nature of such
an impedance characteristic is still unexplained. The impedance locus presented here is almost a semi-circular type except for the high frequency range. In our experiments the transmembrane impedance was measured directly between an internal axial wire and an external electrodes. Taylor\textsuperscript{6)} has already adopted the same measuring system for aiming to avoid the surface dispersion effect which might cause a deformation of the impedance locus. However, the impedance locus he obtained in his high frequency measurements was not a circular arc at all, but was similar to our results. He concluded that the data could be fitted either by the constant phase angle model or by a model having a bounded distribution of time constants. Since the impedance of the axon membrane decreased with frequency and became comparable to that of the electrode system alone, measurements at high frequency contained some uncertainty. However, deviations from a semi-circle at high frequency were evident in each experiment. Even from our experiments covering much lower frequency range we cannot say for sure that the two time constant hypothesis is the only best fit accounting for the deviation from a semi-circle. Anyway, if we adopt $C_1=0.4 \mu F \cdot cm^{-2}$, $C_2=0.9 \mu F \cdot cm^{-2}$, $r=830$ ohm $\cdot cm^2$, $r_1=40$ ohm $\cdot cm^2$, and $r_2=4$ ohm $\cdot cm^2$, a dotted curve such as shown in Fig. 9 which has two time constants, an impedance locus such as shown with dotted curve in Fig. 5 is obtained. The coincidence between this curve and the experimental values (open circles in Fig. 5) seems to be satisfactory. It is worth noting that each parameter in this circuit model remained constant for the large change of frequency at least between 50 Hz and 1 MHz. Such a two time constants model has been introduced by Falk and Fatt\textsuperscript{3)} in the analysis of their longitudinal impedance measurements in the frog muscle fiber. The capacity of lipid bilayer membrane is about $0.4 \mu F \cdot cm^{-2}$ (Hanai et al.\textsuperscript{4}). This value is almost equal to $C_1$ in our equivalent circuit (Fig. 9). Since the capacity of the axon membrane in our experiments is $C_m=C_1+C_2=1.3 \pm 0.3 \mu F \cdot cm^{-2}$ which is the arithmetic mean for 23 axons, we can suppose either that besides the electrostatic capacity there is another capacity component which is arised from

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{schematic.png}
\caption{Equivalent circuit of the squid axon membrane which has two time constants. Dotted curve in Fig. 5 is obtained by adopting $C_1=0.4 \mu F \cdot cm^{-2}$, $C_2=0.9 \mu F \cdot cm^{-2}$, $r=830$ ohm $\cdot cm^2$, $r_1=40$ ohm $\cdot cm^2$, and $r_2=4$ ohm $\cdot cm^2$ respectively.}
\end{figure}
the time depending flow of ions along the surface of fixed charges distributed over the membrane structure, or that the axon membrane is mosaic in its structure. The value of \( C_2 \) distributed from 0.35 to 0.45 \( \mu F \cdot cm^{-2} \) among axons and remained constant for the change in the external \( K^+ \) concentration. On the other hand, \( C_1 \) distributed from 0.7 to 1.2 \( \mu F \cdot cm^{-2} \) in normal sea water, while it decreased by 22-30 per cent in the high external \( K^+ \) concentration media.

TAYLOR\(^6\) concluded that the membrane capacity of the squid axon did not change under deterioration or depolarization down to \(-20 \text{ mV}\) over a frequency range between 10 kHz and 70 kHz. However, the change in \( C_2 \) due to depolarization induced by high external \( K^+ \) concentration was to be observed only with the low frequency measurement below about 2 kHz (FIGS. 7a and 7b). The maximum decrement of the capacity of the axon membrane was 10-25 per cent for the increase in the external \( K^+ \) concentration up to 80-100 mM. The axon membrane is supposed to be full of monovalent cations at such a depolarized state or during excitation (TASAKI\(^7\)). The increase in the monovalent cation concentration in the membrane phase will shield the electrostatic force of negative fixed charge in the membrane structure, causing the decrease of capacity of the axon membrane. Then, it may be also possible that the capacity \((C_1 + C_2)\) of the axon membrane decreases by 10-25 per cent during excitation. However, it is not easy to prove this by the usual bridge method, since the duration of the action potential is of the order of one milli-second. This remains to be proved by another method.

**SUMMARY**

The transmembrane impedance of the squid giant axon was measured for a wide frequency range from 50 Hz to 1 MHz.

The impedance locus was not an exact circular arc, but it showed a clear deviation from it at the high frequency range. Such an impedance locus can be expected, if the axon membrane impedance has at least two time constants. An equivalent circuit model having two time constants was introduced. Each element of the circuit remained constant for the change in frequency.

The capacity of the axon membrane decreased by 10-25 per cent, when the membrane was depolarized by adding 80-100 mM potassium ion externally. Decrease of external sodium ion concentration down to half or increase of external calcium ion concentration from 10 mM to 60 mM had no appreciable influence on the capacity.

We wish to express our many thanks to Drs. T. OHNISHI, T. TAKENAKA, T. HANAI, K. KOBATAKE, H. ASAI, A. IRIMAJIRI and M. TAKAGI for their discussions. Our thanks are also due to the members of Ine Fishermen's Cooperative Association for letting us use one of their rooms and for supplying us squids constantly. This study was supported by a grant for scientific research from the Ministry of Education of Japan.
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