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Measurement of Membrane Capacitance and Resistance of Single Cells Using Two Frequencies

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Summary

A method for the measurement of the membrane capacitance and resistance with two simultaneous sinusoidal frequencies is described. This method combines the advantages of measurements with a single sinusoidal frequency (i.e. low noise – high resolution) and those with rectangular waveform or polyfrequent methods. The mathematical analysis of the impedance as well as the admittance are presented for the evaluation with synchronous detection. Preliminary results are given.

Key words

Patch clamp – Cell membrane capacitance – Cell membrane resistance – Exocytosis – Endocytosis

Introduction

The value of cell membrane capacitance and its variations are often used to determine the changes of the cell membrane. The capacity of biological membranes per unit area is constant (approx. $1\,\mu F/cm^2$) thus its value is directly proportional to the surface of the cell. Membrane capacitance changes during an experiment are, therefore, useful for monitoring of the processes associated with changes in the surface area, for instance exo- and endocytosis. The use of membrane capacitance estimation was first described by Cole (1935) for studying fertilization and exocytosis of cortical granules. Measurement of membrane capacitance using intracellular recording was described by Jaffe *et al.* (1978), Gillespie (1979), Perez and Bernardiny (1985). However, small changes of the membrane capacitance, for instance those accompanying local exocytotic events, were beyond the resolution of the method. The introduction of the whole-cell patch-clamp technique together with a phase-sensitive detector (Neher and Marty 1982) improved the resolving power for the measurement of small membrane capacitance changes. This technique has been applied subsequently in many studies (Neher and Marty 1982, Fernandez *et al.* 1984, Zimmerberg *et al.* 1987, Breckenridge and Almers 1987).

The use of a sinusoidal command potential and a phase-sensitive detector for the measurement of membrane capacitance is accompanied by certain errors. A detailed analysis of this technique was performed by Joshi and Fernandez (1988), and its improvement was described by Fiddler and Fernandez (1989). A further development of the single frequency method was the superposition of a sinusoidal frequency on a DC signal (Lindau and Neher 1988) and a single frequency superimposed onto a slow (2 Hz) rectangular potential (Okada *et al.* 1992) The drawback of these methods is a possible influence on the behaviour of the cell by a DC component and a limited insensitivity to changes of *Rm* and *Rs* (Okada *et al.* 1992). In some experiments the changes to Rm are greater than those of membrane capacitance. This is e.g. the case in measurements of exocytosis of pancreatic cells (Schmid 1993, personal communication).

We tried to develop an AC method of the measurement of cell membrane parameters (capacitance and resistance) using simultaneously two frequencies which, we expect, shall improve the measurement of the membrane parameters. This method let also open the possibility of DC influencing of the cell.

Method

The electrical arrangement for the measurement is shown in Fig. 1. The sinusoidal measuring potential (command potential) is applied to the noninverting input of an operational amplifier connected as a *i/u* converter. Thus the command potential causes a current-flow through the network connected to the inverting input of the amplifier. This current is then monitored as a potential u_{out} at the output of the differential amplifier. The capacitance Cin is formed by the input capacitance of the amplifier and the stray capacitance of the micropipette and connecting wires. This capacitance is assumed to be stable with respect to time and to be compensated. Further analysis will be performed for a network *Cm, Rm* and *Rs*. This network is a dipole containing three elements and its admittance cannot be estimated from a current of single frequency; as a single frequency measurement can give only two values for three variables.

We wanted to maintain the advantages of a narrow-band AC method and therefore we added a second AC channel.

For the mathematical analysis we will first derive an impedance of the network *Cm, Rm* and *Rs*.

The complex impedance is given:

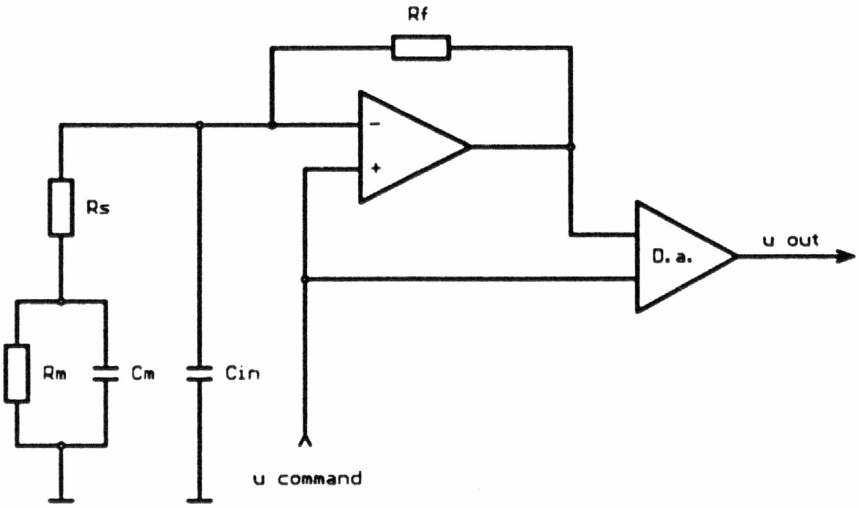

Fig. 1
Arrangement of the measurement. Rm = Cell membrane resistance; Cm = Cell membrane capacitance; Rs = Microelectrode resistance; Ci = Input capacitance; Rf = Feedback resistor; u_{out} = Output potential; D.a. = Differential amplifier.

$$Z = Rs + \frac{\frac{Rm}{j\omega c}}{Rm + \frac{1}{j\omega C}} \qquad \textbf{Eq. 1}$$

Let us separate the real and imaginary part of this impedance for a frequency ω:

$$Re(Z) = Rs + \frac{\frac{1}{Rm}}{\left(\frac{1}{Rm}\right)^2 + (\omega Cm)^2} \qquad \textbf{Eq. 2}$$

$$Im(Z) = \frac{-\omega Cm}{\left(\frac{1}{Rm}\right)^2 + (\omega Cm)^2} \qquad \textbf{Eq. 3}$$

Thus for two frequencies ω and $k\omega$ the equations Eq. 2 and for the second frequency change into:

$$\mathrm{Re}(Zk) = Rs + \frac{\frac{1}{Rm}}{\left(\frac{1}{Rm}\right)^2 + (k\omega Cm)^2} \qquad \textbf{Eq. 4}$$

$$\mathrm{Im}(Zk) = \frac{-k\omega Cm}{\left(\frac{1}{Rm}\right)^2 + (k\omega Cm)^2} \qquad \textbf{Eq. 5}$$

For three variables only three from the equations Eq. 2 through Eq. 5 are sufficient. For further derivation Eq. 4 is not used.

Multiplying Eq. 3 by *k* we obtain:

$$k\mathrm{Im}(Z) = \frac{-k\omega Cm}{\left(\frac{1}{Rm}\right)^2 + (\omega Cm)^2} \qquad \textbf{Eq. 6}$$

Using equations Eq. 5 and Eq. 6 in reciprocal form we can write:

$$\frac{1}{k\mathrm{Im}(Z)} - \frac{1}{\mathrm{Im}(Zk)} = \frac{-\left(\frac{1}{Rm}\right)^2 - (\omega Cm)^2}{k\omega Cm} - \frac{-\left(\frac{1}{Rm}\right)^2 - (k\omega Cm)^2}{k\omega Cm} \qquad \textbf{Eq. 7}$$

Simplification gives:

$$\frac{1}{k\mathrm{Im}(Z)} - \frac{1}{\mathrm{Im}(Zk)} = \frac{k^2-1}{k}\omega Cm \qquad \textbf{Eq. 8}$$

Thus from Eq. 8:

$$Cm = \frac{\mathrm{Im}(Zk) - k\mathrm{Im}(Z)}{\omega \mathrm{Im}(Zk)\,\mathrm{Im}(Z)\,(k^2-1)} = a\,\frac{\mathrm{Im}(Zk) - k\mathrm{Im}(Z)}{\mathrm{Im}(Zk)\,\mathrm{Im}(Z)} \qquad \textbf{Eq. 9}$$

where:

$$a = \frac{1}{\omega(k^2-1)} \qquad \textbf{Eq. 10}$$

a does not change during the measurement.

Rm can be derived from Eq. 3

$$Rm = \sqrt{\frac{\mathrm{Im}(Z)}{\omega Cm(\omega Cm\,\mathrm{Im}(Z)+1)}} \qquad \textbf{Eq. 11}$$

and *Rs* from Eq. 2.

$$Rs = \mathrm{Re}(Z) + \frac{\mathrm{Im}(Z)}{\omega Cm Rm} \qquad \textbf{Eq. 12}$$

To get the relations for admittance we use the following transformation:

$$Z = \frac{1}{A} = \frac{1}{\mathrm{Re}(A)+j\mathrm{Im}(A)} = \frac{\mathrm{Re}(A)-j\mathrm{Im}(A)}{\mathrm{Re}(A)^2+\mathrm{Im}(A)^2} \qquad \textbf{Eq. 13}$$

The real and imaginary components of the complex admittance are:

$$\mathrm{Re}(Z) = \frac{\mathrm{Re}(A)}{\mathrm{Re}(A)^2 + \mathrm{Im}(A)^2} \qquad \textbf{Eq. 14}$$

$$\mathrm{Im}(Z) = \frac{-\mathrm{Im}(A)}{\mathrm{Re}(A)^2 + \mathrm{Im}(A)^2} \qquad \textbf{Eq. 15}$$

After replacing *Re(Z), Im(Z)* in Eq. 9, Eq. 11 and Eq. 12 according to Eq. 14, Eq. 15 and *Re(Zk), Im(Zk)* analogically and after simplification we obtain the following expressions:

$$CM = \frac{k\mathrm{Im}(Ak)\left[1+\left(\frac{\mathrm{Re}(Ak)}{\mathrm{Im}(Ak)}\right)^2\right] - \mathrm{Im}(A)\left[\left(1+\left(\frac{\mathrm{Re}(A)}{\mathrm{Im}(Ak)}\right)^2\right]\right.}{\omega(k^2-1)} \qquad \textbf{Eq. 16}$$

for *Rm*

$$Rm = \sqrt{\frac{\mathrm{Im}(A)}{\omega Cm(\mathrm{Re}(A)^2 + \mathrm{Im}(A)^2 - \omega Cm\mathrm{Im}(A))}} \qquad \textbf{Eq. 17}$$

and finally for *Rs*

$$Rs = \frac{\mathrm{Re}(A) - \frac{\mathrm{Im}(A)}{\omega RmCm}}{\mathrm{Re}(A)^2 + \mathrm{Im}(A)^2} \qquad \textbf{Eq. 18}$$

Despite the fact that the change of *Rs* has no biological interpretation it is useful to monitor it in order to be warned, when the properties of the microelectrode change.

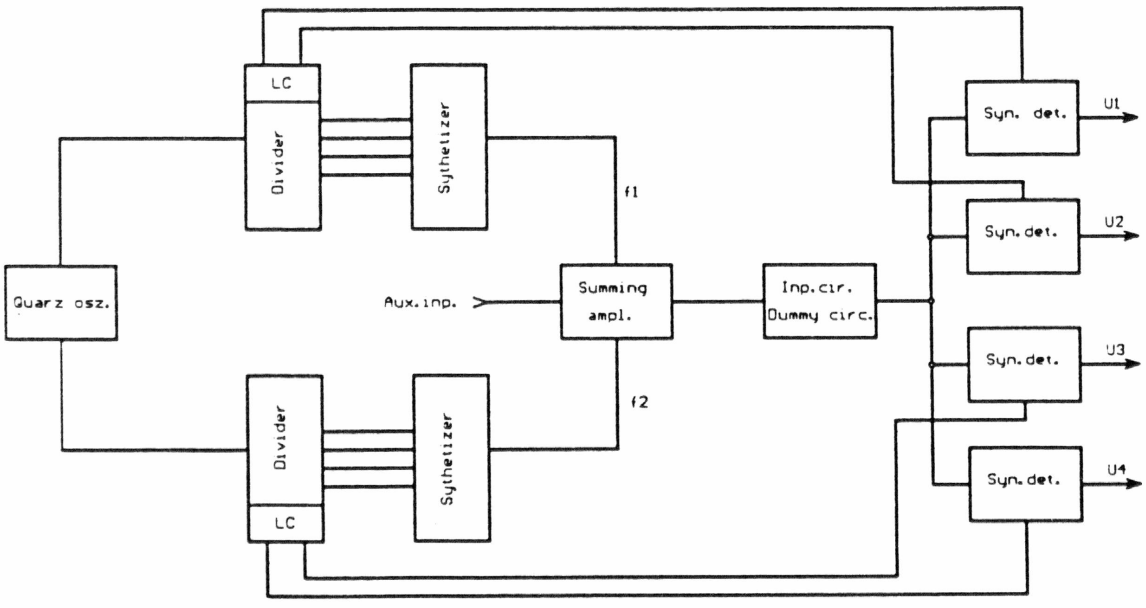

Fig. 2
Experimental set up for preliminary measurements.

Experimental set up

To verify the possibilities of the method we built an experimental set-up (Fig. 2) consisting of:

a) Quartz oscillator which controls all time relations in the device.

b) Dividers. The two dividers generate the waveforms for both the sinus potential synthetizers as well as for the logic circuits (LC) which form the switching potentials for synchronous detectors. The dividing ratio of these dividers can be separately preset to obtain the desired k ratio of both frequencies.

c) The sinus potential synthetizers based on analog multiplexers connected to the resistive network for the approximation of the sinus waveforms. A lowpass filter at the output of each synthetizer rejects all harmonic frequencies generated by the synthesis.

d) Summing amplifier. Before the summing of both potentials takes place the amplitudes and phases can be set. A third auxiliary input for stimulation is prepared.

e) Input *i/u* converter with a dummy circuit. The connection of this part is given in Fig. 1. Values of the dummy circuit are scaled by a factor 100 (resistances divided by 100, capacitance multiplied by 100). There are two reasons for this scaling. 1) It is hardly possible to change *Rm* in the range from 100 megohms to 1 gigaohm without the simultaneous introduction of a different parallel capacity to *Cm*. Not so critical but also with a certain influence would be the capacity error by a change of *Rs*. 2) Capacity changes in the range of femtofarads are difficult to set accurately. Because the amplitude and phase relations remain unchanged this model can be used for the measurement of the sensitivity as well as the influence of changes of *Rm* and *Rs* to *Cm*.

f) Synchronous detectors. The measurements were performed for the k ratio = 2. On the base of the function of the synchronous detection which is (theoretically) fully insensitive to *f.n* and *f/n* (where *f* is the detected frequency and *n* an odd number) there are no bandpass filters necessary for the separation of the two channels.

g) Potentials *U1–U4* are digitalized with 14 bit A/D card and then computed by a PC (HP Vectra RS/20).

Results

The measurements were performed with frequencies 266.6 and 533.2 Hz. The measured values were scaled by 100 therefore the following values are equivalents. Just noticeable difference in *Cm* was found 0.6 fF. For this measurement the accuracy of 14bit digitalization was not sufficient. Therefore the output potentials of the synchronous detectors were measured by a millivoltmeter (Digital multimeter 4660 Simac Electronics, Darmstad Germany) and then evaluated. The influence on the measured capacity *Cm* = 5 pF by a 100 % change of *Rm* was 0.09 % (100–200megohms) and 0.06 % (500–1000 megohms). The influence of the same change of *Rs* was 0.08 % (5–10 megohms) and 0.15 % (10–20 megohms). For *Cm* = 10 pF the reduction of the influence of *Rm* and *Rs* was about 60 % higher. However, such values especially the resolution power for changes of *Cm* can be hardly expected in measurements of real cells especially with respect to the noise.

The one-hour stability, measured after one-hour warming-up time, was 0.12 % *Cm* (*Cm* = 5 pF). The influence of the changes of *Cm* on *Rm* is higher. For the maximal value *Rm* = 1 gigaohm a 100 % change of *Cm* causes an error of 16 % and for *Rm* = 100 megohm 2.4 % The channel separation (crosstalk) was as follows: for channel *A* (smaller frequency) 76 dB and for channel *B* 66 dB. The measurements were performed by switching on and off the other sinus generator. The ratio of the amplitudes was 1:1, *k* was 2.

Discussion

The advantage of this method as compared to the methods using one sinus potential combined with DC or with slow rectangular pulses is the higher reduction of the influence of changes in *RM* and *Rs* to *Cm* and in the possibility of the simultaneous electrical stimulation of the cell. The measurement of *Cm, Rm* and *Rs* does not interfere with a holding potential or an electrical stimulation of the cell and *vice versa*. This method is thus suitable for measurements of single secretory events, such as vesicle fusion with the cellular plasma membrane in exocytotic processes with high accuracy.

The preliminary results have shown that the method is easy for technical realization with relative simple circuits and allows good resolution and accuracy for *Cm* measurement.

As could be expected from mathematical analysis, the error in *Cm* measurements is minimal for small values of *Rs* and high values of *Rm*. Furthermore also in a worst case of the combination of *Cm, Rm* and *Rs*, the error of measurement of *Cm* does not exceed 0.8 %.

The smaller accuracy for the measurement of *Rm* is given by the high ratio of *Rm/Z(Cm)* and can be improved by use of lower frequencies, however with a loss of the accuracy of the *Cm* measurement.

The worst separation of the channel *B* from the channel *A* is evidently due to minute remnants of the second harmonic component in the synthetizer for the smaller frequency.

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Reprint Requests

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