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Theoretical Analysis of Phase Detector Technique for the Measurement of Cell Membrane Capacitance During Exocytosis

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Abstract

Phase detector techique provides a unique probe to membrane recycling phenomenon by enabling dynamic monitoring of cell membrane capacitance. However, it has inherent errors due to constant changes in measurement environments. The present study analyzed several error sources to develope application criteria of this technique, and the following was found based on a theoretical analysis. The initial phase angle has to be appropriately selected to minimize the error due to perturbation of access and membrane conductances. Excitation frequency is also important to determine the initial phase angle. However, deviation of the phase angle from a predetermined initial value during the measurement period does not affect capacitance estimation to a significant degree. Despite an appropriate initial phase selection an error in scaling factor is expected for a large increase in capacitance during exocytosis, which may be overcome by iteratively correcting the scaling factor over the measurement period. These results will provide a useful guideline in practical application of this technique.

Key words:Phase detector technique, patch-clamp technique, whole-cell mode, and membrane capacitance.

I. Introduction

Physiologically important hormones are secreted in the form of discrete granules by fusion with cell membrane(exocytosis) in response to an appropriate stimulus. Since biological membranes have a constant geometry-independent capacitance per unit area of 1 $\mu F/cm^2$, fusion of the macromolecules accompany an

enlarged surface area of the cell, resulting in a corresponding increase in membrane capacitance. Therefore, capacitance measurement is useful to monitor cell surface area during exocytosis.

Amongst several techniques [1-4], a phase sensitive detector in combination with a patch-clamp amplifier enabled to measure microscopic changes in capacitance resulting from the fusion of single exocytotic vesicles[5]. It requires neither complicated signal processing nor mathematical calculation, but is still capable of dynamic on-line monitoring of membrane capacitance changes. However, it has inherent errors, since the phase angle must be initially set and kept over the measurement period while the monitored system(cell parameters) is constantly changing. Although the importance of the initial phase angle setting has been emphasized[6], theoretical basis of criteria allowing an acceptable measurement error has not been established. Therefore, the present study theoretically analyzed the phase detector technique to develope criteria to minimize measurement error. We briefly review the conventional phase detector technique, analyze a few error sources due to the constantly changing environments, and provide application criteria of this technique.

Ⅱ. Analysis

1. Phase detector technique

Single channel currents can be measured by a micropipette electrode attached to a small patch of cell membrane under voltage-clamp conditions[6]. This also can be used to monitor transmembrane current at a whole-cell mode of the patch-clamp technique[7]. Once the stray capacitance of the electrode has been cancelled by the fast compensation circuitry available on a common patch-clamp amplifier at the completion of a giga-seal[8] followed by breaking into the intracellular space, the cell membrane and measurement patch can be represented by a minimal electrical equivalent circuit model shown in Fig. 1. This consists of three parameters:access conductance of the electrode(Gs), membrane conductance(G), and membrane capacitance(C). These parameters are usually voltage-dependent and, in particular, C increases with time during exocytosis, which is of our major concern. When a sinusoidal command voltage signal, small enough to limit the model within a linear operation range, is applied through the electrode, the total admittance(Y) is given by

$$Y(j\omega) = \frac{G + j\,\omega C}{(1+\frac{G}{Gs}) + j\,\omega\frac{C}{Gs}} \quad \cdots\cdots (1)$$

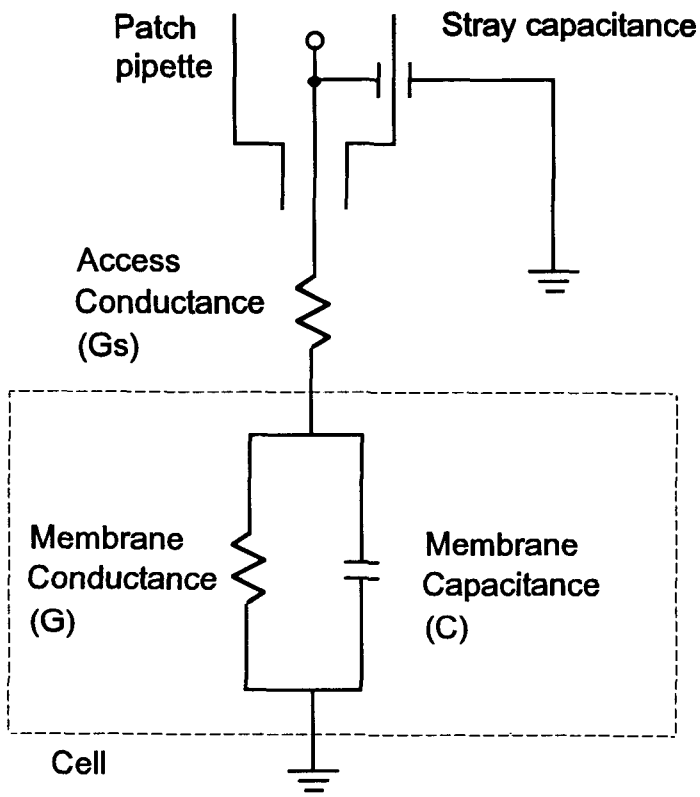

Figure 1. Minimal electrical equivalent circuit model of whole-cell patch-clamp mode. When measurement is made on cell parameters, stray capacitance is compensated by the fast compensation circuitry available on a common patch-clamp amplifier, and excluded from the model.

where j and ω represent imaginary unit and angular frequency, respectively. By introducing phasor notations of the applied voltage(V) and the resultant current (I) signals,

$$I = YV \quad \cdots\cdots (2)$$

For a small change(ΔY) in Y, the corresponding change(ΔI) in current can be linearized as

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$$I = YV \quad \cdots\cdots (2)$$

For a small change(ΔY) in Y, the corresponding change(ΔI) in current can be linearized as

$$\Delta I = V\frac{\partial Y}{\partial G}\cdot\Delta G+V\frac{\partial Y}{\partial C}\cdot\Delta C+V\frac{\partial Y}{\partial Gs}\cdot\Delta Gs \quad \cdots\cdots(3)$$

Note in eq.(3) that each term in the right side represents the contribution of the change in each corresponding circuit parameter to ΔI. Thus, let us define these components as

$$\Delta I_G = V\frac{\partial Y}{\partial G}\cdot\Delta G=VB^2\Delta C \quad \cdots\cdots(4a)$$

$$\Delta I_C = V\frac{\partial Y}{\partial C}\cdot\Delta C=j\,\omega VB^2\Delta C \quad \cdots\cdots(4b)$$

$$\Delta I_{Gs} = V\frac{\partial Y}{\partial Gs}\cdot\Delta Gs=\frac{(G+j\,\omega C)^2}{Gs^2}VB^2\Delta Gs \quad \cdots\cdots(4c)$$

where

$$B^2 = (1+ G/Gs+j\omega C/Gs)^{-2} \quad \cdots\cdots(5)$$

And from eqs.(3 and 4),

$$\Delta I = \Delta I_G + \Delta I_C + \Delta I_{Gs} \quad \cdots\cdots(6)$$

Note in eqs.(4a and 4b) that ΔI_C is orthogonal to ΔI_G, i.e., the change in membrane conductance(ΔG) is not reflected in the component of ΔI parallel to ΔI_C.

Each component in eq.(6) calculates the change in ΔI due to a corresponding parameter change with the other two parameters maintained. The phase angles of ΔI_G and ΔI_{Gs}(α and θ, respectively) relative to the applied sinusoidal voltage (V) are

$$\alpha = \arg(\Delta I_G) = \arg(B^2) = -2\tan^{-1}\frac{\omega C}{G+Gs} \quad \cdots\cdots(7a)$$

$$\theta = \arg(\Delta I_{Gs}) = \arg[B(G+j\omega C)/Gs]^2$$

$$= 2\left[\tan^{-1}\frac{\omega C}{G}-\tan^{-1}\frac{\omega C}{G+Gs}\right] \quad \cdots\cdots(7b)$$

The phase angle difference between ΔI_{Gs} and ΔI_G is

$$\theta - \alpha = 2 \tan^{-1} \frac{\omega C}{G} < \pi \quad \cdots\cdots (8)$$

Based on eqs.(4-8), the relationship of ΔI_G, ΔI_C, and ΔI_{Gs} is graphically presented on the phasor diagram shown in Fig.2. When ω is chosen so that $\theta - \alpha$ is close to π in eq.(8), the change in access conductance(ΔI_{Gs}) would be reflected only to a small degree in the component of ΔI parallel to ΔI_C, since ΔI_{Gs} becomes approximately orthogonal to ΔI_C. In such case, the magnitude of the component of ΔI taken to the direction orthogonal to ΔI_G (or parallel to ΔI_C) would be most sensitive to the change in membrane capacitance(ΔI_C). This component($| \Delta I(\pi/2+\alpha) |$) is calculted from eq.(4) as

$$\begin{aligned} | \Delta I(\pi/2+\alpha) | &= | \Delta I_C | + | \Delta I_{Gs} | \cdot \cos[\theta - (\pi/2+\alpha)] \\ &= | V | \, | B^2 | \, \omega \, [\Delta C + (2GC/Gs^2) \Delta Gs] \quad \cdots\cdots (9) \end{aligned}$$

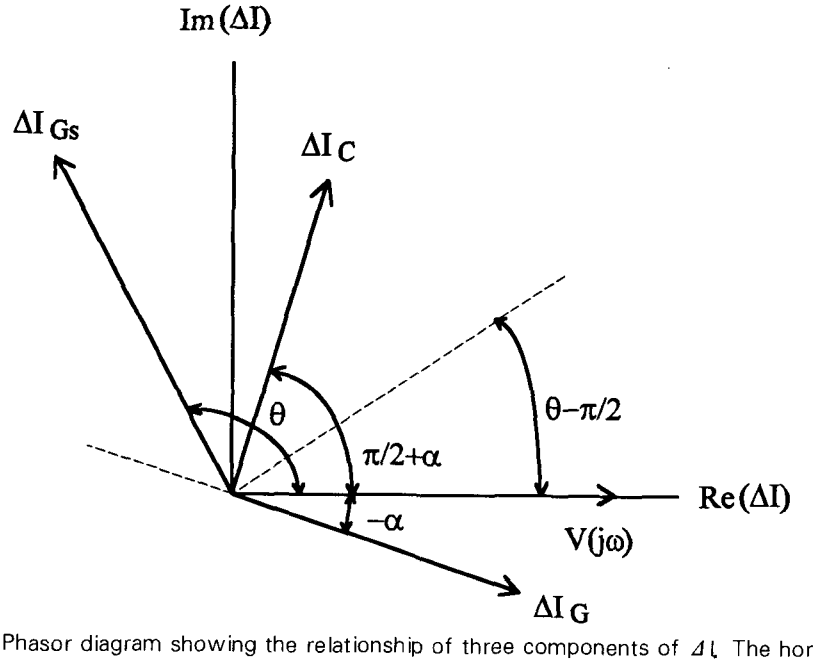

Figure 2. Phasor diagram showing the relationship of three components of ΔI. The horizontal axis is parallel to the phasor of applied voltage(V). Note that ΔI_C is orthogonal to ΔI_G and that $\theta - \alpha$ is less than π.

Note in eq.(9) that $\pi/2+\alpha$ is the phase angle of $\Delta I(\pi/2+\alpha)$ relative to V. For a monotonic voltage perturbation, $V = V_0 e^{jwt}$, eq.(9) is reduced to

$$| \Delta I(\pi/2+\alpha) | = K \cdot \Delta C(1+e_{Gs}) \quad \cdots\cdots(10)$$

where

$$K = \frac{\omega V_0}{(1+G/Gs)^2+(\omega C/Gs)^2} \quad \cdots\cdots(11)$$

and

$$e_{Gs} = \frac{2G}{Gs} \cdot \frac{\Delta Gs/Gs}{\Delta C/C} \quad \cdots\cdots(12)$$

Under the assumption of negligible e_{Gs}(ΔGs/Gs$\ll\Delta$C/C), ΔC can be calculated as

$$\Delta C = \frac{| \Delta I(\pi/2+\alpha) |}{K} \quad \cdots\cdots(13)$$

Estimation of membrane capacitance change(ΔC) according to eq.(13) is experimentally performed as follows. In a whole-cell patch-clamp mode, parameters(G, C, and Gs) are initially estimated by the slow compensation circuitry available on a patch-clamp amplifier[5] or directly on time-domain[9] followed by the calculation of $\pi/2+\alpha$ and K according to eqs.(7a and 13), respectively. Then sinusoidally varying command voltage(V) signal is applied and the current change(ΔI) also sinusoidally varying at the same frequency is resulted. The magnitude of the current component parallel to the direction of initially determined phase angle($\pi/2+\alpha$) relative to V is obtained by a phase-locking amplifier connected to the patch-clamp amplifier, thereafter the membrane capacitance is continuously monitored by eq.(13). While the measurement is performed, the initial phase angle cannot be adjusted unless another estimation of cell parameters is made, which disrupts the capacitance measurement. However, this technique has been successfully conducted with a reasonable accuracy[5].

2. Error sources

Phase detector technique described above is to continuously monitor the magnitude of sinusoidal current component($| \Delta I(\pi/2+\alpha) |$) having the phase angle, $\pi/2+\alpha$, relative to the sinusoidal command voltage signal followed by

scaling with K(eqs.(11) and (13)) enabling dynamic measurement of membrane capacitance. Since the phase angle, $\pi/2+\alpha$, cannot be adjusted in the middle of the measurement, its value is initially set and kept throughout the measurement. Since the circuit parameters(G,C, and Gs) continuously change during exocytotic process, these changes cause measurement error in estimating ΔC(eq.(13)) as follows.

Although $\pi/2+\alpha$ has been accurately determined orthogonal to ΔI_G to prevent the undesirable effect of changes in membrane conductance(ΔG), the change in access conductance(ΔGs) inevitably affects the measurement(e_{Gs} in eqs.(10 and 12)). When e_{Gs} is not negligible, $|\Delta I(\pi/2+\alpha)|$ is no longer proportional to ΔC and eq.(13) would provide erroneous ΔC value. While ΔGs is caused by access condition of the micropipette electrode and might be minimized by creating a well-conditioned membrane patch, C shows a consistent increase during exocytosis. Appropriate selection of excitation frequency(ω) could make a phase angle difference between ΔI_{Gs} and ΔI_G close to π(eq.(8)), and under such condition, $\theta-\pi/2$ (orthogonal to ΔI_{Gs}) would be approximately parallel to ΔIc. Therefore, selecting the phase angle, $\theta-\pi/2$, instead of $\pi/2+\alpha$ and using the resultant current component, $|\Delta I(\theta-\pi/2)|$, instead of $|\Delta I(\pi/2+\alpha)|$ in eq.(13), would also enable ΔC measurement. When $\theta-\pi/2$ is chosen as an appropriate phase angle, an error(e_G) due to ΔG analogous to e_{Gs} in eq.(10) is resulted, since $\Delta I(\theta-\pi/2)$ is no longer orthogonal to ΔI_G. Selecting $\theta-\pi/2$ as an initial phase angle may provide a better estimate of ΔC if e_G is smaller than e_{Gs}. This possibility has been tested to be true in a model study[10]. However, this is not always true and we present a criterion which phase angle to select through a rigorous analysis in the subsequent section.

While the measurement goes on with either initial phase angle setting, changes in circuit parameters could make the initial value deviate from being orthogonal to ΔI_G or ΔI_{Gs}, resulting in a further measurement error. This type of error, however, is very small and not considered here (explained in dicussion).

The last type of error in ΔC etimation is caused by a change in scaling factor(K in eq. (13)) due to the changes in circuit parameters. This will also be considered in the subsequent section.

3. Phase angle selection criteria

As previously mentioned, an initial phase angle orthogonal to either ΔI_G or ΔI_{Gs} is selected to obtain the current component, the magnitude of which is approximately proportional to ΔC. Conventional phase detector technique selects $\pi/2+\alpha$ orthogonal to ΔI_G, while $\theta-\pi/2$ orthogonal to ΔI_{Gs} has been suggested to be more accurate in a model study[10]. We compare the expected errors of both

methods as follows.

When the phase angle of $\theta-\pi/2$ is selected, the magnitude of the corresponding current component $|\Delta I(\theta-\pi/2)|$ is calculated similarly to eq.(9) from eqs. (4) and (6) as

$$|\Delta I(\theta-\pi/2)| = |\Delta I_G| \cos[\alpha-(\theta-\pi/2)] + |\Delta I_C| \cos[(\pi/2+\alpha)-(\theta-\pi/2)] \quad (14)$$

From eq.(7) and with the condition of $G \ll \omega C$, eq.(14) is approximated to

$$|\Delta I(\theta-\pi/2)| = K \cdot \Delta C(1+e_G) \quad (15)$$

where

$$e_G = \frac{2G^2}{\omega^2 C^2} \cdot \frac{\Delta G/G}{\Delta C/C} \quad (16)$$

Dividing eq.(12) by (16) gives

$$\frac{e_{Gs}}{e_G} = \frac{\omega C/CS}{G/\omega C} \cdot \frac{\Delta Gs/Gs}{\Delta G/G} \quad (17)$$

Since it is reasonable to assume $\Delta Gs/Gs = \Delta G/G$(explained in discussion), eq. (17) is reduced to

$$\frac{e_{Gs}}{e_G} = \frac{\omega C/Gs}{G/\omega C} = \begin{cases} <1 \text{ for } \omega C < \sqrt{GGs} & (18a) \\ >1 \text{ for } \omega C > \sqrt{GGs} & (18b) \end{cases}$$

Eq.(18) provides a criterion which initial phase angle to select, i.e., taking $|\Delta I(\theta-\pi/2)|$ is superior only if $\omega C > \sqrt{GGs}$. Since access conductance is usually much larger than membrane conductance($G \ll Gs$) at a whole-cell mode, and with the aforementioned asumption of $G \ll \omega C$, this criterion is reduced to

$$G \ll \sqrt{GGs} < \omega C \quad (19)$$

Therefore, a high enough excitation frequency of ω has to be chosen to use $\theta-\pi/2$ as a better initial phase angle, otherwise $|\Delta I(\pi/2+\alpha)|$ provides a more accurate

estimate of ΔC. However, too high frequency introduces an error in the scling factor of K in eq.(11), limiting the accuracy of ΔC estimation. This is described below.

4. Scaling factor consideration

Since usually, G≪Gs, eq.(11) is reduced to

$$K = \frac{\omega Vo}{1+(\omega C/Gs)^2} \quad \cdots\cdots(20)$$

When ω is selected to be $\omega C \ll Gs$,

$$K = \omega V_o \quad \cdots\cdots(21)$$

which is independent of the cell parameters. Thus the scaling factor does not introduce any measurement error if $G \ll \omega C \ll Gs$. Combined with the condition of eq.(19), $|\Delta I(\theta-\pi/2)|$ is a better estimate of ΔC only if both $G \ll \omega C \ll Gs$ and $\omega C > \sqrt{GGs}$ are satisfied. Even with $G \ll \omega C \ll Gs$, $|\Delta I(\pi-2+\alpha)|$ is more accurate if $\omega C < \sqrt{GGs}$. In other words, $\omega C = \sqrt{GGs}$ is a border line of which estimate has to be selected or a guide line of an appropriate frequency for either phase angle. When $\omega C \ll Gs$ cannot be satisfied, eq.(13) consistently underestimates ΔC, since capacitance increases with time during exocytosis[9]. Consistent underestimation of ΔC may be minimized by interatively correcting the scaling factor according to the estimated value of capacitance. At each instant, ΔC is estimated and added to the previous value to obtain the current capacitance and to provide the correction for the next scaling factor. When this correction procedure is performed by an analog circuit or as often as possible, the error due to erroneous scaling factor should be minimized(discussed later).

Ⅲ. Discussion

Monitoring membrane capacitance is currently a unique dynamic probe to membrane recycling phenomenon. It is based on a very well defined specific capacitance($1\mu F/cm^2$) of biological membranes independent of cell type or size. Phase detector technique has been a very useful way of measuring capacitance, and as a result, studying exocytosis[5,11-13], since it enables size discrimination of secretory granules as well as dynamic on-line measurement[5,11].

However, this technique is exposed to inherent errors, since the cell under

observation is constantly changing while the measurement procedure cannot be accordingly adjusted. The basic assumption is that both access conductance(Gs) and membrane conductance(G) do not change with time and the scaling factor(K) is insensitive to changes in C, while C is the only variable consistently increasing with time during exocytosis. When these assumptions are approximately met, dynamic monitoring of membrane capacitance is possible.

Constancy of Gs and G depends on access patch environment and ionic channel characteristics, respectively. Once measurement condition is established, some changes in both variables are inevitable. The resultant errors are directly reflected to the proportionality of the monitored current components with ΔC to different degrees (eqs.(10,12,15, and 16)). Therefore, appropriate phase angle selection is of primary importance. Joshi and Fernandez[10] noted the fact that $\theta-\alpha$ gets close to π when ω is selected such that $G \ll \omega C$. They maintained that selecting the phase angle orthogonal to ΔI_{Gs} instead of ΔI_G in current component calculation may be more accurate as long as ωC is limited to meet $\omega C \ll Gs$, which is a necessary condition for a constant scaling factor(K). However, their conclusion was based on a typical model experiment and different conclusion could be drawn depending on different combinations of cell parameters. We reviewed the phase detector technique and proved that the necessary condition of $G \ll \omega C \ll Gs$ does not guarantee the superiority of the phase angle of $\theta-\pi/2$. Our analysis demonstrates that another condition should be added to select $\theta-\pi/2$, which is $\omega C > \sqrt{GGs}$, otherwise $\pi/2+\alpha$ used in conventional phase sensitive detector technique is more accurate(eq.(18)). $\omega C=\sqrt{GGs}$ is a deflection point, determining a better estmate of ΔC. It is interesting that $\omega C=\sqrt{GGs}$ is the geometric mean of G and Gs. We think that it is a reasonable result, since G and Gs are theroretically possible lower and upper -limits of ωC, respectively. In the evaluation of this criterion of $\omega C=\sqrt{GGs}$, we assumed that the relative changes of both G and Gs are equal to each other(eqs.(17 and 18)). This is the most reasonable assumption, since G and Gs should be maintained constant in an ideal preparation, and since actual changes of these parameters are impossible to predict. Therefore, we consider our criterion valid. When ΔC is to be monitored by phase detector technique during exocytosis, the initial phase angle on the phase-locking amplifier should be set at $\theta-\pi/2$ if ω can be selected to satisfy both $G \ll \omega C \ll Gs$ and $\omega C > \sqrt{GGs}$, otherwise $\pi/2+\alpha$ has to be chosen as usual.

Although the initial phase angle has been appropriately and accurately determined for current component calculation as described above, the consistent increase in C expected during exocytosis affects α and/or θ in eq.(7) while the phase angle ($\pi/2+\alpha$ or $\theta-\pi/2$) cannot be adjusted from the initial value over the measurement period. And this might introduce an error.

During exocytosis, the change in C should be the primary error source, since C

is the only parameter in consistent change. However, a change in $\omega C/Gs$ from 0 to 0.4, which is considered a fairly large value, error in α is expected to be approximately 6^0 to a 15% change in C. The upper bound of an error in the calculation of the current component magnitude becomes approximately 10% (see Appendix). In other words, the technique itself is insensitive to the phase angle change once an appropriate initial phase angle is selected as previously described. Therefore, we do not consider this one of the major error sources.

Despite the above discussion, a progressive increase in $\omega C/Gs$ during exocytosis could result in an error in the scaling factor K in eq.(11), thus in capacitance estimation by eq.(13). This can occur when $\omega C \ll Gs$ cannot be satisfied. Since K is given by eq.(20) under a usual patch condition($G \ll Gs$), an increase of 0.5 in $\omega C/Gs$ during a progressive exocytosis would result in an approximately 20% error in capacitance estimation($1/(1+0.5^2)=0.8=20\%$ error), which is a significant underestimation. This may be compensated by iteratively correcting K as

$$K_n = \frac{\omega V_0}{1+(\omega Cn - 1/Gs)^2} \quad \cdots\cdots(22)$$

where C_{n-1} is the previous capacitance measurement($C_n = C_{n-1} + \Delta C_n$) and K_n is the scaling factor to currently apply. This was tested as follows. In a rat mast

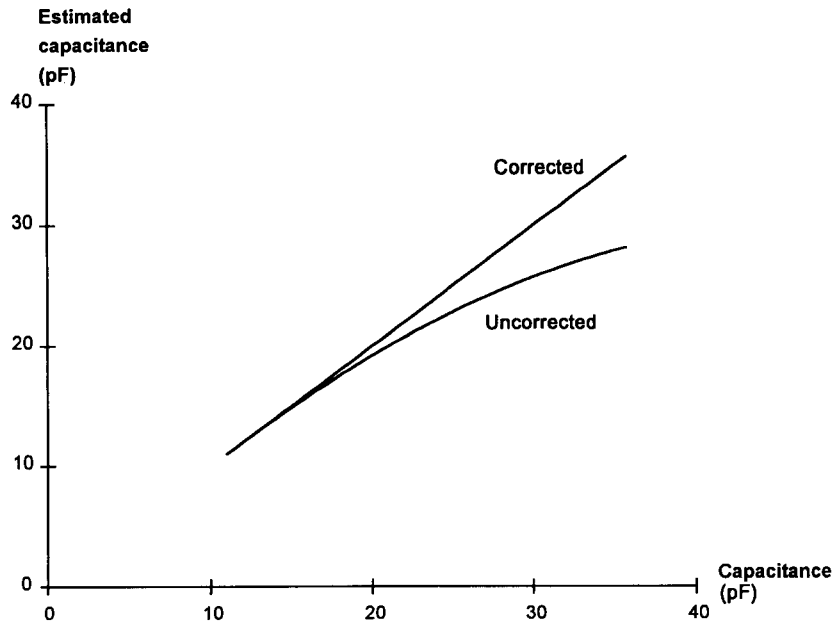

Figure 3. Underestmation of capacitance(C) with a constant scaling factor($K=K_0$) for a large capacitance change observed in the rat mast cell. When K was corrected at each steps of 250 fF, estimated change in C was indistinguishable with true C change.

cell, the cell parameters have been measured before and after exocytosis: $G_s=2.5x10^{-7}S$, $G=1x10^{-9}S$(before and after), C=11 pF(before), and C=36 pF(after) by Joshi and Fernandez[10]. C before exocytosis was taken as an initial estimate of C and was increased in successive steps of 250 fF. The resultant $|\Delta I(\pi/2+\alpha)|$ was calculated by eqs.(1,2, and 7a) and ΔC was calculated by eqs.(11 and 13) with and without the correction procedure of eq.(22). While this correction procedure at each step caused a negligible error in C, approximately 23% underestimation of C was resulted at the final value(after exocytosis) when K was kept at the initial value as shown in Fig.3. This is an obvious result, since the correction in K has been made at every calculation steps. In a practical situation, analog compensation feedback circuitry would be able to accomplish this in corporation with the phase detector set-up.

Ⅳ. Summary

Phase detector technique was reviewed to develope application criteria in the measurement of membrane capacitance during exocytosis. Several error sources were considered in the analysis and practical ways to minimize measurement error were presented. It was found that the excitation frequency and initial phase angle have to be carefully selected depending on the access and membrane conductances and the initial membrane capacitance of a whole-cell patch clamp mode. Since membrane capacitance increases during exocytosis, a method to correct the expected error in scaling factor was suggested. Since this technique is currently in a wide use to study exocytosis, the present study will provide a useful guideline in practical application of this technique.

Ⅴ. Appendix

To estimate an error due to the phase angle change as a result of capacitance increase during exocytosis, let us assume that the initial phase angle($\pi/2+\alpha$) was accurately selected and a change of $\Delta\alpha$ has been resulted from the increase in capacitance, ΔC. From eq.(7a),

$$\alpha = -2tan^{-1}\frac{\omega C}{G+Gs} = -2tan^{-1}\frac{\omega C}{Gs} \quad \cdots\cdots (A1)$$

assuming $G \ll Gs$, which is usual under a normal patch-clamp environment. For an increase in capacitance by ΔC, the corresponding change in α is calculated as

$$\Delta\alpha = \frac{\partial \alpha}{\partial C} \cdot \Delta C = -2 \cdot \frac{\omega C/Gs}{1+(\omega C/Gs)^2} \cdot \frac{\Delta C}{C} \quad \cdots\cdots (A2)$$

When the current, ΔI, has been measured, the magnitude of the component to the direction of $\pi/2+\alpha$ (parallel to ΔI_c) is

$$\begin{aligned} f(\alpha) &= |\Delta I(\pi/2+\alpha)| = |\Delta I| \cos[\arg(\Delta I) - (\pi/2+\alpha)] \\ &= |\Delta I| \sin[\arg(\Delta I) - \alpha] \quad \cdots\cdots (A3) \end{aligned}$$

When $\arg(\Delta I)$ represents the phase angle of ΔI relative to the excitation voltage. When α has been changed to $\alpha+\Delta\alpha$,

$$\frac{f(\alpha+\Delta\alpha)}{f(\alpha)} = \frac{\sin[\arg(\Delta I)-(\alpha+\Delta\alpha)]}{\sin[\arg(\Delta I)-\alpha]} \quad \cdots (A4)$$

For a small change of $\Delta\alpha$, eq.(A4) is reduced to

$$\frac{f(\alpha+\Delta\alpha)}{f(\alpha)} = 1 - \frac{\Delta\alpha}{\tan[\arg(\Delta I)-\alpha]} \quad \cdots\cdots (A5)$$

Since $\theta-\alpha$ in eq.(8) is less than π, $\arg(\Delta I)$ is less than $\pi/2+\alpha$(see Fig.1) and also would be approximately parallel to ΔI_c for a ususal range of $G<\omega C<Gs$.

Therfore, it can be assumed

$$\pi/4+\alpha < \arg(\Delta I) < \pi/2+\alpha \quad \cdots\cdots (A6)$$

and

$$\tan[\arg(\Delta I)-\alpha] > 1 \quad \cdots\cdots (A7)$$

From eqs.(A2,A5,A6, and A7), the relative error of $f(\alpha)$ is

$$\left|\frac{f(\alpha+\Delta\alpha)-f(\alpha)}{f(\alpha)}\right| < |\Delta\alpha| \cdot 2 \cdot \frac{\omega C/Gs}{1+(\omega C/Gs)^2} \cdot \left|\frac{\Delta C}{C}\right| \quad \cdots\cdots (A8)$$

Eq.(A8) sets the upper bound of the error in currnet component calculation due to the phase angle deviation resulting from capacitance increase during

exocytosis. For a very large value of $\omega C/Gs=0.4$ and 15% change in ΔC, this upper bound is approximately .10%. And the corresponding deviation of the phase angle from the initial value is only 6^0.

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세포외 분비시 막 캐패시턴스를 측정하기 위한 위상감지법(phase detector technique)의 이론적 분석.

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위상감지법(phase detector technique)은 세포의 막 캐패시턴스(membrane capacitance)를 실시간적으로 측정할 수 있는 유일한 방법이나 측정이 행해지는 동안 세포의 상태가 끊임없이 변화하기 때문에 피할 수 없는 측정오차가 존재한다. 본 연구는 이 오차의 근원을 분석하여 위상감지법의 실용한계를 규정하고자 하였다. 이론적 분석에 기초하여 다음과 같은 사실을 밝힐 수 있었다. 1) access conductance와 membrane conductance의 변화에 기인하는 측정오차를 줄이기 위해서는 초기 위상치를 올바로 선택하여야 한다. 2) 이 때 세포를 여기시키기 위해 인가하는 전압의 주파수를 알맞게 선택하여야 한다. 3) 그러나 초기 위상치가 정해진 이후의 위상 변화는 막 캐패시턴스의 측정에 큰 영향을 미치지 않는다. 4) 초기 위상을 적절히 선택하였다 하더라도 세포외 분비시 막 캐패시턴스가 크게 증가하는 경우에는 비례상수에 오차가 발생한다. 이 때 발생하는 오차는 측정기간 동안 비례상수를 되풀이하여(iteration) 보정함으로써 방지할 수 있다. 이상의 결과는 향후 위상감지법을 사용할 때 유용한 실용한계를 제공하리라 생각된다.

찾아보기 낱말:위상감지법(phase detector technique), Patch-clamp technique, Whole-cell mode, 막 캐패시턴스(membrane capacitance).