

Effects of pipette geometry on the time course of solution change in patch clamp experiments

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ABSTRACT The time course of change in current through K_{ATP} channels in inside-out membrane patches, after step change of permeant ion (K^+) concentration, was measured. A simple model of the patch as a membrane disc at the base of a cone with the apex removed, was able to describe the time course of channel activity after step change of $[K^+]$. By measuring pipette geometry and using jumps of [permeant ion], it was then possible to estimate the time course of concentration at the membrane for jumps of any other ion or gating ligand. A simple channel block mechanism was used to simulate experiments with concentration jumps of a blocking ligand. The rate constants for ligand-channel interaction were extracted by least-squares fitting of computed mass action responses to those observed in simulated experiments. The simulations showed that even with diffusion delays of hundreds of milliseconds (as may occur in inside-out patch experiments), ligand association and dissociation rates of up to $1,000\text{ s}^{-1}$ could be accurately extracted by this approach. The approach should be generally applicable to the analysis of ligand concentration jump experiments on any ion channel whose activity is modulated by intracellular ligand.

INTRODUCTION

The excised patch clamp technique (Hamill et al., 1981) allows measurement of the ionic current flowing through single ion channels in a small patch of cell membrane. The effect of ionic substitutions and ligand modulation of channel behavior can be studied by changing the solution surrounding the patch pipette. Methods for changing the solution at the tip of the pipette involve either moving the pipette into the new solution or switching the solution flowing past the pipette. The second method does not usually allow determination of the time of solution change at the pipette tip and is therefore more suitable for examination of the steady-state effects of the solution change. When the pipette is physically moved into a new solution, the exact time at which the pipette meets the new solution may also be unknown, but the speed at which the solution changes at the membrane is probably greater.

The uncertainties in the exact timing of the solution change appear to have been removed by the "oil gate" technique, developed by Qin and Noma (1988). In this technique, the different bathing solutions are separated by a partition and the pipette is moved through the partition into the new solution via a small gap which is filled with oil. The oil prevents the different bathing solutions from mixing and, because of the high resistance of the oil, causes a current artifact on the patch clamp record when the pipette meets the new solution. However, the patch of membrane in the tip of the pipette may be some distance from the orifice of the

pipette (Sokabe and Sachs, 1990) and the time course of the solution change within the pipette itself will be governed by diffusion.

To examine the possible delays associated with the diffusion of a new solute into the pipette, we have analyzed the effects of pipette geometry on the time course of solution change at the patch of membrane within the pipette. Our data suggests that the time course of solution change at the membrane may seriously complicate interpretation of the kinetics of the response of single channels to changes in modulator concentration and suggest that the results of such experiments be interpreted with caution. We present an approach which may be used to overcome some of the kinetic limitations associated with this method. A preliminary presentation of these findings has been made to the Biophysical Society (Cannell and Nichols, 1991).

METHODS

Experimental

Single ventricular myocytes were enzymatically dissociated from adult rat hearts (see Lederer and Nichols, 1989). Experiments were performed at room temperature, in an oil-gate chamber based on the design of Qin and Noma (1988), described in Lederer and Nichols (1989). Microelectrodes (2–6 $M\Omega$) were pulled from borosilicate glass (1.5-mm o.d. No. 1B150F-6, WPI Inc., New Haven, CT) on a horizontal puller (BB-CH Mechanex, Geneva, Switzerland). Micropipettes were sealed onto cells by applying light suction to the rear of the pipette. This typically resulted in a seal resistance of $>10\text{ G}\Omega$.

Inside-out patches were obtained by lifting the electrode and then passing the electrode tip through the oil-gate.

The pipette (extracellular) solution used in these experiments had the following composition (mM): NaCl 140, KCl 4, CaCl₂ 1, Na-Hepes 5; pH 7.2. The bath (intracellular) solution contained (mM): Cl⁻ 140, HEPES 10, EGTA 1; pH 7.25. Total Na⁺ plus K⁺ was 140 mM.

Patch-clamp currents were measured using a Dagan Model 8900 Patch Clamp (Dagan Corp. Minneapolis, MN) with a 10-GΩ head-stage, and filtered at 1 kHz. Signals were digitized at 22 kHz (Neurocorder, Neurodata, NY, NY) and stored on video tape. Experiments were replayed through a 1-kHz 8-pole Bessel filter and redigitized at >10 kHz by a microcomputer (Compaq Computer Corp., Houston, TX; Data Translation, Marlborough, MA) using custom-written software.

Numerical simulation

The geometry of the pipette was modeled as a right cone (half angle Θ) with the apex removed (see Fig. 1). The patch of membrane within the pipette formed a reflective barrier for diffusion at a distance L_{PIP} from the pipette tip whose radius was R_{TIP} . We are not aware of any analytical solution to the diffusion equation describing this geometry which does not require numerical integration so a purely numerical approach was adopted. The length of the pipette between the tip and the membrane was split into n elements of thickness dx . The flux of solute (F) across the boundary between two elements was given by

$$F = D \cdot \pi \cdot (R_{TIP} + [(L_{PIP} - x_i) \tan \{\Theta\}])^2 (C_i - C_{i+1}) / dx, \quad (1)$$

where C_i is the concentration of solute in the i^{th} element, x_i the position of that element from the patch, and D the diffusion coefficient. The model was tested by simulating diffusion in a cylinder (Crank, 1975). The tests indicated that large numbers of elements would be needed if the element thickness dx were constant. This would increase the time needed to compute the solution, as well as creating stability problems when attempting to fit experimental records to derive L_{PIP} (because the size of elements could vary over a large range during the fitting process). By placing more elements near the tip of the pipette (where dC/dx is largest immediately after a solution change) the numerical solution to the diffusion equation could achieve the desired accuracy with fewer elements. Making dx linear in i^2 reduced the number of elements needed, but an even more rapid convergence was achieved by making dx linear in $i^{1.75}$. This nonlinear discretization helped reduce errors in the solution, as well as achieving stability for $n = 50$ when L_{PIP} was varied from 2 to 40 μm . Because the volume of the elements

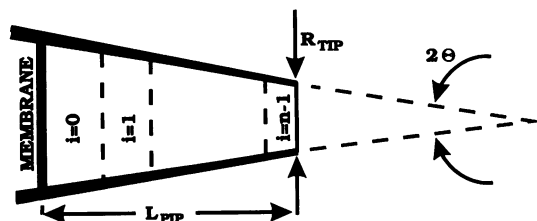


FIGURE 1 The geometry of the pipette was modeled as a right cone (half angle Θ) with the apex removed. The membrane patch within the pipette formed a reflective barrier for diffusion at a distance L_{PIP} from the pipette tip whose radius was R_{TIP} . The length of the pipette between the tip and the membrane was split into n elements of thickness dx .

changed with position along the pipette, the net flux into each element (across both its boundaries) was calculated for all elements before being divided by the volume of the element to give dC_i/dt .

An 80386/80387 microcomputer was used to run a program for the solution of stiff differential equations (FACSIMILE, Chance et al., 1977) which integrated the equations describing dC_i/dt . Starting integration, step sizes were of the order of 10^7 ms and after a change in solute concentration at the tip of the pipette, the integrator required ~ 300 steps before a new steady state was achieved. Mass conservation was achieved to better than 1 part in 10^6 , indicating that truncation errors in the system used were negligible.

Consideration of the dimensions of typical patch pipettes and the time scale over which diffusion gradients existed (see results) suggested that the results be presented in units of μm and ms. Time-course graphs are presented in units of ms for a diffusion coefficient of $10^5 \text{ cm}^2/\text{s}$ (the rate at which calcium diffuses in free solution) and may be used to derive the time taken for diffusion of other solutes by multiplying the time axis by $10^{-5} (\text{cm}^2/\text{s})/D_{\text{solute}}$.

Single Channel Simulation

To simulate single-channel records, a simple ion blocking scheme was used:



where C is the closed state, O the open state and B a blocked state (state C was included to make the model more realistic, by ensuring open state noise in the absence of blocker). k_{BO} , the unblocking rate was varied between 10 and $1,000 \text{ s}^{-1}$ and k_{OB} was varied similarly to keep the blocker dissociation constant at 20 μM . Single-channel records were simulated by solving the equation:

$$R = \int_0^{T_c} \exp(-k) \cdot dt, \quad (3)$$

where R was a random number between 0 and 1, T_c the time of the next transition to a new state and k the sum of the rate constants leaving the current state. A computer program was written in Turbo C (Borland International Inc., CA) to solve this equation and generate a sequence of channel states and transition times. The resulting single-channel records were digitally filtered at 500 Hz with a conventional FIR method.

RESULTS

Fig. 2A illustrates the time course of change in current through ATP-sensitive potassium channels (Noma, 1983) in the absence of ATP, when the electrode was moved from a solution containing 140 mM K⁺ to a solution containing 4 mM K⁺ (Na⁺ substituted), through an oil gate. On entering the oil, patch current falls to zero due to the high resistance of the oil. The emergence of the electrode tip into the 4 mM K⁺ solution results in an electrical artifact that provides an accurate (<1 ms) time mark for exit from the oil. The patch current decreases due to the change in electrochemical driving

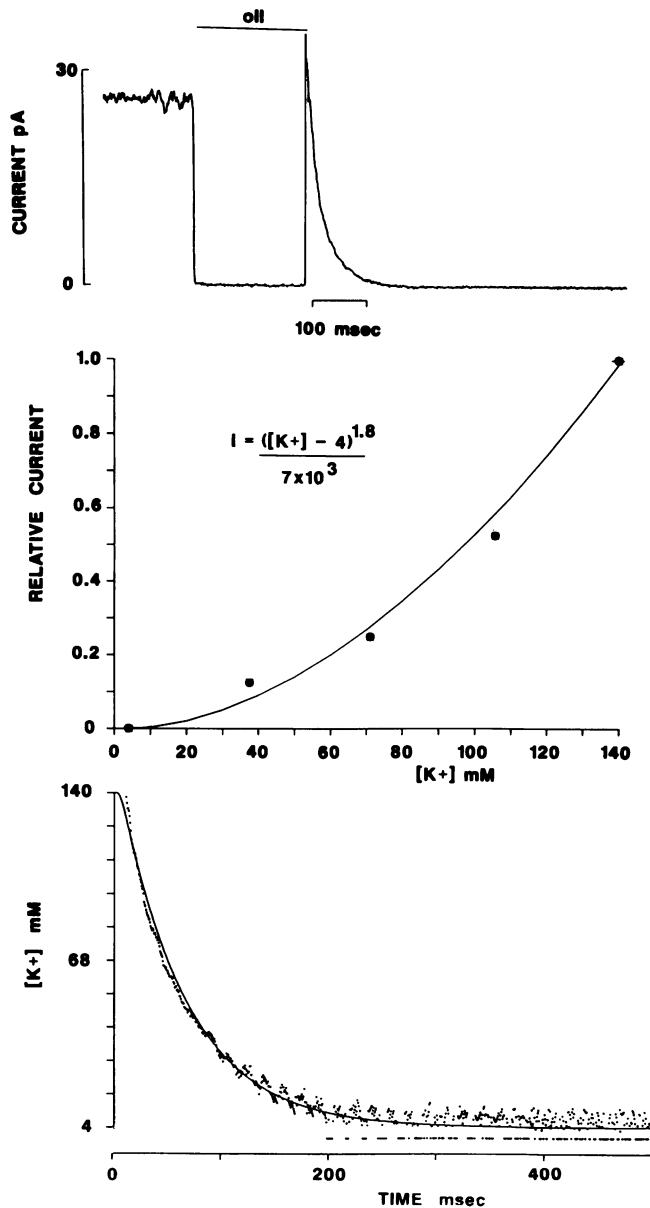


FIGURE 2 The time course of diffusion into the pipette tip. (A) The time course of change in current through ATP-sensitive potassium channels (in zero ATP) when the electrode was moved from 140 mM K^+ to 4 mM K^+ (Na^+ substituted), through an oil gate (oil). (B) Measured steady-state $[K^+]$ -dependence of patch current (I -normalized to maximum current observed in 140 mM $[K^+]$). Circles show means of 3–4 experiments. Solid line is fit to empirical relationship shown. (C) Estimated $[K^+]$ at the patch (dots), as a function of time, using empirical relationship derived in (B). Pipette tip radius (R_{TIP}) and cone angle (Θ) were measured optically with a microscope and eyepiece graticule after the experiment. The distance between the pipette tip and the membrane was the only free parameter and was varied in the model (Fig. 1). The model (smooth curve) accurately reproduced the observed time course of change in $[K^+]$ (dots), when L_{PIP} was set to 9.8 μm .

force. However, patch current does not instantaneously attain the final level, but decreases approximately exponentially. Similarly, on switching back to the high K^+ solution, the patch current increases approximately exponentially to the final level. These changes in current reflect changes in the electrochemical driving force across the membrane patch ($E_m - E_k$) and the $[K^+]$ -dependence of single-channel conductance. Knowing the dependence of channel current on $[K^+]$ would permit the time course of change of $[K^+]$ to be inferred from the time course of change of patch current. Fig. 2 B shows the measured steady-state $[K^+]$ dependence of patch current. Using the empirical relationship shown in Fig. 2 B, Fig. 2 C shows the calculated $[K^+]$ at the patch, as a function of time. It is clear that the $[K^+]$ across the membrane patch does not change instantaneously, despite the rapidity of solution change at the tip of the pipette (Qin and Noma, 1988) and this is due to the patch of membrane being some distance from the pipette tip (Hamill et al., 1981; Sokabe and Sachs, 1990). This diffusion-limited change in patch current was simulated with the model described in the methods. The pipette tip radius (R_{TIP}) and cone angle (Θ) were measured optically with a microscope and eyepiece graticule after the experiment. With the constraints imposed by measurement of R_{TIP} and Θ , the distance between the pipette tip and the membrane was the only free parameter. As shown by the smooth curve in Fig. 2 C, the model accurately reproduced the observed time course of change in K^+ , when L_{PIP} was set to 9.8 μm . In eight similar experiments the best estimate of L_{PIP} ranged between 3.2 and 23.0 μm , in good agreement with the range of values measured optically (Sokabe and Sachs, 1990; Sakmann and Neher, 1983).

Time course of solution change: effects of pipette geometry

The simulated time course of solution change (Fig. 2 C) is not a simple exponential but can reasonably be approximated by an exponential decline after a delay. This delay increases with increasing L_{PIP} , and is due to a nonzero pipette cone angle (Θ) coupled with a finite tip radius. However, the time course of solution change becomes approximately exponential once the concentration has reached 50% of its final value.

Fig. 3, A and B show the effect of varying L_{PIP} , Θ and R_{TIP} on the time taken for 50 and 90% of the solution change to occur respectively (for $D = 10^5 \text{ cm}^2 \text{ s}^{-1}$). The pipette dimensions illustrated in these simulations cover the majority of tip radii and cone angles that are likely to be pulled by standard micropipette pullers from borosilicate glass (Sakmann and Neher, 1983). As is to be expected, increasing the distance between the mem-

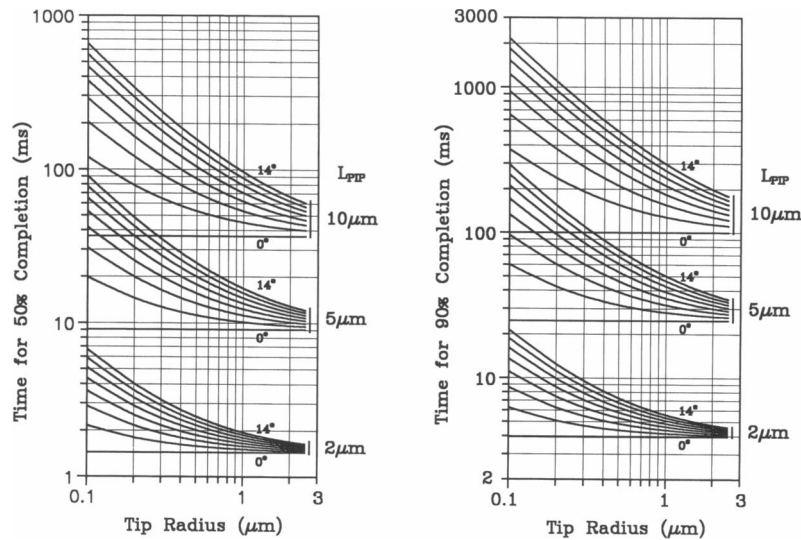


FIGURE 3 The effect of L_{PIP} , R_{TIP} and Θ on diffusion time. (A and B) The effect of varying L_{PIP} , Θ and R_{TIP} on the time taken for 50% (A) and 90% (B) of solution change to occur (for $D = 10^{-5} \text{ cm}^2\text{s}^{-1}$, diffusion coefficient of calcium).

brane and the tip of the pipette increases the time taken for the solution change to occur. When Θ is ϕ° , the pipette is a cylinder and the tip radius (R_{TIP}) has no effect on the time taken for the solution change and the solution change is 90% complete in $\sim (L_{PIP})^2$ ms (L_{PIP} in μm). When Θ is 14° , decreasing the tip radius from 2.0 to 0.2 μm causes an approximately three-fold increase in the time taken for 90% completion of the solution change. At constant Θ (14°) and L_{PIP} (10 μm), varying the tip radius over the same range (2.0 to 0.2 μm) now results in an approximately five-fold increase in diffusion time. It is apparent that the time taken for the solution change to occur is highly dependent on the geometry of the pipette and for reasonable ranges of L_{PIP} and R_{TIP} can vary by more than two orders of magnitude. It should be noted that L_{PIP} may be larger than 10 μm in some experiments (Sokabe and Sachs, 1990) which will result in solution changes taking several seconds to attain 90% completion. However, the time taken for the solution change will be minimized by using pipettes with large R_{TIP} and small Θ .

Correcting for diffusion delays

Diffusion delays of >1 ms will occur for most substances, even when the patch of membrane is formed only a few microns from the pipette tip. The situation is further complicated by the fact that the solution to the diffusion equation is not a simple exponential (except at late times) for typical pipette geometries. However, as illustrated in Fig. 2 it is possible to simulate the time course of solution change at the membrane and thereby

derive the effective distance of the membrane from the pipette tip. If R_{TIP} and Θ can be measured optically before or after an experiment, then estimating the time course of the concentration change of permeant ions¹ in response to a step change in concentration at the tip of the pipette can provide a convenient method to determine the effective L_{PIP} . Once this is done, the time course of concentration change of any other modulator of channel activity can be predicted by multiplying the time axis of observed activity changes by the factor $D_{\text{permeant ion}}/D_{\text{modulator}}$ (because the rate of diffusion is directly proportional to the diffusion coefficient).

The measured time course of change in channel activity in response to a step change of [modulator] *at the tip of the pipette* arises from the convolution of the time course of the change in [modulator] *at the membrane* with the kinetics of modulator-channel interaction (a function of membrane [modulator] and time). Because permeant ion concentration jumps can be used to predict $d[\text{modulator}]/dt$ at the membrane, it may be possible to deconvolve the observed channel response to modulators and derive the kinetics of modulator-channel interaction. Given a model for the interaction of a channel with a diffusing modulator, then it is straightforward to add the relevant equations to those describing the diffusion of the modulator and obtain the time course of channel response. The rate constants describing the interaction of the channel with the modulator

¹The validity of this approach is based on the assumption that, in the time-scale with which we are concerned (milliseconds), ion-channel flux is an instantaneous function of driving force.

can then be altered to minimize the difference between the observed and simulated channel response and thereby obtain best estimates of the model rate constants.

However, the ability of this simulation method to extract the rate constants associated with the reaction of the modulator and the channel will be limited by the stochastic noise associated with channel activity. To examine these points further, a simple ion channel blocking model was used (see methods).

The predicted time course of response (P_0) of the channel to a step change in concentration of blocker for the model in Fig. 4 is rapid (*dotted line*). However, with the membrane patch situated $5 \mu\text{m}$ from the tip of the patch pipette ($\Theta = 10^\circ$, $R_{\text{TP}} = 0.5 \mu\text{m}$), the time course of the change in channel activity is considerably slower. The delay associated with diffusion of the blocker into the patch pipette results in the response of the channel changing from an exponential to a sigmoidal time

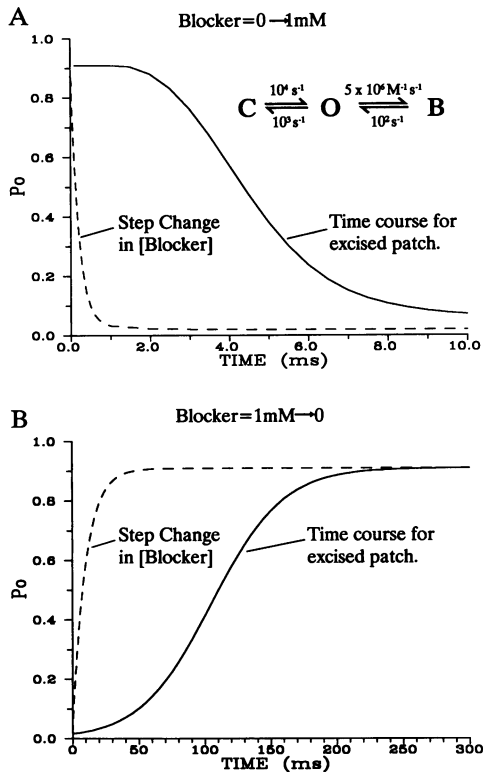


FIGURE 4 The time course of channel current following [blocker] jumps. The predicted time course of response (P_0) of the channel to a step change in concentration of blocker is quite rapid (*dotted line*). However, with the membrane patch situated $5 \mu\text{m}$ from the tip of the patch pipette ($\Theta = 10^\circ$, $R_{\text{TP}} = 0.5 \mu\text{m}$), the time course of the change in channel activity is considerably slower (*solid line*). The delay associated with diffusion of the blocker into the patch pipette results in the response of the channel changing from an approximately exponential to a pseudosigmoidal time course.

course. It is clear that the relation between the observed time course of channel response and that expected from the kinetics of channel interaction with the blocker alone is complex.

Fig. 4 shows responses calculated from mass action (i.e., an infinite number of channels), and represents ideal records from which to deconvolve the channel kinetics. However, the limited number of channels in a patch (or number of times the response can be averaged) will introduce stochastic noise that will decrease the ability to extract the rate constants of channel interaction with the blocker. Fig. 5 shows simulated records of channel activity for 1, 10, and 100 channels (the records have been filtered at 500 Hz). The time course of the response calculated from mass action is shown as the smooth line in each plot, and only a few channel transitions occur during the change in open probability from 0.1 to 0.9. However after averaging 100 records, the time course of channel open probability becomes clearer. Averaging 100 records results in a further reduction in stochastic noise and there is close

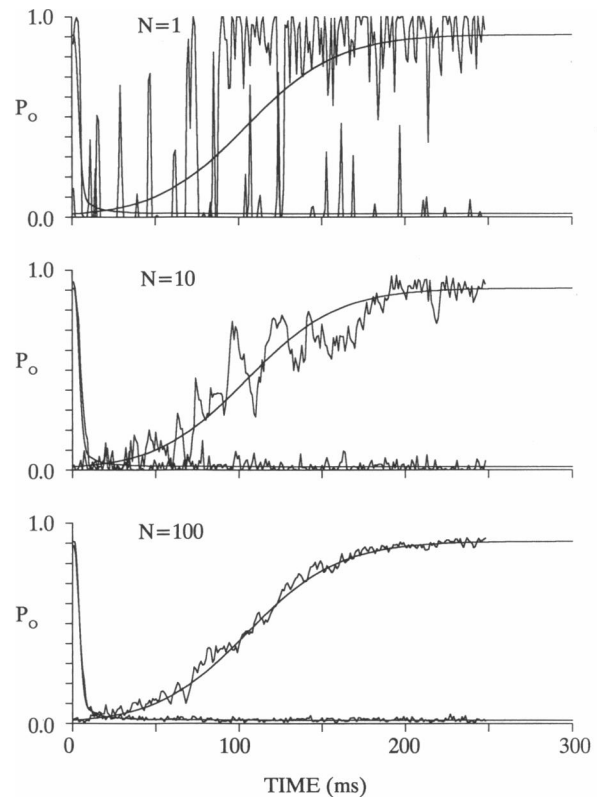


FIGURE 5 The effects of stochastic noise introduced by the limitation of a finite number of channel in a patch. Simulated records of channel activity for 1, 10, and 100 channels (the records have been filtered at 1 kHz). The time course of response calculated from mass action is shown as the smooth lines in each plot.

agreement between the mass action response and the observed time course of channel open probability.

The ability to extract rate constants from such data was examined by performing 10 independent simulations with either 20, 50, or 100 channels in the patch (or equivalent number of averages of single channel records) with rate constants for channel unblock of 10 s^{-1} , 100 s^{-1} , and $1,000 \text{ s}^{-1}$. The blocking rate constant was altered similarly to keep the binding constant for the blocker the same ($5 \times 10^4 \text{ M}^{-1}$). Rate constants were estimated by performing a least squares fit of the computed mass action response to the simulated patch records. As shown in Fig. 6, good estimates of the rate constant for channel unblock were obtained until the off-rate constant approached $1,000 \text{ s}^{-1}$, when a systematic overestimate developed, resulting in an overestimate of the unblock rate constant. As might be expected, an increase in the number of channel records averaged results in a reduction in the error of the estimate of the unblock rate constant. In contrast to the development of systematic errors in the estimation of the rate constants, systematic errors in the estimate of the binding constant of the blocker for the channel did not occur and the

binding constant was well estimated for all block and unblock rate constants examined (data not shown).

DISCUSSION

This manuscript deals with two conceptually different, though related, questions: (a) How rapidly can solution changes be achieved in excised patch-clamp experiments? (b) Is it possible to correct for the effects of diffusion delays associated with solution changes in the analysis of patch-clamp experiments? We have shown that a considerable diffusion delay can exist in inside-out patch experiments that will confound simple extraction of reaction rate constants. In fact, for most geometries likely to be experienced during excised patch clamp experiments, between 10 and 100 ms will be required for 90% of the solution change to be completed. It should be pointed out that in outside-out configurations, the membrane does not appear to be recessed into the electrode, and diffusion delays should not then be determined by patch geometry.

For inside-out patch experiments, change at the

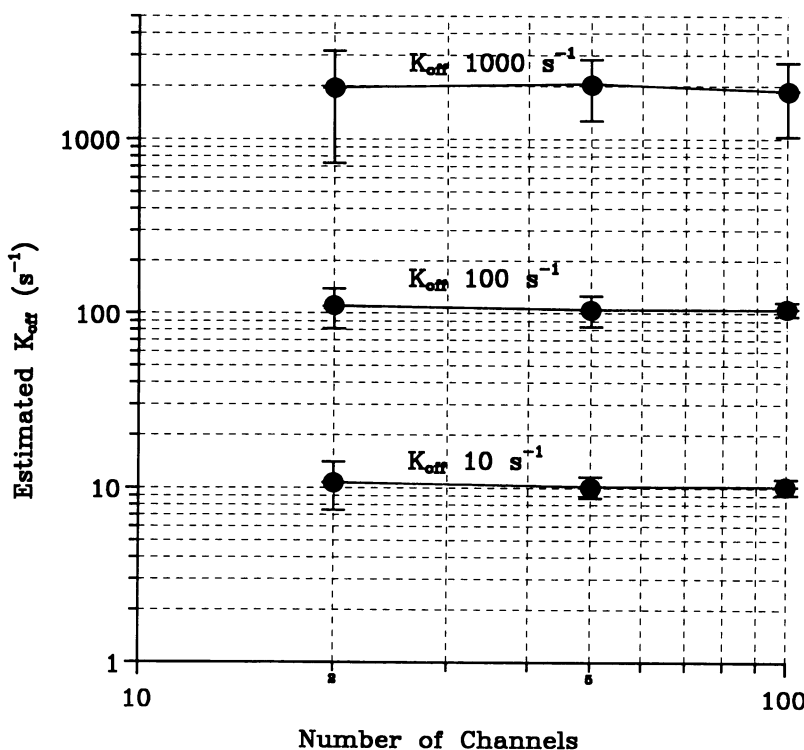


FIGURE 6 Errors in the estimation of rate constants. Estimated k_{off} versus number of channels in the patch for three different true k_{off} values as shown. The blocking rate constant was altered similarly to keep the binding constant for the blocker the same ($5 \times 10^4 \text{ M}^{-1}$). Rate constants were estimated by performing a least-squares fit of the computed mass action response to the simulated patch records. Symbols show means \pm S.E. for $n = 10$ in each case.

membrane is likely to be at least an order of magnitude slower than could be achieved in, for example, a stopped-flow machine, despite the rapidity with which the solution at the tip of the pipette may be changed. The results argue that caution needs to be exercised in interpreting the time course of channel activity during solution changes.

Nevertheless, it is possible to estimate the time course of solution change at the membrane by using jumps in the concentration of permeant ions¹. We have shown that these data can then be used to estimate the effective distance of the patch from the pipette tip and model the time course of solution change at the patch. With such a description of the time course of solution change it is possible to simulate changes in channel activity in response to the changing concentration of channel modulator and thereby extract rate constants for the interaction of the modulator with the channel (assuming a suitable model) by fitting simulated records to the observed channel behavior. The approach we have adopted is not as numerically intensive as the method recently described by Magleby and Weiss (1990) which performs maximum likelihood fits of individual single-channel records. Such a method could also be used to fit kinetic models to changes in modulator concentration, although it is not clear whether the precision of the fitting procedure will be improved by using simulated single-channel records rather than the mass action response of the channel.

Advantages of the simulation method

The rate constants associated with ion channel activity can be estimated by measuring the activity of single channels and fitting exponentials to dwell time distributions (Colquhoun and Hawkes, 1983). This approach requires quite large numbers of transitions and long periods of steady-state recording may be needed. For example, the mean burst duration for the simulation in Fig. 5, with an unblocking rate of 100 s^{-1} at $20 \text{ } \mu\text{M}$ [blocker] (i.e., where half maximal block would occur) would be 1 s. Several minutes of uninterrupted recording would therefore be required to obtain a large enough number of bursts (100–1,000) for estimation of the model rate constants. With the simulation method presented here, an estimate of the rate constant can be obtained in a few seconds, although for rapid rate constants (i.e., when $k_{\text{unblock}} > 1,000 \text{ s}^{-1}$) examination of steady-state dwell-time distributions may be a superior method.

During a solution change, the channel is exposed to a wide range of modulator concentrations and this enables rapid determination of the binding constant of the

channel for the modulator. By comparison, estimation of the binding constant from steady-state single-channel records may require long periods of recording at several different modulator concentrations.

Finally, this method may provide insight into non-steady state channel behavior that is not provided by examination of steady-state dwell-time distributions. For example, suppose a modulator activates the channel and then causes a slower inhibition of the channel. Steady-state analysis may not resolve the initial interaction of the modulator without the complication of slow inactivation. By analogy with the utility of pulse protocols in voltage clamp experiments, the measurement of the responses of ion channels to rapid change of [modulator] may allow determination of the rate constants for both activation and inactivation. In addition, the oil-gate/simulation method may help to circumvent the problems of channel run down or slow irreversible modification of channel behavior that may obviate long recordings necessary for dwell-time histogram analysis.

Limitations in the estimation of model rate constants

The simulation method extracts rate constants for the interaction of the blocker with the channel by deconvolving the observed time course of channel activity and the estimated time course of [modulator] following a step change of [modulator] at the tip of the pipette. As shown in Fig. 6, a systematic error in the determination of the rate constants is observed when the unblocking rate constant was $1,000 \text{ s}^{-1}$. This error results from the effects of stochastic noise on the fitting process. If the rate constants describing the interaction of the modulator with the channel were infinite, then channel activity would be described by the steady-state dependence on [modulator] (M), as M changes. For finite rate constants, the observed time course of channel activity must lag behind the activity that would be predicted by the steady-state dependence of activity on M ($f[M]$). Determination of the rate constants is effectively dependent on detection of the time lag in experimental records. The stochastic noise in records of channel activity will sometimes result in the observed activity temporally preceding $f[M]$. This situation becomes more likely as the rate of channel association with the modulator increases and the time lag decreases. During the fitting process, the computer is unable to reproduce a time course of channel activity which ever leads $f[M]$ because this would require rate constants larger than infinite. Hence, there is a discontinuity in the parameter space which results in an overestimate of the rate constants as the values of the parameters approach the discontinuity.

Despite this problem, the simulation method seems well able to estimate the channel unbinding rate constant for values up to $1,000\text{ s}^{-1}$. Considering that the model solution change took $>80\text{ ms}$ for 90% completion, the ability of the simulation method to extract such a rapid rate constant is notable.

The estimate of the rate constants for blocker-channel interaction was improved by increasing the number of channels in the patch or averaging individual responses. Whereas it is not unusual to obtain multiple channels in a patch, alternately averaging multiple responses is facilitated with the oil-gate technique because the artifact which occurs on the current record as the pipette leaves the oil gate provides a precise marker of the onset of solution change (Qin and Noma, 1988). If the movement of the pipette through the oil gate were mechanized, large numbers of repetitive solution changes might be possible in a short time. Recent evidence suggests that the depth of the patch within the pipette may even change during an experiment (Sokabe and Sachs, 1990). This potential problem could be overcome by also repeating the permeant ion concentration jump after each jump in modulator concentration.

Conclusions

The results presented in this paper argue that caution should be applied in the analysis of concentration jump experiments to determine kinetic constants for ligand-gated ion channels. A considerable diffusion delay can exist in inside-out patch experiments that will confound simple extraction of reaction rate constants. For most geometries, tens of milliseconds will be required for 90% completion of the solution change. However, we have shown that, by measuring pipette geometry, and using jumps of [permeant ion], it is possible to estimate the time-course of [ligand] change at the membrane. Thus, it becomes possible to deconvolve the true ligand-dependent rate constants by least-squares fitting of computed mass action responses. The approach that we have developed should be generally applicable to the analysis of ligand concentration jump experiments on

any ion channel whose activity is modulated by intracellular ligand.

This work was supported by Grants from the National Institutes of Health (HL 45742) and American Heart Association (Maryland Affiliate) to C. G. Nichols.

Received for publication 11 April 1991 and in final form 25 July 1991.

REFERENCES

- Cannell, M. B., and C. G. Nichols. 1991. "Stopped flow" time resolution in excised patch clamp experiments? *Biophys. J.* 59:76a. (Abstr.)
- Chance, E. M., A. R. Curtis, I. P. Jones, and C. R. Kirby. 1977. FACSIMILE: a computer programme for flow and chemistry simulation, and general initial value problems. Computer Science and Systems division, Atomic Energy Research Establishment (AERE) Harwell, Oxfordshire, Her Majesty's Stationary Office, London.
- Colquhoun, D., and A. G. Hawkes. 1983. The principles of stochastic interpretation of ion-channel mechanisms. *In* Single Channel Recording. B. Sakmann, and E. Neher, editors. Plenum Publishing Corp., New York.
- Crank, J. 1975. The Mathematics of Diffusion 2nd ed. Oxford University Press, Oxford.
- Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch. Eur. J. Physiol.* 391:85-100.
- Lederer, W. J., and C. G. Nichols. 1989. Nucleotide modulation of the activity of rat heart ATP-sensitive K^+ channels in membrane patches. *J. Physiol. (Lond.)* 419:193-211.
- Magleby, K. L., and D. S. Weiss. 1990. Estimating kinetic parameters for single channels with stimulation: a general method that resolves the missed event problem and accounts for noise. *Biophys. J.* 58:1411-1425.
- Qin, D., and A. Noma. 1988. A new oil-gate concentration jump technique applied to inside-out patch-clamp recording. *Am. J. Physiol.* 255:H980-H984.
- Sakmann, B., and E. Neher, 1983. Geometric parameters of pipettes and membrane patches. *In* Single Channel Recording. B. Sakmann, and E. Neher, editors. Plenum Publishing Corp. New York.
- Sokabe, M., and F. Sachs. 1990. The structure and dynamics of patch-clamped membrane: a study using differential interference contrast microscopy. *J. Cell Biol.* 111:599-606.