Development and Application of Analysis Software for Ion Channel Kinetics.

Yukio HOSOYA, M.D., Ph.D.

Abstract : The author has developed original software for analyzing ion channel kinetics. This program was designed to perform single-channel analysis used for patch-clamp experiments. The source code of the program was written in the C and IGOR programming languages. The software supports both Microsoft Windows and Macintosh computers. To evaluate the performance of the program, simulated ion channel data generated by an ion channel simulator were analyzed. Both open time and closed time histograms could be well fitted with the theoretical curves. These measured time constants were nearly identical to those predicted theoretically from the simulated model. Currents of a cardiac inwardly rectifying potassium ion (I_{K1}) channel or ATP-sensitive potassium ion (K_{ATP}) channel were recorded with a patch-clamp technique using single ventricular myocytes of a guinea pig heart. The I_{K1} and K_{ATP} channel currents were analyzed with the program. The software could perform automated event detection, even if there were multiple ion channels with baseline drift or background noise. It was confirmed that the analysis program was reliable and useful for patch-clamp experiments.

Key words : patch clamp technique, ion channel, computer software, single-channel analysis, kinetics

Introduction

Patch-clamp recordings of single-channel activity are measurements of the current flowing through an ion channel over time[1-4]. These recordings show sudden transitions between two well-defined levels of current flow, i. e., closed and open states of an ion channel. The main function of single-channel analysis software is to detect, quantify and store these opening and closing events automatically, and reconstruct an idealized waveform. The major aim of the analysis program is to extract a model specific information from the event lists, and to clarify the kinetics of an ion channel.

Several software packages for the single-channel analysis are commercially available, e. g., FETCHAN[5,6] (Axon Instruments, Inc., Foster City, CA, USA), and TAC[7, 8] (Bruxton Corporation, Seattle, WA, USA). FETCHAN supports only MS-DOS (Microsoft disk operating system; Microsoft Corporation, Redmond, WA, USA) on IBM-PC/AT compatible computers, while TAC runs on both Microsoft Windows and Macintosh computers (Apple Computer, Inc., Cupertino, CA, USA). Some other analysis programs have been developed in various laboratories, however these are not commercially available.

The author developed original software for continuous data acquisition of ion channel currents[9]. To analyze the acquired ion channel data, the author also has written an analysis program for ion channel kinetics. This analysis software supports both Microsoft Windows and Macintosh computers.

Methods

Development of the analysis program

Methods for developing the software were

Department of Nursing, Faculty of Health Science,
Yamagata Prefectural University of Health Science.
260 Kamiyanagi, Yamagata, Yamagata 990-2212, Japan.

described in detail in a previous paper[9]. The single-channel analysis system was built using IGOR Pro[10, 11] (WaveMetrics, Inc., Lake Oswego, OR, USA). IGOR Pro is an extensible graphing, and programming tool for scientists. The source codes used for the analysis program consisted of two components: (1) main analysis program as the core of this software written in the C programming language, and (2) programs for user-interface written in the IGOR's programming language[11]. The source program written in IGOR's language consisted of about 14,000 lines of codes. These codes were used for user-interface part of the programming, for example, displaying dialog boxes to prompt for input values, displaying windows, and creating publication-quality graphs of the traces.

The C source codes of the main analysis program were used for number crunching, especially for, procedures necessary to iterate through each point in a wave. Because these routines should be executed as fast as possible in order to perform high-speed analysis, source codes of the main analysis program were written in the C programming language. The source program consisted of about 10,000 lines of C codes. There are two sets of source programs for Windows and Macintosh versions of the program. The source codes, such as calculations were completely platform-independent. Some routines, such as dialog-related, menu-related, and file-related routines are written in a platform-dependent manner.

These source files were compiled along with the source files supplied by IGOR XOP Toolkit[12] (WaveMetrics, Inc.), under the development system. For Macintosh computers, CodeWarrior Professional[13] release 5 (Metrowerks, Inc., Austin, TX, USA) was used as a development system, while for Microsoft Windows computers, Microsoft Visual C++[14] ver.6.0 was used to compile the C source files. The compiler is software that translates a program code written in a high-level specific programming language (e.g., C language) into a low-level machine code, which can be directly executed by the computer's CPU (central processing unit). Because the main analysis program was compiled to the machine code, it runs faster than IGOR's built-in programming language, and can directly access the operating system calls. However there are Windows and Macintosh versions of the analysis program, the functionality is almost identical. The program supports Microsoft Windows 95, 98, Me, 2000, NT 4.0, and Apple Macintosh operating system (MacOS) running System 7.1 or later. For Macintosh computers, both PowerPC and 680x0 processors are supported.

Patch-clamp experiments

Single ventricular myocytes of the guinea pig heart were obtained by an enzymatic dissociation method described previously[15-19]. The heart was retrogradely perfused through the coronary arteries on a Langendorff apparatus with nominally Ca^{2+} free solution containing collagenase. To isolate single ventricular cell, a small piece of ventricular tissue was dissected and agitated in the recording chamber. Currents of a cardiac inwardly rectifying potassium ion (I_{K1}) channel were recorded in cell-attached configuration of the patch-clamp technique[1-4], while currents of a cardiac ATP-sensitive potassium ion (K_{ATP}) channel were recorded in the inside-out patch configuration. All patch-clamp experiments were performed in the laboratory of Yamagata University School of Medicine.

Electrophysiological recordings

The I_{K1} or K_{ATP} channel currents were recorded using a patch-clamp amplifier (EPC-7 ; HEKA Elektronik, Lambrecht/Pfalz, Germany), and stored on a video tape through a PCM (pulse code modulator) converter system (VR-10B ; Instrutech Corp., Port Washington, NY, USA). For analysis, data were reproduced, low-pass filtered (Programmable Filter 3625 ; NF Electronic Instruments, Yokohama, Japan), digitized by an analog to digital (A/D) converter (ITC-16 ; Instrutech Corp.), and acquired continuously on a computer with the acquisition program[9] developted by the author.

Simulated ion channel currents were generated by an ion channel simulator[20] (Quantipore Stochastic Simulator Model QS-1 ; Instrutech Corp.), and directly digitized by an A/D converter, then continuously acquired on a computer.

Results

Event detection

Figure 1 represents instrument-like front panels of the single-channel analysis software using some pop-up menus and buttons. Users can perform single-channel analysis with these panels, menus, and dialog boxes. The major aim of single-channel current analysis is to reconstruct the idealized current waveforms from which information about the mechanisms of ionic channel function is derived[21, 22]. For single-channel analysis, an event is defined as a sudden change in current as the result of the opening or closing of an ion channel. Trace *A* of Fig. 2 shows an ion channel current of the patch-clamp recording. The dashed line (1) represents the current level when the ion channel is closed (i.e., zero current level), while line (3) represents the level when the ion channel is open. The deference between these two current levels represents single channel current amplitude. A transition between states of a channel occurs when the current amplitude passes through a threshold between the two levels[21, 22]. In this program, the threshold for judging the transition was set at half of the single-channel amplitude. The line (2) shows the 50% threshold current level.

By using the automated event-detection algorithm, the current from the ion channel was idealized as a rectangular waveform (Fig. 2B), with rapid transitions between closed channel level and open channel level. The program calculates each open time and closed time duration, and builds a table of opening and closing current events. The event table contains an event level index, and a dwell time duration for each transition.

Analysis of the simulated ion channel currents

To evaluate the quality and capabilities of the analysis software, kinetics of simulated ion channel currents generated by the ion channel simulator were analyzed by this program. Figure 3 illustrates simple three-state kinetic model tested in this study. In the kinetic model, there are two closed states, i.e., Closed(2) and Closed(1), and one open state (Open). Each parameter (k_{20}, k_{02}, k_{01}, and k_{10}) indicates the rate constant of the corresponding transition. In the study, each rate constant was set as follows. $k_{20} = 1.85s^{-1}$, $k_{02} = 7.40s^{-1}$, $k_{01} = 22.2s^{-1}$, and $k_{10} = 955s^{-1}$. Because there is just a single open state in the model, the frequency distribution of the open dwell times is theoretically single exponential[23, 24]. Since the open state has two exits, the time constant of open time is

τ (O) = $1/(k_{02}+ k_{01})$ = 33.8 msec. (Equation 1)

The probability density function for all shut periods should be a sum of two exponentials. The time

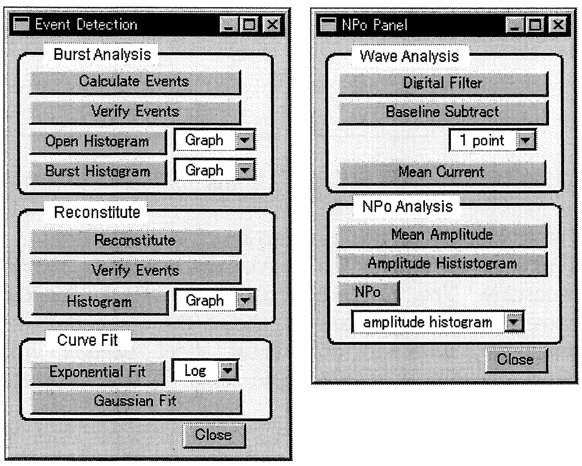

Fig. 1 Front panels of single-channel analysis software
Users can perform single-channel analysis using these panels, menus, and dialog boxes.

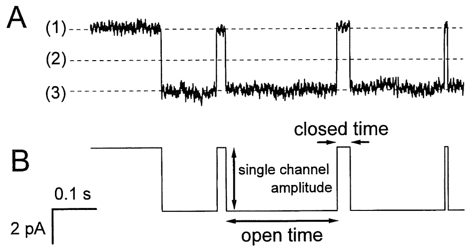

Fig. 2 Methods of event detection
Trace *A* shows an original ion channel recording. Inward currents were shown downward. Dashed line (1) represents zero current level, while line (3) shows the current level when ion channel is open. Dashed line (2) represents 50% threshold current level. The analysis program performed event detection by half amplitude threshold method, and produced an idealized trace (*B*).

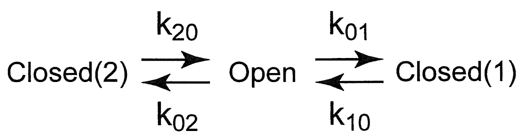

Fig. 3 Kinetic model tested in this study
In the kinetic model, there are two closed states, i. e., Closed (2) and Closed (1), and one open state (Open). Each parameter (k_{20}, k_{02}, k_{01}, and k_{10}) indicates rate constant of the corresponding transition.

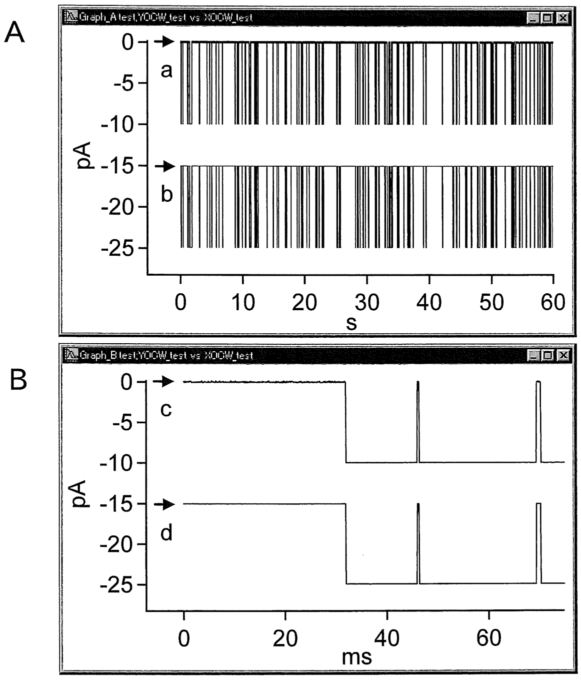

Fig. 4 Idealization of simulated ion channel current
Trace *a* represents the original simulated ion channel recording. The analysis program created idealized current (trace *b*). Panel *B* shows the same data as panel *A*, whereas only initial short portion was displayed on an expanded time scale. Arrows indicate the zero current level.

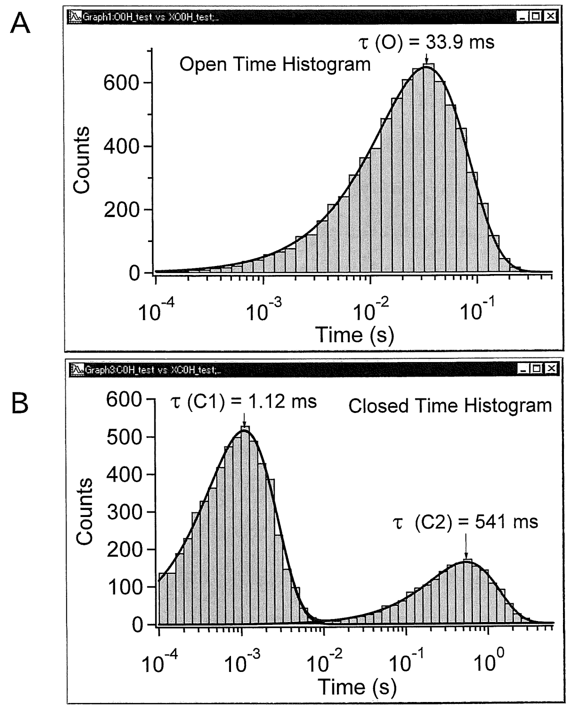

Fig. 5 Dwell time histograms of simulated ion channel data
The open time and closed time histograms of the simulated ion channel data in Fig. 4 were plotted against logarithmic bin widths. There are 10 equally spaced logarithmic bins per decade. Each histogram was fitted with a theoretical probability density function.

constants of short and long closed times are respectively

τ (C1) = $1/k_{10}$ = 1.05 msec, and τ (C2) = $1/k_{20}$ = 540 msec. (Equation 2)

The parameters of this kinetic model were set to the ion channel simulator, and artificial ion channel signal was acquired to a computer with the acquisition program[9].

Figure 4 shows an evaluation of simulated data. Trace *a* of the figure represents the original simulated ion channel recording. The analysis program performed automated event detection based on the threshold methods, and created idealized current waveform (trace *b*). Panel *B* shows the same data as panel *A*, whereas only initial short portion was displayed with an expanded time scale. The trace containing over 1.3×10^7 points could be analyzed automatically within two minutes by the program. It was confirmed that idealization procedure of the program was reliable from the results.

The second step in the analysis procedure is the extraction of a model specific information from the events list. Figure 5 represents the open time and closed time histograms of the simulated ion channel data in Fig. 4. Because the number of events is usually large, it is convenient to sort the event data into bins. Each dwell time will then fall into one of the bins, and the count in the appropriate bin is incremented. To represent a wide distribution of event duration accurately, the analysis program can construct dwell time histograms using logarithmic bin widths[25] on the time axis, i.e., the bin width was increased logarithmically. Bins have constant widths on the logarithmic time axis as shown in Fig. 5. In the present study, 10 bins were spaced per decade. Such bins have constant relative width; for example, one bin might represent durations from 1.00 to 1.26 msec, while another might represent durations from 100 to 126 msec. The number of counts per bin is plotted on the ordinate, with time plotted logarithmically on the abscissa. In the case of a single exponential distribution, the probability density function peaks at its time constant in the logarithmic histograms[25].

Event analysis program builds duration (dwell-time) histograms, and these histograms can be fitted to theoretical curves. The program performs an automatic fitting to a theoretical curve such as a sum of exponential terms by using the least-squares methods. As shown in Fig. 5A, the open time histogram could be well fitted with a single exponential curve with a time constant of 33.9 msec. On the other hand, the closed time histogram could be well fitted with a sum of two exponential terms with time constants of 1.12 and 541 msec, respectively (Fig. 5B). These measured time constants were nearly identical to those predicted theoretically from the simulated model (equations 1 and 2). It was verified that the analysis program could produce reliable dwell time histograms, and the automatic fitting procedure was also acceptable.

Baseline detection

The accuracy of the threshold method for event detection depends on a stability of the baseline current level. The baseline current, that is, the current level corresponding to a closed channel, may drift slowly over the course of an experiment. The analysis program uses an event-detection technique that is insensitive to the baseline variations. It measures the absolute current level prior to and following each transition. The difference between the two levels is the amplitude of the transition. The absolute current level following the transition is used as a starting point for detecting subsequent transitions. This technique does not depend on baseline stability over long periods. The program can identify the baseline as it changes. The program averages the most recent closed-or open-channel current level, and compares it to the old baseline level, and then determines a new baseline current level. Because the analysis program can track changes in baseline during the idealization process, events detection analysis can be fully automated with continuous tracking of baseline drift.

Trace *a* in Fig. 6 represents the simulated ion channel current with baseline drift. Trace *a* was produced as the summation of trace *a* in Fig. 4A and artificial sine curve. The analysis program could perform event detection of the simulated ion channel data with baseline drift, and reconstructed the idealized current waveform (trace *b*). The idealized trace was nearly identical to trace *b* in Fig. 4A, and it was confirmed that the baseline detection of the analysis program was reliable.

Single-channel analysis of I_{K1} channel

To evaluate the abilities of the program to analyze an observed ion channel data, I_{K1} channel currents were recorded and analyzed. Patch-clamp recordings usually contain substantial background noise. Trace *a* in Fig. 7 shows I_{K1} channel currents,

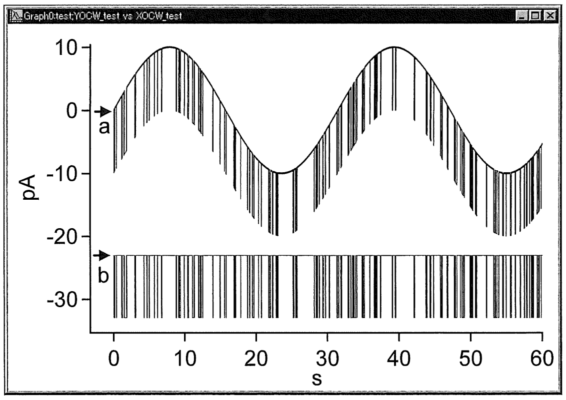

Fig. 6 Idealization of simulated ion channel data with baseline drift
Trace *a* in the figure shows the simulated ion channel current with baseline drift. Trace *a* was produced as the summation of trace *a* in Fig. 4 and artificial sine curve. Trace *b* represents idealized current data calculated from trace *a*. Events detection analysis can be fully automated with continuous tracking of baseline drift.

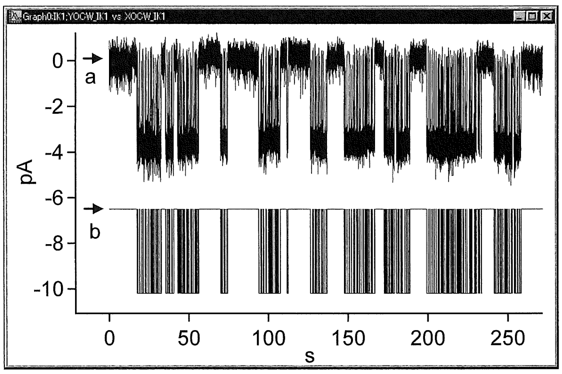

Fig. 7 Analysis of I_{K1} channel
Trace *a* represents I_{K1} channel currents, which was recorded in the cell-attached configuration of patch clamp technique. Trace *b* represents the idealized current waveform reconstructed from trace *a*. Inward currents were shown downward. Arrows indicate zero current level.

which was recorded in the cell-attached configuration of the patch clamp technique. Trace *b* represents the idealized current waveform reconstructed from trace *a*. The program could idealize an ion channel data, even if there was some background noise.

Figure 8 shows the review window of the program. Upper trace represents original I_{K1} channel current, while lower trace shows idealized current waveform plotted on the expanded time scale. After the automatic idealization process was finished, users can review the detected transitions and accept the events, or reject them, and remove those caused by artifacts.

Figure 9 illustrates the open time and closed time histograms of I_{K1} channel data in Fig. 7. The open time histogram was well fitted with a single exponential curve with a time constant of 284 msec (panel *A*). The closed time histogram was also well fitted with a single exponential curve with a time constant of 15.1 msec (panel *B*).

Trace *a* in Fig. 10 shows I_{K1} channel current with a baseline drift. Trace *a* was produced as a summation of trace *a* in Fig. 7 and artificial sine curve. The analysis program could perform event detection of the I_{K1} channel data with baseline drift, and created the idealized current waveform (trace *b*). The idealized trace was nearly identical to trace *b* in Fig. 7, and it was verified that the baseline detection of the analysis program was reliable, even if there was substantial background noise.

Analysis of patch-clamp recording with multiple channels

Patches of cell membrane do not always conveniently contain a single ion channel. Trace *a* in Fig. 11 shows K_{ATP} channel currents, which were recorded in the inside-out configuration of the patch clamp technique. In this recording, multiple ion channels are active in a patch. The analysis program could perform automatic event detection of the patch-clamp recordings showing multiple channels, and the idealized current waveform (trace *b*) was reconstructed. Panel *B* shows the same data as panel *A*, whereas only short portion was plotted on an expanded time scale.

Baseline detection of patch-clamp recording with multiple channels

Trace *a* in Fig. 12 shows a K_{ATP} channel current

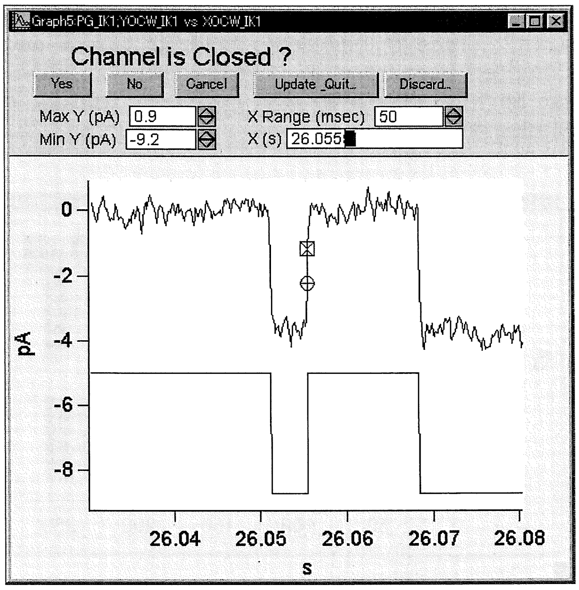

Fig. 8 Review window
Upper trace shows original I_{K1} channel current, while lower trace represents idealized current waveform on an expanded time scale. Users can review the detected transitions, and can accept or reject each event.

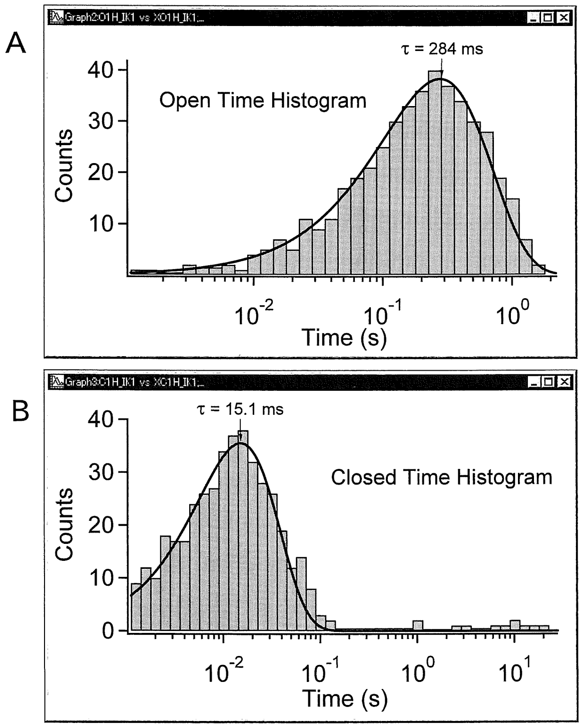

Fig. 9 Dwell time histograms of I_{K1} channel current
The figure illustrates open time and closed time histograms of I_{K1} channel data in Fig. 7.
Logarithmic bin histograms were plotted against a logarithmic time axis. Both dwell time histograms were well fitted with single exponential curves.

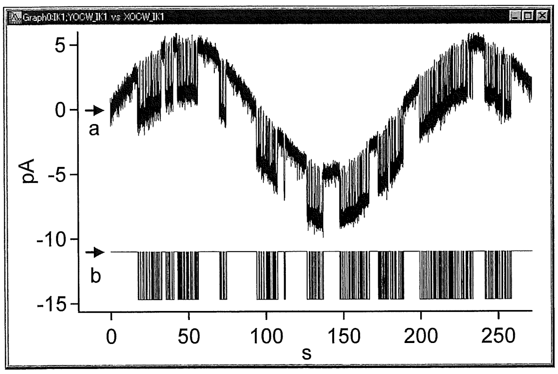

Fig.10 Idealization of I_{K1} channel data with baseline drift
Trace *a* in the figure shows the I_{K1} channel current with baseline drift. Trace *a* was produced as the summation of trace *a* in Fig. 7 and artificial sine curve. Trace *b* represents idealized current waveform reconstructed from trace *a*.

with a baseline drift. Trace *a* was produced as the summation of trace *a* in Fig. 11 and artificial sine curve. The analysis program could perform automated event detection of the K_{ATP} channel data with baseline drift, and reconstructed the idealized current waveform (trace *b*). The analysis program can track changes in baseline during the idealization process. Trace *c* represents the baseline current waveform reconstructed by the automated baseline detection algorithm of the program. The idealized trace was almost identical to trace *b* in Fig. 11, and it was confirmed that the baseline detection of the analysis program was reliable and useful, even if there were multiple channels with background noise.

Discussion

Event detection

In the present study, half amplitude threshold method was used for the event detection. This method is simple and do not require correction of event durations, because the effect of filter delay on the time lag between the actual channel transition and the measured time at which the current signal crosses the threshold is the same for openings and closings[4). However, this automated method requires a good signal-to-noise ratio, and has some limitations. If the record contains background noise or artifacts higher than the threshold level, the program judges these artifacts as channel opening or closing. The program provides the function of digital low-pass filter. If the

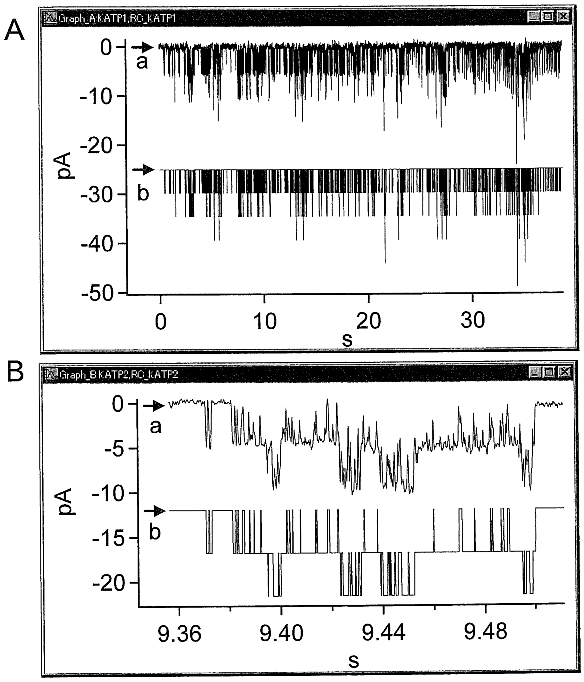

Fig.11 Idealization of patch-clamp recording with multiple channels
Trace *a* represents K_{ATP} channel currents, which were recorded in the inside-out patch configuration. In this recording, multiple ion channels are active in a patch. By the analysis program, idealized current waveform (trace *b*) was reconstructed. Panel *B* shows the same data as panel *A*, whereas only short portion was displayed with an expanded time scale. Inward currents were shown downward. Arrows indicate zero current level, at which all ion channels in a patch were closed.

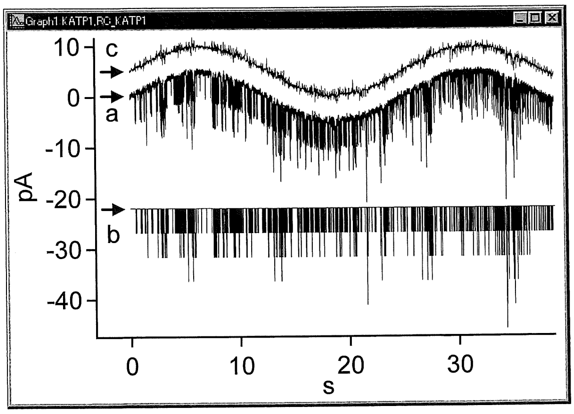

Fig.12 Baseline detection of patch-clamp recording with multiple channels
Trace *a* represents a K_{ATP} channel current with baseline drift. Trace *a* was produced as the summation of trace *a* in Fig. 11 and artificial sine curve. The analysis program could perform automatic event detection, and reconstructed the idealized current waveform (trace *b*). Trace *c* shows the baseline current waveform reconstructed by the program.

background high-frequency noise is significant, the user can reduce the noise level by using digital Gaussian filter before the event detection analysis. After the analysis, the user can review the idealized currents and remove some events due to artifacts (Fig. 8).

Baseline detection

There are several algorithms for automated baseline detection; (1) defining a new baseline which maximizes the number of the times that the current signal crosses it during the closed state[26] ("zero crossing" methods), (2) defining a new baseline at the peak of the histogram of the baseline current amplitude[27], (3) averaging the baseline current level to get a new baseline[28].

In this analysis program, new different methods were used for automated baseline detection. The analysis program averages the most recent closed-or open-channel current level, compares it to the old baseline level, and then determines a new baseline current level. Other algorithms for automatic baseline detection were also tested, however, it was confirmed that this method was most reliable and efficient.

Dwell time histograms

There are several methods for displaying dwell-time histograms. (1) The most common way is to use a linear bin histogram, which has constant bin widths plotted on a linearly scaled abscissa for duration. (2) To accurately represent a wide distribution of durations, a logarithmic bin histogram is also used. In the case of very simple two-state model (i.e., open-closed model), the distribution of open or closed time should be fitted with a single exponential curve. In this case, both the linear and logarithmic bin histograms can be used to fit with a single exponential curve.

On the other hand, in the case of more complex model with multiple open and closed states, the distribution of dwell times will be fitted with a sum of multiple exponential terms. These terms might have time constants that differ by orders of magnitude. If two or more exponential components exist, logarithmic bin histogram is more useful to represent a wide distribution of durations accurately and to simulate the theoretical curves compared with the linear bin histogram. Under the transformation to a logarithmic abscissa, the probability density function corresponding to each exponential component is not monotonic but has a peak at the value of the time constant[25]. This analysis program can create both linear and logarithmic bin histograms.

Development of the analysis program

Although several software packages for single-channel analysis are commercially available, these programs are not sufficient in some cases. The commercial software cannot be modified easily, because generally software companies do not supply the source code of the commercial program to the users. On the other hand, it is easy to change the program with original software. One can add some analysis functions or can alter the program for the particular experiments. In some cases it may even be necessary, or at least advantageous to execute such changes for each experiment.

Conclusions

Single-channel analysis software for patch-clamp experiments has been developed. To evaluate the capability and reliability of the software, both simulated data and ion channel current data were recorded and analyzed. It was confirmed that this analysis software was reliable and useful for patch-clamp experiments.

References

1) Neher E., Sakmann B. : Single channel currents recorded from membrane of denervated frog muscle fibers. *Nature* 260:779-802, 1976.

2) Hamil O. P., Marty A., Neher E., Sakmann B., Sigworth F. J. : Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfügers Archiv: European Journal of Physiology* 391: 85-100, 1981.

3) Penner R. : A practical guide to patch clamping. In "Single-Channel Recording, Second Edition", Sakmann B., Neher E. (Eds.), New York, Plenum Publishing Corporation, 1995.

4) Heinemann S. H. : Guide to data acquisition and analysis. In "Single-Channel Recording, Second

Edition", Sakmann B., Neher E. (Eds.), New York, Plenum Publishing Corporation, 1995.

5) Fetchan ver.6 User's Guide : Axon Instruments, Inc., 1997.

6) Data analysis. In "The Axon Guide", Foster City, Axon Instruments, Inc., 1995.

7) TAC ver.4.0 User's Manual : Bruxton Corporation, 1999.

8) Single-channel analysis : an introduction. In "Monographs", Seattle, Bruxton Corporation, 1998.

9) Hosoya Y. : Development and application of the original software for continuous high-speed data acquisition of ion channel current. *Yamagata Journal of Health Science* 3, 59-67, 2000.

10) IGOR Pro Version 3.1, User's Guide, WaveMetrics, Inc., 1999.

11) IGOR Pro Version 3.1, Programming and Reference Manual, WaveMetrics, Inc., 1999.

12) IGOR XOP Toolkit Version 3.1, Reference Manual, WaveMetrics, Inc., 1999.

13) CodeWarrior Professional release 5, User's Guide, Metrowerks, Inc., 1999.

14) MSDN Library Microsoft Visual Studio 6.0, Microsoft Corporation, 1999.

15) Yamada M., Jahangir A., Hosoya Y., Inanobe A., Katada T., Kurachi Y. : G_K^* and brain $G_{\beta\gamma}$ activate muscarinic K^+ channel through the same mechanism. *Journal of Biological Chemistry* 268: 24551-24554, 1993.

16) Terzic A., Findlay I., Hosoya Y., Kurachi Y. : Dualistic behavior of ATP-sensitive K^+ channels towards intracellular nucleoside diphosphates. *Neuron* 12:1049-1058, 1994.

17) Ito H., Hosoya Y., Inanobe A., Tomoike H., Endoh M. : Acetylcholine and adenosine activate the G protein-gated muscarinic K^+ channel in ferret ventricular myocytes. *Naunyn-Schemiedeberg's Archives of Pharmacology* 351:610-617, 1995.

18) Hosoya Y., Yamada M., Ito H., Kurachi Y. : A functional model for G protein activation of the muscarinic K^+ channel in guinea pig atrial myocytes : Spectral analysis of the effect of GTP on single-channel kinetics. *Journal of General Physiology* 108:485-495, 1996.

19) Hosoya Y., Kurachi Y. : Functional analyses of G-protein activation of cardiac K_G channel. In "Potassium Ion Channels : Molecular Structure, Function, and Diseases" *Current Topics in Membranes* 46 : 355-369, San Diego, Academic Press, 1999.

20) Sigworth F. J. : QS-1 Instruction Manual. Instrutech Corp. 1986.

21) Colquhoun D., Hawkes A. G. : The principles of the stochastic interpretation of ion-channel mechanisms. In "Single-Channel Recording, Second Edition", Sakmann B., Neher E. (Eds.), New York, Plenum Publishing Corporation, 1995.

22) Colquhoun D., Sigworth F. J. : Fitting and statistical analysis of single-channel records. In "Single-Channel Recording, Second Edition", Sakmann B., Neher E. (Eds.), New York, Plenum Publishing Corporation, 1995.

23) Sakmann B., Trube G. : Voltage-dependent inactivation of inward-rectifying single-channel currents in the guinea-pig heart cell membrane. *Journal of Physiology* 347, 659-683, 1984.

24) Colquhoun D., Hawkes A. G. : On the stochastic properties of ion channels. *Proceedings of the Royal Society of London* -Series B : 211:205-235, 1981.

25) Sigworth F. J., Sine S. M. : Data transformations for improved display and fitting of single-channel dwell time histograms. *Biophysical Journal* 52, 1047-1054, 1987.

26) Sachs F. : Automated analysis of single-channel records. In "Single-Channel Recording", Sakmann B., Neher E. (Eds.), New York, Plenum Publishing Corporation, 1983.

27) Aldrich R. W., Yellen G. : Analysis of nonstationary channel kinetics. In "Single-Channel Recording, Sakmann B., Neher E. (Eds.), New York, Plenum Publishing Corporation, 1983.

28) Colquhoun D. : Practical analysis of single channel records. In "Microelectrode techniques", Standen N. B., Gray P. T. A., Whitaker M. J. (Eds.), Cambridge, Company of Biologist Ltd., 1987.

Received : October 30, 2000.
Accepted : February 5, 2001.