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Gating kinetics of potassium channel and effects of nerve growth factors in PC12 cells analyzed with fractal model[1]

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KEY WORD PC12 cells; potassium channels; nerve growth factors; fractals; patch-clamp techniques; kinetics

ABSTRACT

AIM: To study the gating kinetics of voltage-dependent K^+ channels in clonal pheochromocytoma (PC12) cells and the effect of nerve growth factor (NGF) on it. **METHODS**: Outward currents of K^+ channel were recorded in PC12 cells cultured with or without NGF using cell-attached patch-clamp technique. The kinetic features of K^+ channel and the effect of NGF on them were analyzed based on the fractal model. **RESULTS**: The fractal dimension D for the closed durations of K^+ channel in PC12 cells cultured without NGF was proportional to the absolute value of the pipette potential (V_p). Whereas the fractal dimension D' was inversely proportional to it. Under pipette potentials of 0, -30, -50, and -70 mV, the values of D were 1.75, 1.88, 1.95, and 2; and of D' were 1.51, 1.40, 1.34, and 1.23. After the addition of NGF the changes in the fractal dimensions became more complex. At the same pipette potential, fractal dimensions for K^+ channel in PC12 cells cultured with NGF for both the open and closed durations were not greater than those in PC12 cells cultured without NGF. The kinetic setpoints for both the close and open durations did not vary with the pipette potential with values as follows: A(without NGF) = 0.29 ± 0.05, A(with NGF) = 0.71 ± 0.06, A'(without NGF) = 0.110 ± 0.020, and A'(with NGF) = 0.38 ± 0.08. **CONCLUSION**: The voltage dependence of D(without NGF) increases the probability of the channel for remaining closed for long durations as the patch is depolarized, and that of D'(without NGF) decreases the probability of the channel for remaining open for long durations as the patch is depolarized. The addition of NGF in the PC12 cell culture accelerates the dynamic process of the K^+ channel occurring over long time scales.

INTRODUCTION

Single channel recording gives one a unique opportunity to study the gating kinetics of single ion channels[1,2]. One aim of such a study is to enable characterization of, for example, interactions between neurotransmitters and their target receptor-channels. However, before such molecular interpretations may be made with any degree of certainty, it is essential that the relevant gating model be employed to interpret the observed kinetics. For a long time, most of the researchers have studied the kinetics of ion channels with Markov models, which assume that the channel has a few discrete conformational states such as closed, open, and inactivated. And it is also assumed that the transition probabilities per unit time between these states, the kinetic rate constants, are independent of the history of the previous sequence of states[3,4]. However, since the properties of channel proteins may not be similar to the physical properties assumed by the Markov models, the parameters of these models do not provide physically meaningful information about ion channels. Many proteins have large numbers of conformational states that are separated by only small energy barriers. Moreover, changes in protein conformation occur over many time scales. Thus, a channel would be expected to have many conformational states, rather than a few discrete states, and have a continuous distribution of time scales of kinetic rate, that is, channel protein switciing between conformational states is fractal in time[5-7].

The fractal model of channel kinetics, consistent with these ideas, was proposed previously[8] and recently these ideas have been interpreted further[9]. The studies carried out previously suggest that the spontaneous fluctu-

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ation in the conformation of the ion channel protein may be better described by a fractal model[8]. However, there are few reports about the fractal analysis of ion channel kinetics so far.

Clonal pheochromocytoma (PC12) cells, developed from a pheochromocytoma tumor of the rat adrenal medulla, have become currently a very important model for the study of neuronal differentiation, and they are the premiere tools for the study of nerve growth factor (NGF). NGF is thought to play an important role in neuronal survival, growth, differentiation and development. This article used the fractal model to analyze and interpret the single channel currents recorded from K^+ channels in PC12 cells and the effect of NGF on them.

MATERIALS AND METHODS

Experimental materials and techniques PC12 cells and NGF were provided by Dr ZHANG Mao-Bin of Department of Biochemistry, Medical College of University of California in Los Angels. The passage cultured PC12 cells were used. The cells were plated on glass coverslips 1 – 7 d before being used for the experiments, and NGF 100 mg/L was added 1 – 3 d before the experiment.

At depolarizing pipette potentials of 0, −30, −50, and −70 mV, the outward currents of K^+ channel of sorts in PC12 cells cultured with or without NGF during the culture were recorded in a cell attached configuration of patch clamp method (Fig 1). The single channel conductance determined from the slope of the I – V curve was 51 pS ± 5 pS ($n = 5$). Using a CEZ-2200 patch clamp amplifier (Nihon Konden, Japan), currents were filtered with a four-pole Bessels filter, and recorded in PCH digital videocassette recorder. Current amplitude, channel open durations, channel closed durations etc. were measured by using PCLAMP software in an IBM PC/AT computer equipped with a TI-1-125 interface with 125 kHz LABMASTER DMA data acquisition system. Some kinetic properties of those channels were also reported previously[10-14].

The fractal model of ion channel kinetics In the fractal model

$$\text{closed} \underset{k_c}{\overset{k_o}{\rightleftharpoons}} \text{open}$$

the kinetic rate constant k_o or k_c for leaving the closed or open states is actually a function of the time scale, t, at which they are observed such that $k_o(t) = At^{1-D}$ and $k_c(t) = A't^{1-D'}$, where A, A' are kinetics setpoints, and

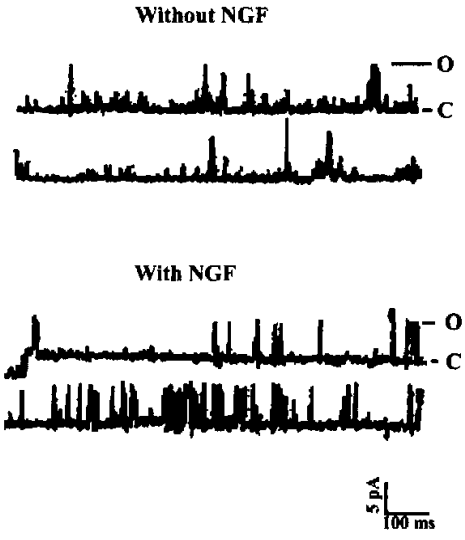

Fig 1. Representative K^+ current records from untreated and NGF-treated PC12 cells at $V_p = -30$ mV. NGF = 100 mg/L. Openings are upward deflection.

D, D' are the fractal dimensions. Thus, the closed and open states are each represented as a continium of many conformational states. In the present study we have reviewed the closed durations. The equations for the open duration are analogous with $k_o(t)$ replaced by $k_c(t)$. If $p(t)$ be the probability for which the channel remains closed for the duration $[0, t]$. It can be shown that

$$p(t) = \exp[(-At^{2-D})/(2-D)]$$

and the close-time probability density function can be given as

$$f(t) = -\mathrm{d}p(t)/\mathrm{d}(t)$$
$$= At^{1-D}\exp[(-At^{2-D})/(2-D)]$$

The rate of channel openings depend inversely on the time scale, such that $D \geqslant 1$. The restriction that $p(0) = 1$ requires that $D < 2$. Thus the fractal dimension D is restricted to the range $1 \leqslant D < 2$.

Let the smallest time interval that can be resolved define the effective time scale t_{eff}. We can only detect channel closings of duration $t > t_{eff}$. Thus, the channel kinetics can be meaningfully measured as the effective kinetic rate constant, k_{eff}, which follows the conditional probability that a channel that has been closed for at least a duration of t_{eff} is bound to open. Hence we can find that

$$k_{eff}(t_{eff}) = f(t_{eff})/p(t_{eff})$$
$$= -\mathrm{d}[\ln p(t)]/\mathrm{d}t \mid t = t_{eff}$$
$$= At_{eff}^{1-D}$$

This effective kinetic rate constant summarizes the information about the processes that happen at many different time scales. The fractal dimension D determines the relative contribution of various processes at different time scales and the kinetic setpoint A determines whether all the processes take place slowly or rapidly.

The method of the fractal analysis To judge if the kinetics of an ion channel can be described by a fractal model, we must determine the effective rate constant k_{eff} measured at different time scales t_{eff}. If $k_{eff} = At_{eff}^{1-D}$, then the kinetics of the channel is fractal. Liebovitch *et al* have proposed a practical method to determine whether the kinetics of ion channel is fractal. Any complex stochastic process can be approximated by an infinite number of adjacent Markov processes. For a two-state closed $\rightleftharpoons$ open Markov process, that is, the fractal model with $D = 1$, there exist $p(t) = \exp(-k_o t)$, $f(t) = k_o \exp(-k_o t)$, and $k_o = -\mathrm{d}[\ln p(t)]/\mathrm{d}t$ where k_o is a constant. Paying attention to the fact that this form for ko is similar to that for the effective kinetic rate constant $k_{eff} = -\mathrm{d}[\ln p(t)]/\mathrm{d}t \mid t = t_{eff}$, the kinetics of any channel, over a small range of time, is a form of a close $\rightleftharpoons$ open Markov process with $k_{eff} = k_o$. Thus, k_{eff} is equal to minus the slope of $\ln[f(t)]$ *vs* t evaluated over a small range of closed duration. To do this, the data is sampled at one frequency and closed time histograms are constructed with different bin size t_b. The bin size t_b determines the effective time scale of the analysis and thus $t_b = t_{eff}$. To maintain the local analysis for k_{eff}, we must exclude the first time bin (which includes the closings $t << t_b$ which are much smaller than the time scale $t - b$) and the longer time bins (which includes the closings $t >> t_b$ that are much longer than the time scale t_b). Then, for the semilogarithmic plot of each histogram, least squares fit was used to determine the slope over the second through fourth bins. This procedure thus determines k_{eff} as a function of t_{eff}. It has been shown that if there are multiple plateaus on the plot of $\log(k_{eff})$ *vs* $\lg(t_{eff})$, and then the kinetics of the channel can be well described by a Markov process. However, if the kinetics of the channel are fractal, this plot will be a straight line in the form of $\lg(k_{eff}) = (1 - D)\lg(t_{eff}) + \lg A$ (see Fig 6 in reference 8). Thus, the two parameters of the fractal model, D and A, can be determined from the slope and the intercept of the straight line. It has also been shown that based on this procedure D is overestimated on the average by 10 percent[8]. So D was reduced by 10 percent in our reported results. Once the fractal dimension D and the kinetic setpoint A were determined, $N(t)/\triangle t$ *vs* t could be plotted for certain $\triangle t$, and N_T *vs* t could be plotted on the same plot, where $N(t)$ was the number of closings of duration t to $t + \triangle t$, and N_T was the total number of closed times. Since it is difficult to determine N_T accurately, N_T has been regarded as an adjustable parameter. The parameter shifts the closed duration histogram up and down, but does not change its shape. Thus, we have compared the experimental data to the fractal model based on the equation $N(t) = N_T \triangle t f(t)$.

Statistical analysis Data were expressed as $\bar{x} \pm s$. Statistical significance was determined by a paired t-test.

RESULTS

The Markov model of gating kinetics of K^+ channels in the PC12 cells Using Markov process, the gating kinetics of K^+ channels in PC12 cells was modeled[13,14]. Regardless of the open or closed time distributions of the channels, at least two exponentials were required to fit them. These distributions were explained in terms of the following kinetic model

$$\text{closed} \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} \text{closed} \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} \text{open} \underset{k_{43}}{\overset{k_{34}}{\rightleftharpoons}} \text{open}$$

Both the open and closed durations instead of histograms were directly used to estimate rate constants k_{ij} $(i, j = 1, 2, 3;\ i \neq j)$. However, Markov models can not be uniquely determined from only the open and closed duration distributions. Moreover, Markov models involve too many adjustable parameters. Thus, it needs a large number of experimental data to make good estimations. Too many parameters are also inconvenient for the evaluation of the effects of medicines on the channels.

Determining the fractal feature of K^+ channels in PC12 cells The open and closed durations was used, which had been measured from the single channel records to construct histograms of bin sizes of 0.5, 1, 2, 4, 8, 16, 32, and 64. Only histograms with monotonically decreasing numbers for both the open and closed durations in the first four bins were used for further analysis. For PC12 cells cultured with or without NGF, two samples of these histograms were shown respectively in Fig 2A and Fig 2B. The lines on each histogram were obtained from least squares fits using the second through fourth bins. The effective rate constants k_{eff} were the negative values of the slopes of these lines. With or

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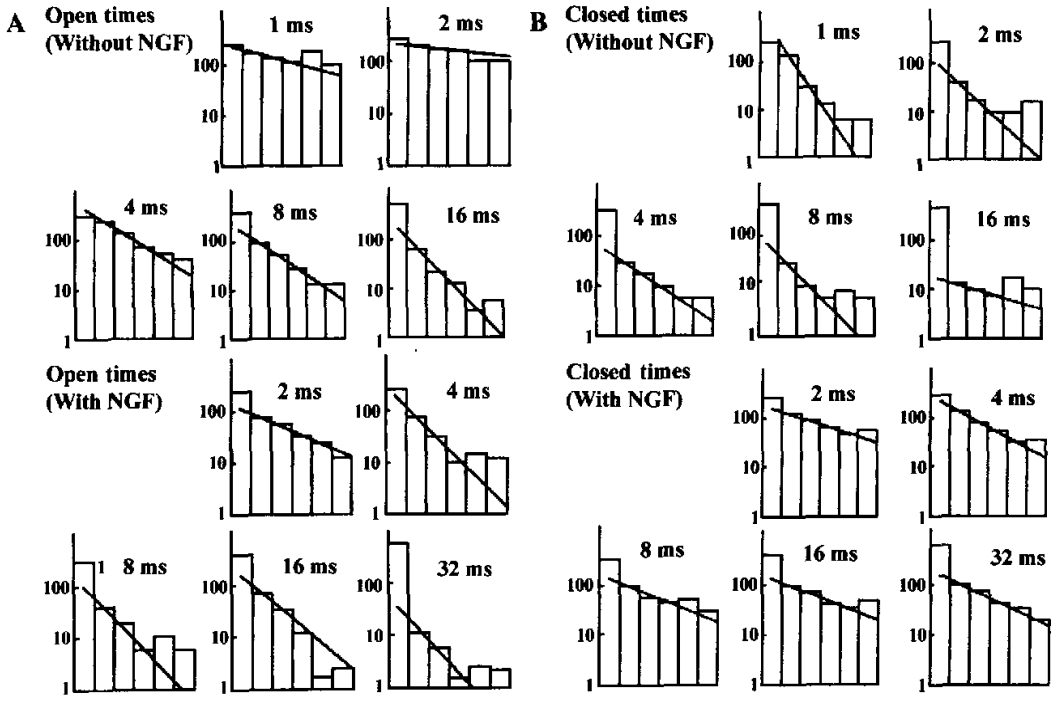

Fig 2. Semilogarithmic plots of the open(A) and closed(B) time histograms of bin size 1, 2, 4, 8, 16, and 32 ms at the $V_p = -50$ mV. The lines were obtained from least squares fits using the second through fourth bins. The negative of the slope of the line equals the effective kinetic rate constant k_{eff} for leaving the open(A) and closed(B) state at the effective time scale t_{eff} equal to the bin size.

without NGF, the plots of $\lg(k_{eff})$ *vs* $\lg(t_{eff})$ for both the open and closed durations did not have the plateaus which could indicate the existence of multiple discrete states as predicated by Markov models but instead were approximately straight lines, which indicated that these channels could be represented by a model using fractal kinetics. Four samples of these plots were shown in Fig 3.

Voltage dependence of the fractal dimensions The fractal dimensions D for the closed durations and D' for the open durations measured from the single channel records of PC12 cells cultured without NGF were both dependent on the pipette potential. D was proportional to the absolute value of the pipette potential, but the D' was inversely proportional to it. Under pipette potentials of 0, −30, −50, and −70 mV, the values of the D were 1.75, 1.88, 1.95, and 2; and the values of the D' were 1.51, 1.40, 1.34, and 1.23 (Fig 4).

For PC12 cells cultured with NGF, a complex picture emerged regarding the pipette potential dependence of the fractal dimensions D and D'. The changing tendencies of the D and D' were just contrary (Fig 5). In spite of that, under the same pipette potential the fractal dimensions for both the open and closed durations of K^+ channel in PC12 cells cultured with NGF were not larger than those in PC12 cells cultured without NGF.

Effect of NGF on kinetic setpoint The kinetic setpoints for both the open and closed durations measured from single channel records of PC12 cells cultured with or without NGF did not vary with the pipette potential (Fig 6). The following observations were made: A(without NGF) = 0.29 ± 0.07, A'(without NGF) = 0.110 ± 0.020 and A(with NGF) = 0.71 ± 0.06, A'(with NGF) = 0.38 ± 0.08.

Although the kinetic setpoints for both the open and closed durations measured from the single channel records of PC12 cells cultured with or without NGF were independent of the pipette potential, there was a difference between their values due to the effect of NGF. A(with NGF) was greater than A(without NGF) ($P < 0.05$), and A'(with NGF) was also greater than A'(without NGF) ($P < 0.05$). After the fractal dimensions and the kinetic setpoints had been determined, the experimental

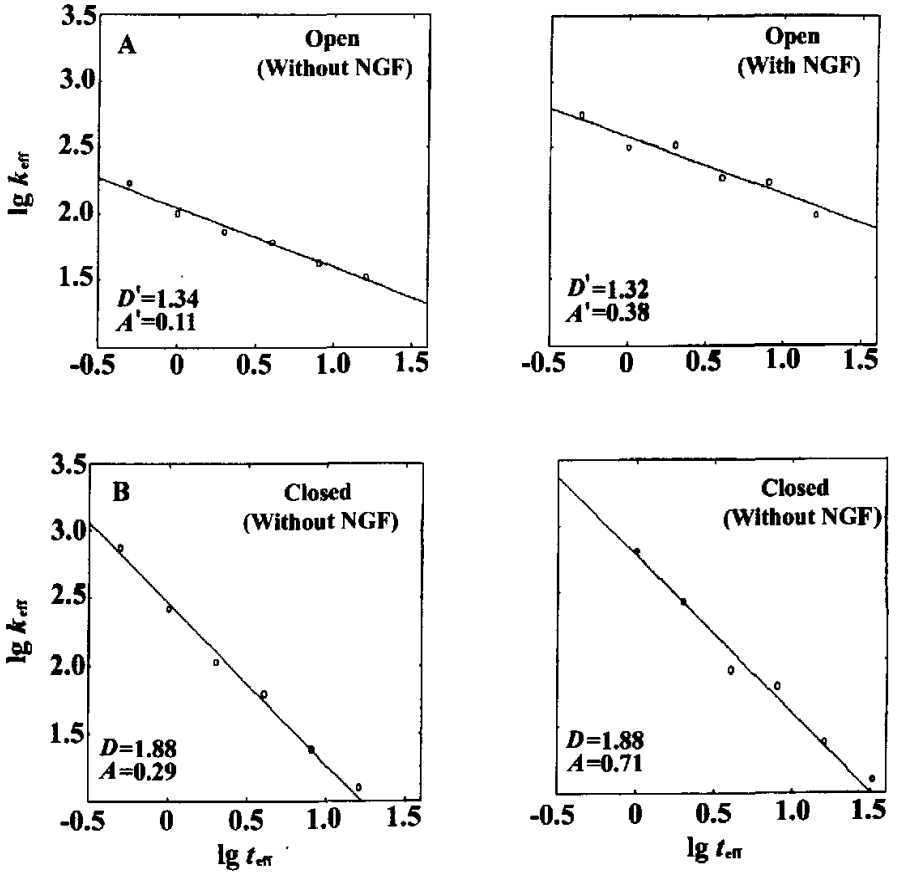

Fig 3. A) Plot of $\lg(k_{eff})$ *vs* $\lg(t_{eff})$ for the open durations at $V_p = -50$ mV. B) Plot of $\lg(k_{eff})$ *vs* $\lg(t_{eff})$ for the closed durations at $V_p = -30$ mV (k_{eff} in Hz and t_{eff} in ms). The slopes and intercepts of the lines in these plots determine the fractal dimensions and the kinetic setpoints.

data was compared to the fractal model, and the open and closed durations were both well represented by the fractal model. An example of fitting the probability density function $f(t) = At^{1-D}\exp\{-[A/(2-D)]t^{2-D}\}$ to the experimental data was given in Fig 7.

DISCUSSION

The present study indicated that it was suitable to model the gating kinetics of K^+ channel in PC12 cell with a fractal model. The results give evidence that the spontaneous fluctuation in the conformation of the ion channel protein may be a continuous process, which was well described by a fractal model.

Modeling gating kinetics of K^+ channel in PC12 cell with Markov process is based on two steps. First, a Markov model was chosen and specified by the number of states and their connectivity is very difficult in practice. Secondly, the parameters of the model were estimated. This needed a much more complicated mathematical analysis than required to estimate the parameters of the fractal model. Furthermore, there are so many parameters in the Markov process that the data here is insufficient to estimate them under certain pipette potentials. This makes it difficult to evaluate dynamic properties and the effect of NGF on the behaviors of channels, quantitatively. But for the fractal model, under all pipette potentials, good estimations of parameters can be produced. This makes it possible to describe the gating kinetics of ion channel quantitatively and makes it easy to distinguish the behaviors of K^+ channels in PC12 cells cultured with or without NGF respectively.

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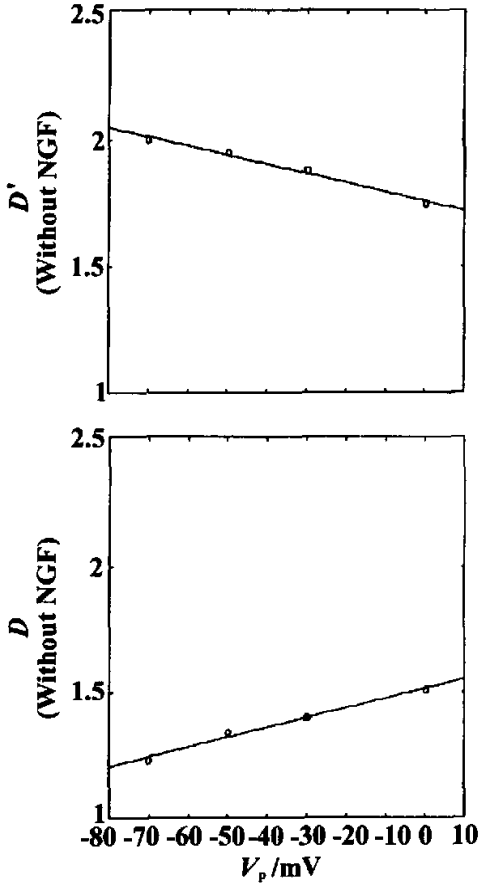

Fig 4. The dependence of the D (without NGF) and D' (without NGF) on pipette potential. NGF = 100 mg/L.

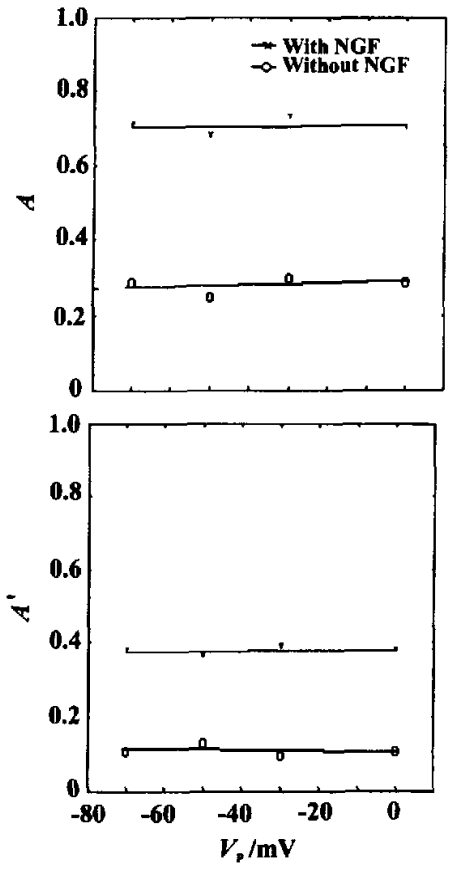

Fig 6. The independence of the kinetic setpoint on pipette potential.

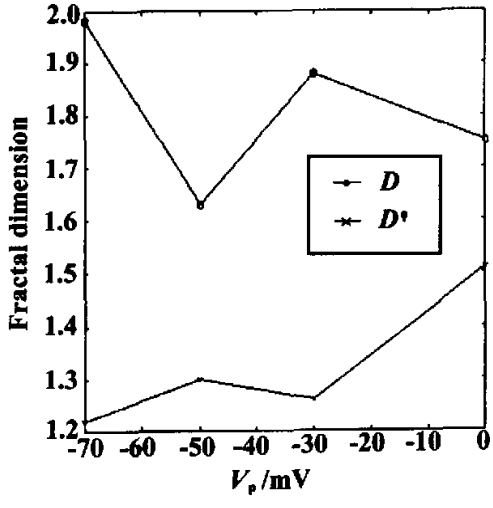

Fig 5. The dependence of D (with NGF) and D' (with NGF) on pipette potential. NGF = 100 mg/L.

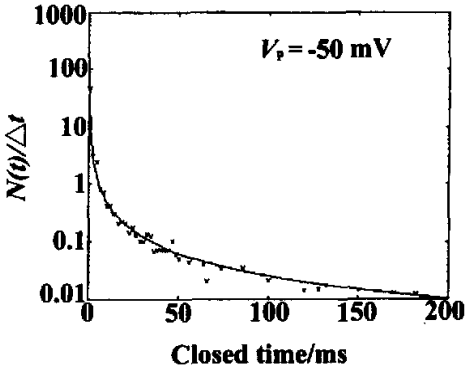

Fig 7. Fitting the fractal model to the experimental histogram at N_T = 10000, $\triangle t$ = 2 ms, D = 1.95, and A = 0.288.

From kinetic rate constants $k_o(t) = At^{1-D}$ and $k_c(t) = A't^{1-D'}$ where t is the time for which the channel remains in its current state and kinetic rate constant is the transition probability per unit time out of that state, and A(without NGF) is a constant, the voltage dependence of D(without NGF) increases the probability of the channel to remain closed for long durations when the patch is depolarized. On the contrary, voltage dependence of D' (without NGF) decreases the probability of the channel to remain open for long durations when the patch is depolarized. The kinetic setpoint determines if all the processes take place slowly or rapidly. Under the same pipette potential the fractal dimension satisfies D(with NGF) $\leq D$ (without NGF) and D' (with NGF) $\leq D'$ (without NGF). Based on the above two points we concluded that A (with NGF) $> A$ (without NGF) and A' (with NGF) $> A'$ (without NGF) meaning that for K^+ channels in PC12 cells cultured with NGF, the dynamic processes occurring over long time scales happened more rapidly than those without NGF.

It is very interesting to compare the results of this study with those of Liebovitch and Sullivan's[15]. In their results, when the patch was hyperpolarized, the kinetic setpoint for both the open and closed durations of the K^+ channel from cultured mouse hippocampal neurons depended greatly on the voltage, but the fractal dimension was independent of the hyperpolarizing voltage[15]. The difference between our observations and those of Liebovitch's may be caused by different polarities of the pipette potential, or may be due to the different characteristic of the channels themselves or due to both. We tend to believe that such a difference may be caused by the structural difference between two sort of K^+ channels.

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应用分形模型研究 PC12 细胞钾离子通道门控动力学及神经生长因子对其影响[1]

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关键词 PC12 细胞；钾通道；神经生长因子；分形；膜片箝技术；动力学

目的：研究 PC12 细胞上的钾离子通道门控动力学及神经生长因子(NGF)对其影响. **方法**：用膜片箝技术以细胞附贴式记录 PC12 细胞钾通道外向电流，用分形模型分析钾离子通道的门控动力学特征和 NGF 对其影响。**结果**：未经 NGF 处理的 PC12 细胞钾通道的关闭持续时间的分形维 D 与吸管位势的绝对值成正比，开放持续时间的分形维 D' 与其成反比，在吸管位势 0，−30，−50 和 −70 mV 下，D 的值分别是 1.75，1.88，1.95 和 2；D' 的值分别是 1.51，1.40，1.34，1.23，在相同的吸管位势下，用 NGF 处理后，钾通道开、关持续时间的分形维不大于经 NGF 处理前钾通道开、关持续时间的分形维. 开放与关闭持续的动力学设定点不随吸管位势变化，它们的值为 A(无 NGF) = 0.29 ± 0.05，A(有 NGF) = 0.71 ± 0.06，A'(无 NGF) = 0.110 ± 0.020 和 A'(有 NGF) = 0.38 ± 0.08. **结论**：随着片膜被去极化，D(无 NGF)的电压依赖性增加了通道保持长时间关闭的概率，D'(无 NGF)的单调性减少了通道保持长时间开放的概率. 在细胞培养液中增加 NGF 后，加速了在较长时间尺度上发生的钾通道的动力学过程.

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