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Existence of memory in ion channels[1]

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AIM: To explore the existence of memory in Ca^{2+}-dependent K^+ channels of cultured aortic smooth muscle cells (ASMC) and voltage-dependent K^+ channels of clonal pheochromocytoma (PC12) cells. **METHODS**: Calculating the sample auto-correlation functions based on the digitized signals or the 0—I series corresponding to closing and opening of the channels after routine evaluation, rather than the sequence of sojourn times. **RESULTS**: The sample auto-correlations showed a decreasing trend with elapse of time, stable to repeated observations under the same conditions and sensitive to treatments. **CONCLUSION**: The attribute of memory exists in some single channels as an intrinsic feature of them, independent of any extrinsic assumptions on missing observations due to limited time resolution.

KEY WORDS ion channels; memory; thoracic aorta; vascular smooth muscle; PC12 cells; nerve growth factors; potassium

The time series of ionic current measured by the patch clamp technic has a quantal character, indicating the channel status, opening and closing. Statistical inference for single channel recording is usually focused on the sequence of sojourn times and inevitably meets the problem of time interval omission[1,2]. Approaches directly based on the noisy digitized data, thus avoiding such a problem, have appeared recently[3,4]. To develop a rather simple stochastic model and intuitive interpretation, the statistic properties of the digitized signals have been explored in our databank and an attribute of memory is found in single channels to varying extent. The purpose of this paper is to report such a phenomenon since the existence of memory, if any, suggests a set of alternative indicators for experimental study as well as a significant aspect for theoretical consideration on the gate kinetics of ion channels.

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MATERIALS AND METHODS

Ca^{2+}-dependent K^+ channels in cultured thoracic aortic smooth muscle cells The thoracic aortic cells were cultured[5]. The depolarization-induced Ca^{2+}-dependent outward K^+-currents were recorded in the cells from Wistar-Kyoto rat with normal blood pressure (WKY, systolic pressure <17.3 kPa) and from spontaneously hypertensive rat (SHR, systolic pressure >21.3 kPa) respectively by patch clamp technic in the way of cell attached with pipet potentials ranged from -10 to -120 mV. The channel conductance was about 40 ps. The effects of cyclopiazonic acid (CPA) on the probability of opening were reported previously[6].

Voltage-dependent K^+ channels in clonal pheochromocytoma (PC12) cells The depolarization and superpolarization-induced voltage-dependent K^+-currents were recorded in clonal PC12 cells from rat adrenal, with or without nerve growth factors (NGF) during the culture, by patch clamp technic in the way of cell attached and inside-out, respectively. The pipet potentials ranged from -80 to 80 mV. The channel conductances ranged from 35 to 55 ps. Some kinetic properties of those channels were also reported previously[7].

Auto-correlation function Let k is the concerned time span evaluated with the number of time points and $x_i (i=0,1,2,\ldots n)$ is either the value of the current at i-th time point in the digitized raw data (DAT) or the value of 0 or 1 at i-th time point depending on the status of closing or opening in the restored series (EVL) through routine evaluation by well experienced experimenter. Then, for $k=0,1,2,\ldots$, the sample auto-correlation function $r(k)$ is calculated as

$$r(k)=\sum_{i=0}^{n-k}B(i,k)/\left(\sum_{i=0}^{n-k}B(i,0)\sum_{i=0}^{n-k}B(i+k,0)\right)^{1/2}$$

where

$$B(i,k)=(X_i-\bar{X}_{k1})(X_{i+k}-\bar{X}_{k2})$$

$$\bar{X}_{k1}=\sum_{i=0}^{n-k}\frac{X_i}{n-k+1},\quad \bar{X}_{k2}=\sum_{i=0}^{n-k}\frac{X_{i+k}}{n-k+1}$$

It is used to measure the memory with respect to time span in the series DAT or EVL. Obviously, $r(0)=1$ and $-1\leqslant r(k)\leqslant 1$ for any $k\neq 0$.

Statistical significance of auto-correlation Denote the population auto-correlation function with $\rho(\tau)$ for time span τ. It can be proved that for white noise, $\rho(\tau)=0, \tau\neq 0$, such that when the length of the series is n ($n+1$ time points), there will be arround 95.5 % of the time points having their sample auto-correlation falling into the band between $\rho=\pm 2n^{-1/2}$. Therefore, if there are far more than 4.5 % of the time points having their sample auto-correlation out of the band, the population auto-correlation function is regarded as significantly different from $\rho(\tau)=0, \tau\neq 0$, hence the attribute of memory exists.

Simple descriptive indices for auto-correlation Two intuitive indices are used: the value of $r(\tau)$ and the integral $\int_0^\tau r(t)\,dt$, where τ is any fixed time point selected. The former refers to the height of the auto-correlation curve at τ and the latter refers to the algebraic sum of the area between the curve and the time axis. The bigger values of $r(\tau)$ and $\int_0^\tau r(t)\,dt$ indicate a stronger memory.

RESULTS

The sample auto-correlation functions $r(k)$ of more than 40 series of patch clamp recordings for cultured aortic smooth muscle cells and clonal PC12 cells in our databank have been calculated such that all show the existence of memory with $r(k)$ decreasing slowly.

For a cultured aortic smooth muscle cell from SHR, 3 recordings under the same experimental conditions ($V_p=-10$ mV) showed the status of the single channel switching actively between opening and closing. One can hardly see the similarity among the original signals due to the random feature, but their auto-correlation functions based on EVL showed a well-consistant pattern (Fig 1).

For a cultured aortic smooth muscle cell from WKY, 2 recordings under the same experimental conditions accepting CPA or not had different patterns of auto-correlation functions (based on EVL). The one with CPA showed weaker memory than that without CPA in terms of $r(\tau)$ and $\int_0^\tau r(t)\,dt$ given any value of τ (Fig 2 A & B).

For a PC12 cell cultured with NGF, 2 recordings under $V_p=+60$ mV and $V_p=-60$ mV showed different patterns of auto-correlation (based on DAT). The latter, depolarization one, showed a stronger memory (Fig 2 C & D).

For another PC12 cell cultured without NGF, 2 recordings under $V_p=+60$ mV and $V_p=-60$ mV also showed different patterns of auto-correlation (based on DAT). The depolarization one showed a weaker memory (Fig 2 E & F).

Comparing the recordings under $V_p=+60$ mV of 2 PC12 cells cultured with and without NGF, the one with NGF showed a much weaker memory (Fig 2 C & E). Comparing the other 2 recordings under $V_p=-60$ mV, the similar difference also existed (Fig 2 D & F).

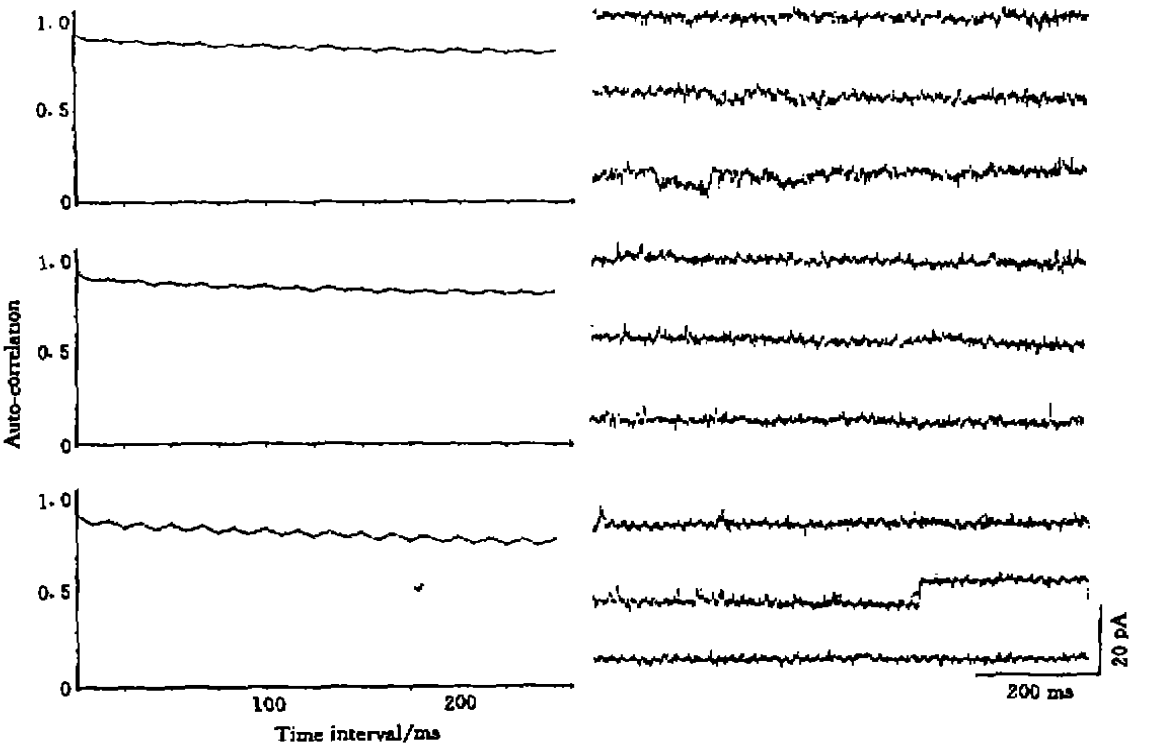

Fig 1. Three repeated K^+ channel recordings from a cell of SHR at $V_p = -10$ mV and their auto-correlation functions.

DISCUSSION

A stable feature of patch clamp recordings Although it is too early to evaluate the sampling variability of the above mentioned sample auto-correlation functions among repeated observations under same experimental conditions, incorporating the past experience[8], the phenomena such as Fig 1 gives a rough impression that the inferences based on auto-correlation are relatively stable to the noisy signals.

An alternative feature to distinguish different situations Adding of CPA had increased the probability of opening in the concerned channel of WKY[6], and here it seems that CPA may weaken the memory in the same channel (Fig 2 A & B). The kinetics of concerned channels of PC12 cells was reported before as a voltage-dependent one in terms of the distributions of sojourn time (either open or close) and the probability of opening, and here it seems that the attribute of memory may also voltage dependent (Fig 2 C-F). The auto-correlations based on the signals from PC12 cultured with and without NGF show different patterns and suggest a possibility that the one cultured with NGF has weaker memory under either $V_p = +60$ mV or $V_p = -60$ mV. Although it is not enough by now to draw any specific conclusion on the above impressions, conceptually, the auto-correlation is a feature close related to the rigorously ordered sequence rather than aggregated ones such as the marginal distributions or probabilities with the successive "order" ignored.

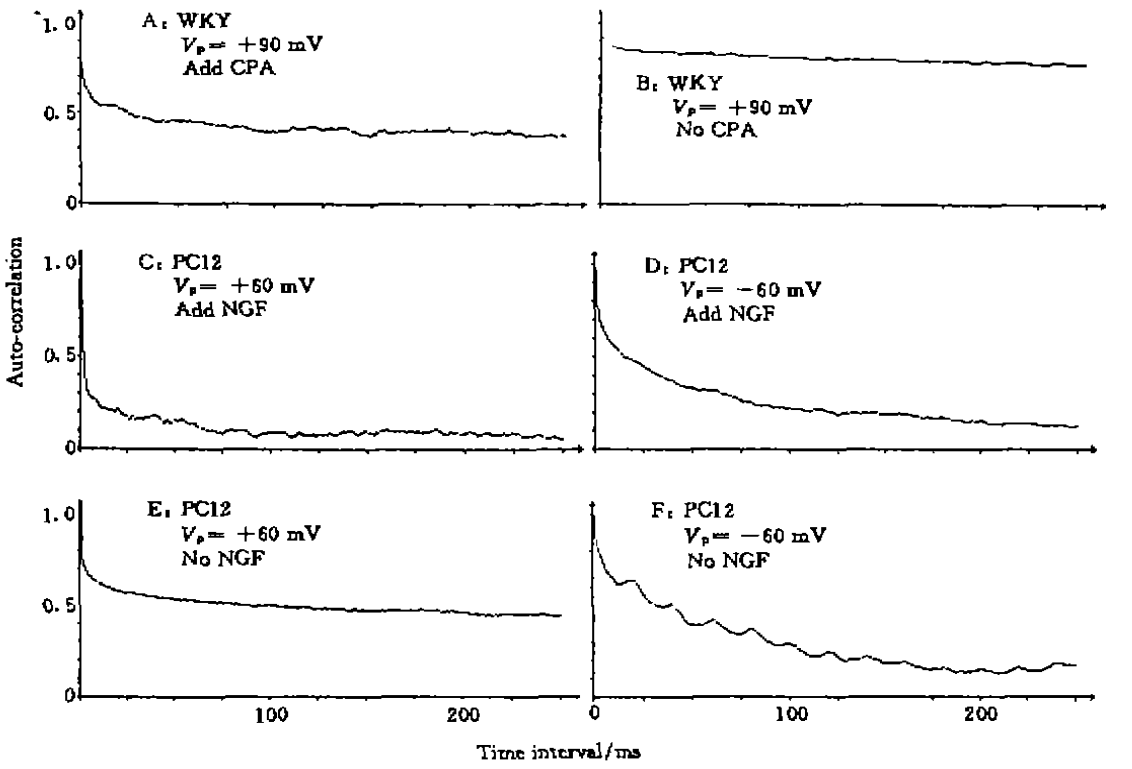

Fig 2. Auto-correlation functions based on K^+ channel recordings from cells of WKY rat aorta and PC12.

An intrinsic feature released from the problem of time omission The sample auto-correlatons are calculated based on the signal point by point in time span. Especially, if the raw data are used, it objectively reflects the essential pattern of memory in the channels without any extrinsic assumptions for missed observed part between successive time points. It may be affected in precision but not distorted in whole picture by the time resolution.

An alternative route and open questions The existence of memory in various ion channels and its change with treatments open an alternative route for further explorations. As a specific attribute that has not been widely noticed, it may exist in some channels under certain conditions and not exist in others under any condition. If it does happen to certain channels, how to identify and classify systemeticly, how to incorporate it in modeling of ion channel kinetics? And finally, what is the physical interpretation and possible machanism of memory in ion channels?

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REFERENCES

1 Ball FG, Sansom MSP. Single-channel autocorrelation functions: the effects of time interval omission. Biophys J 1988; **53**: 819—32.

2 Wilson DL, Brown AM. Effect of limited interval resolution on single channel measurements with application to Ca channels. IEEE Trans Biomed Eng 1985; BME—**32**: 786—97.

3 Chung SH, Moore JB, Xia L, Premkumar LS, Gage PW. Characterization of single channel currents using

digital signal processing techniques based on hidden Markov models.
Philos Trans R Soc Lond [Biol] 1990; 329: 265-85.
4 Fredkin DR, Rice JA. Maximum likelihood estimation and identification directly from single-channel recordings. Proc R Soc Lond [Biol] 1992; 249: 125-32.
5 Kwan CY, Lee RMKW. Changes of β-adrenoceptors in the aortic muscle cells of spontaneously hypertensive rat. Can J Physiol Pharmacol 1990; 68: 1461-68.
6 Guan YY, Zhang J, Lee RKW, Kwan CY, Daniel EE. Cyclopiazonic acid enhanced the Ca^{2+}-dependent outward K^+ currents in cultural aortic smooth muscle cells from SHR and WKY. Chin Pharmacol Bull 1993; 9: 257-60.
7 Ye JH, Fan J, Chen ZH, Chen PX. Kinetic properties of a delayed rectifier potassium channels in PC12 cells. Acad J Sun Yat-Sen Uni Med Sci 1991; 12: 250-53.
8 Ball FG, Rice JA. Stochastic models for ion channels: introduction and bibliography. Math Biosci 1992; 112: 189-206.

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离子通道中记忆性的存在[1]

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目的：探索血管平滑肌培养细胞 Ca^{2+} 依赖性 K^+ 通道和肾上腺髓质瘤无性繁殖细胞电压依赖性 K^+ 通道的斑片钳记录中记忆的存在性。**方法**：基于原数字化信号或对应通道开关的0－1序列而非基于持续时间的序列，计算样本自相关函数。**结果**：样本自相关函数具有随时间跨度下降的趋势，对重复观察有稳定性，对不同处理有敏感性。**结论**：某些单离子通道可能存在记忆性，作为信号的一种内在特性，独立于对有限时间分辨力引起的疏漏观察所作的任何外在假定。

关键词 离子通道；记忆；胸主动脉；血管平滑肌；PC12细胞；神经生长因子；钾

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Inhibition of left ventricular hypertrophy and expression of proto-oncogenes c-*myc* other than c-*fos* in myocardium by early captopril treatment in SHR rats[1]

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AIM: To explore the mechanisms by which angiotensin converting enzyme inhibitor (ACEI) prevents the development of left ventricular hypertrophy (LVH). **METHODS**: Captopril (Cap 100 $mg \cdot kg^{-1} \cdot d^{-1}$) was given orally to ♀ spontaneously hypertensive rats from intrauterine period to 16 wk of age. Experiments were performed at 40 wk of age. SBP, left ventricular weight to body weight ratio (LVW/BW) were assessed. The levels of c-*myc* and c-*fos* mRNA in the left ventricle were measured by Northern blot. **RESULTS**: Early-onset Cap therapy significantly decreased SBP. After discontinuance of treatment for 24 wk, SBP of SHR_{cap} was still maintained at a lower level. LVW/BW in SHR_{cap} was markedly reduced. The expression of myocardial c-*myc* mRNA was decreased by

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