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Conditional open and delay time histograms of sodium channels

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Currents through single sodium channels were recorded in neuroblastoma cells. Open time histograms were constructed from openings which appeared between 2.0 and 5.0 ms after the onset of the depolarization. Histograms constructed from openings which were not preceded by other openings showed a maximum at $t > 0$ in contrast to those, which were preceded by other openings. Time constants of delay time histograms fitted by the sum of two exponentials were different for the first, second and third records of runs. The results support the view that sodium channels have multiple open and closed states and the transition probabilities among the states depend on local conditions of the membrane.

Open time histograms constructed for the 0–5 ms range of the depolarization showed a maximum at $t > 0$ in contrast to those, which were constructed for the 5–40 ms range [1]. Patlak and Ortiz [2] reported that sodium channels can be interconverted into different modes of gating. The present paper reports further changes of open as well as delay time histograms depending on the previous history of the membrane.

Single sodium channel currents were measured in neuroblastoma cells, N1E 115. The experimental technique has been described earlier [1,3]. Single-channel currents were recorded in cell-attached configuration [4]. Patches were hyperpolarized by 80 mV (relative to the cells' resting potential of about −40 mV) to remove resting inactivation. 40 ms long depolarizing pulses were applied every second. Pipette and bath solutions contained (in mM): 140 NaCl, 5.0 KCl, 1.8 $CaCl_2$, 0.8 $MgCl_2$, 20 Hepes, 20 glucose. The pH was adjusted to 7.3 and the temperature was 8°C.

Details of the measuring system were described by Hof [5]. Pulse generation and data acquisition were made by a DEC LSI 11/23 microcomputer. Analog signals were filtered at 2 kHz (−3 dB) by a four-pole low-pass filter and digitized at 10 kHz. The dead time of the measuring system was < 0.1 ms [1] and the signal-to-noise ratio was > 8.3. Open times were measured on records which did not display overlapping openings. Experiments with more than 2500 depolarizations were used for the present calculations. Less than 1500 depolarizations were not sufficient for detailed statistical analysis.

Figs. 1a and 1b show open time histograms constructed under different conditions. Both histograms were constructed from openings which appeared between 2.0 and 5.0 ms after the onset of the depolarization. The histogram in Fig. 1a contains events appeared between 2.0 and 5.0 ms, which were not preceded by channel openings. By contrast, Fig. 1b shows the histogram of those openings which were preceded by other openings.

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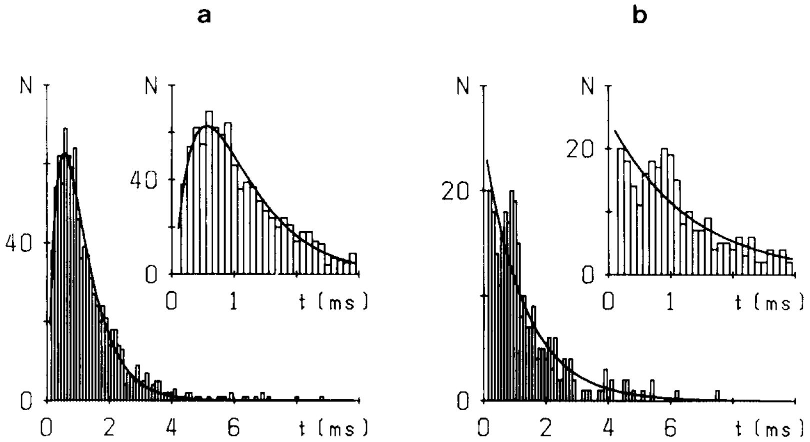

Fig. 1. Conditional open time histograms constructed from openings in the 2.0–5.0 ms range of the depolarization. The histograms include events which were not preceded (a) and were preceded (b) by openings at 0–2.0 ms. In (a) the histogram was best fitted by the difference of two exponentials resulting in $\tau_1 = 0.39$ ms and $\tau_2 = 0.71$ ms, chi-square (df) = 18.14 (26), $P = 0.87$. The histogram in (b) was fitted by a single exponential; $\tau = 1.30$ ms, chi-square (df) = 22.86 (18), $P = 0.19$. The mean open times (OT) and the number of events (ΣN) were OT = 1.29 ms, $\Sigma N = 940$ for (a) and OT = 1.32 ms, $\Sigma N = 289$ for (b). V_H = RP − 80 mV, V_m = RP.

The intervals for selection of events (2.0 to 5.0 ms) and for conditioning (0 to 2.0 ms) were chosen to see a maximum in the open time histogram [1] and to find sufficient events for both conditions. The two histograms are clearly different in the early phase. The plot in Fig. 1a has a maximum at approx. 0.6 ms similar to the histogram reported earlier for this time range of the depolarization [1]. This histogram could be best fitted by the difference of two exponentials. The time constants $\tau_1 = 0.39$ ms, $\tau_2 = 0.71$ ms are similar to those obtained earlier for the unconditional open time histograms, which were $\tau_1 = 0.34$ ms and $\tau_2 = 0.83$ ms for the 2.0–6.5 ms time range of the depolarization (see Table II in Ref. 1). In contrast, the plot in Fig. 1b does not show a clear character in the early phase. It was approximated by a single exponential with $\tau = 1.30$ ms.

The difference between Figs. 1a and 1b suggests that the open conformations or the transition probabilities between the open and closed states (see Ref. 1) may be influenced by previously opened channels. It follows that the first, second, etc. openings on a record might have different open times. The mean open time of the ith opening (OT_i) calculated from the same experiment as Fig. 1 was: $OT_1 = 1.30$ ms ($n = 894$), $OT_2 = 1.43$ ms ($n = 292$), $OT_3 = 1.62$ ms ($n = 91$), $OT_4 = 1.73$ ms ($n = 32$). Although the increase of the mean open time with the order number is clear, the interpretation is difficult. The larger the order number the longer the delay of the opening. Therefore, the increase of the open time with the order number might be enhanced by a factor due to the time dependence of the open time [1,6]. (It is necessary to note that channels open only once during a depolarization (see also Ref. 7). Patches contained four to six channels. The probability for reopening was 0.05 calculated after Kunze et al. [8], which value is about 10-times smaller than the probability for first opening of a channel. Therefore, the second, third, etc. openings on a record are mainly first openings and not reopenings.)

Previous results indicated that the open time histograms (and so the open conformations, or the transition probabilities) change with increasing time measured from the onset of the depolarization [1]. For the present analyses it was supposed that certain changes of histograms may occur on longer time scale, i.e. from depolarization to depolarization, too. It was shown already by Patlak and Ortiz [2] that sodium channels can have different modes of gating and that the modes can be interchanged. It was also reported [9] that consecutive depolarizations elicit groups of records with and without openings. The clustering of these groups in the present experiments was highly significant (the parameter for clustering, $Z > 2.24$,

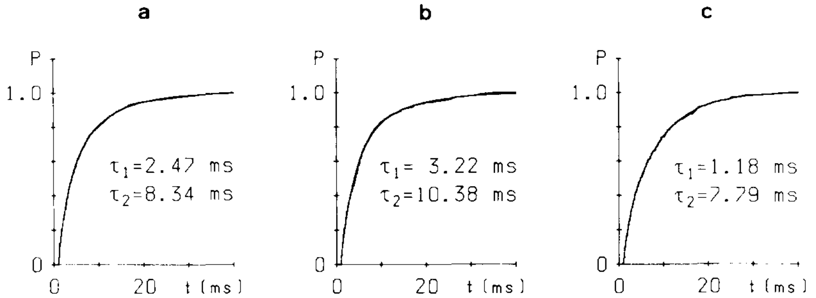

Fig. 2. Cumulative delay time histograms (the probability of finding openings at time $< t$) constructed from the first (a), the second (b) and the third (c) records of runs having openings. Fits were obtained by the sum of two exponentials. The time constants ($\pm$deviations) are (in ms): for (a) $\tau_1 = 2.47 \pm 0.07$, $\tau_2 = 8.34 \pm 0.11$; for (b) $\tau_1 = 3.22 \pm 0.10$, $\tau_2 = 10.38 \pm 0.35$; for (c) $\tau_1 = 1.18 \pm 0.06$, $\tau_2 = 7.79 \pm 0.03$.

the significance level, $\alpha < 0.01$; for definitions see Ref. 9) offering the possibility to study whether the kinetic properties of channels are changing from record to record during an active group, so called run. Therefore, open and delay time histograms were constructed from openings of the first, second and third records of runs.

Open time histograms changed only moderately as already reported [1]. The mean open times were 1.12 ms, 1.13 ms and 1.36 ms measured on the first, second and the third records of runs. Selections of openings for further different conditions (like time intervals of pulses), however, resulted in insufficient events for statistical analysis.

Cumulative delay time histograms, i.e. the probability of finding openings at times $< t$ constructed for the first, the second and the third records of runs are shown in Figs. 2a–c, respectively. These probabilities were fitted by the sum of two exponentials, resulting in time constants τ_1 and τ_2. Both time constants change with the record number of runs. τ_1 and τ_2 are larger for the second records than for the first records of runs, but they are smaller for the third records. These results suggest that the closed-to-open time constant, i.e. the activation time course may change during a run.

The results show that (a) the open time histograms are not only time dependent [1,6], but depend also on the previous history of the membrane and (b) that the activation parameter is also variable during the course of a run.

The dependence of the open time on previous openings could be explained by channel–channel interaction. The local conditions around a channel may be changed by other channel openings. It follows that the distance between channels must be short (see Ref. 9). (It is worthy of note that the time dependence of the open time histograms and the dependence of the open time on the previous openings depend probably on the membrane potential (V_m) too. However, both types of calculations can only be studied in a very limited potential range (about RP ± 10 mV), because at stronger depolarization openings appear shortly after the onset of the pulse preventing detailed analyses.)

The change of the time constants of the delay time histograms calculated for consecutive records of runs suggests a slow conformational change of the closed and resting states. Or else the transition probabilities may change during the interpulse intervals of runs. This might be a particular property of the channel-forming protein or elicited by cytoplasmic processes.

These findings provide further evidence for multiple open and closed conformations of sodium channels. The results also support the idea of the 'micro states' (i.e. kinetically distinct sub-conformations of the main states) of sodium channels, first suggested by Patlak (unpublished results) and raise the question, how to describe the function of the sodium channel on microscopic level.

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