Stationarity of Sodium Channel Gating Kinetics in Excised Patches from Neuroblastoma N1E 115

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ABSTRACT Na channel gating parameters in a number of preparations are translated along the voltage axis in excised patches compared to cell attached or whole cell recording. The aim of this study is to determine whether these changes in gating behavior continue over an extended period or, rather, develop rapidly on excision with stationary kinetics thereafter. Average currents were constructed from single-channel records from neuroblastoma N1E 115 at various times after excision, excluding the first 5 min, in eight inside-out excised patches. Single exponentials were fitted to the current decay of the average records, and the mean time constant for each patch was determined. Values were plotted as the percentage difference from these means for each patch against time from excision. Collected results show no obvious trend in values from 5 min to 2 h. Kinetics are stationary, and shifts in Na channel gating parameters along the voltage axis seen in excised as compared to whole cell configuration in neuroblastoma must be complete by the first few minutes after excision. Raising the internal Na concentration reduced the single channel current amplitude, confirming that these are Na channels.

INTRODUCTION

Excised patches offer a number of advantages for single-channel recording. For example, inside-out excised patches permit full and rapid control of the solution bathing the cytoplasmic face of the membrane. A question with excised patches is whether single-channel gating kinetics hold stationary for recording periods lasting several hours.

There are a number of reports that Na channel gating parameters are translated along the voltage axis in excised as compared to cell attached or whole cell recording configurations (Fenwick et al., 1982; Cachelin et al., 1983; Fernandez et al., 1984; Vandenberg and Horn, 1984; Kunze et al., 1985; Kohlhardt et al., 1987). This same effect was found in neuroblastoma N1E 115 (Nagy et al., 1983; Aldrich and Stevens, 1987). Less clear is the time course over which the shift in parameters occurs. Most studies reported only a single comparison of gating kinetics with one observation made before or at the time of excision and a second sometime after. Kunze et al. (1985), working on rat ventricular myocyte Na channels, did note that kinetics shifted rapidly on patch excision and were stable thereafter, and Vandenberg and Bezanilla (1991) reported that Na channel kinetics recorded in patches excised from slit-open squid axons remained similar for several hours. The issue remained unclear for neuroblastoma N1E 115. Aldrich and Stevens (1987) noted that Na channels in excised patches from these cells tended to change their behavior over time, but did not specify the time course of these changes. The present experiments address this question in neuroblastoma.

I find that Na channel gating kinetics in inside-out excised patches from neuroblastoma do remain stationary from 5 min (the shortest interval examined) to at least 2 h after excising. A preliminary report of some of these results has been made (Goldman, 1994).

MATERIALS AND METHODS

Cell culture

Neuroblastoma N1E 115 cells were grown in Dulbecco's modified Eagle's medium (GIBCO/BRL, Gaithersburg, MD) containing 14.4 mM $NaHCO_3$, 17 mM NaCl, 19.4 mM glucose, and supplemented with 10% fetal calf serum (GIBCO/BRL) in a humidified atmosphere of 5% CO_2 in air at 37°C. Cells were used 1–3 days after plating in culture dishes.

Electrical recording methods

Single-channel currents were recorded using the inside-out excised patch configuration (Hamill et al., 1981). Patch pipettes were pulled from borosilicate glass capillaries (TW 150–6; World Precision Instruments, Sarasota, FL), Sylgard coated (Sylgard 182, Dow Corning Corp., Midland, MI), and fire polished. The external recording (pipette) solution contained 150 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$, and 10 mM HEPES. pH was adjusted to 7.30 ± 0.05 with NaOH. Except where noted the solution bathing the cytoplasmic face of the patch (internal solution) contained 145 mM CsF, 10 mM CsCl, 5 mM Cs-EGTA, and 10 mM HEPES. pH was adjusted to 7.30 ± 0.05 with CsOH. Pipettes had resistances of 6–12 MΩ when measured in external recording solution.

Culture dishes were placed in a recording chamber arranged with continuously flowing solution and temperature control. To minimize changes in the dish solution level, solution was drained from the culture dish by a D.C. pump (N-07002–33 and N-07002–39; Cole-Palmer, Niles, IL) whose pumping rate was controlled by means of a float/piezoresistive sensor arrangement (AE801; Sensonor, Horton, Norway) and a feedback circuit, built in-house, as described by Cannell and Lederer (1986). Temperature was maintained by means of electrically controlled Peltier-effect heat exchangers (801-1001-02-00-00, Intercon-

Received for publication 20 March 1995 and in final form 16 August 1995.
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0006-3495/95/12/2364/05 $2.00

nection Products, Pompano, FL), which cooled the solution before it entered the culture dish. Temperature ranged from 6°C to 10°C, but varied by less than 1°C during any individual experiment. Seals ranged from about 10 to more than 100 GΩ, but were generally in the range of 20–50 GΩ. After seal formation, the external recording solution in the culture dish was replaced with internal solution before excising. An Ag-AgCl ground lead was coupled to the bath with a KCl-agar bridge.

Single-channel currents were recorded with an Axopatch-1B patch clamp (Axon Instruments, Foster City, CA). Voltage clamp pulses were formed and data were collected using a TL-1-125 interface (Axon Instruments) controlled with an IBM AT-compatible PC. Data were sampled at 100-μs intervals and low-pass filtered at 2 kHz (−3 dB). Pulses were presented at 1/s.

Data analysis

Leak and capacity currents were reduced by analog subtraction during the experiment and eliminated by digital subtraction of the average of traces with no channel openings. Data acquisition and analysis was performed with pCLAMP (Axon Instruments).

RESULTS

Stationarity of kinetics

Fig. 1 presents eight selected records, during a 45-ms step to −30 mV, from an inside-out excised patch. Channel openings are shown as downward deflections. The top trace indicates the pulse protocol. Holding potential for all experiments was −120 mV. The bottom trace in Fig. 1 is the average current constructed from 40 consecutive traces. These records were obtained 1 h after excising the patch.

Fig. 2 (top trace) again shows the average current from Fig. 1. The middle trace shows another average current obtained 30 min later from the same patch. The peak current is reduced as indicated by the expanded current scale for the middle trace. This was a general feature of these experiments. Channel activity always decreased over the lifetime of the patch. The bottom traces again show the top trace, now with the middle trace superimposed. The time courses are identical, indicating no detectable change in gating kinetics over this 30-min period.

To quantify any possible effects of time from excision on channel kinetics, single exponentials were fitted to the decay of the average currents. Fig. 3 shows one example (same record as in Fig. 1). The current decay could always be well described by a single exponential. Aldrich et al. (1983), Aldrich and Stevens (1987), and Quandt (1987) also found that the current decay in neuroblastoma N1E 115 could be well described by a single exponential (however, see Nagy et al., 1983).

In eight different patches, single-channel currents were recorded and average currents constructed at various times after excision. Exponentials were fitted to each of the average current records, and the time constants were determined. These time constants are here called τ_h, even though at the potentials of −30 and −40 mV at which these experiments were conducted, the current decay will be determined by the rate of recruitment of new open channels

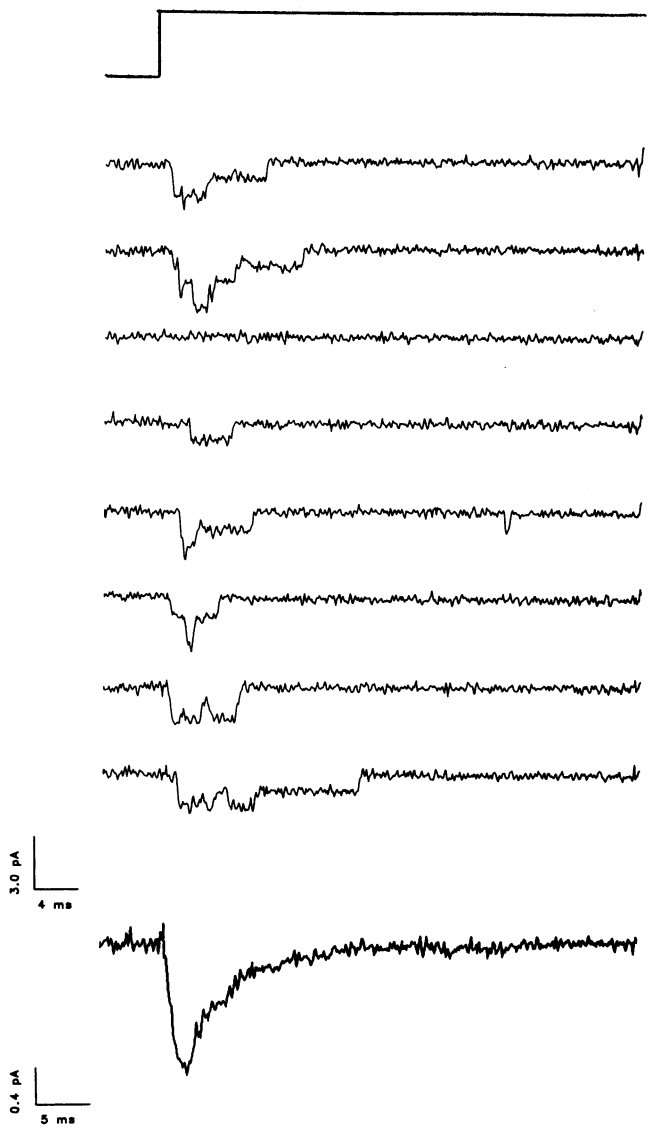

FIGURE 1 Selected single-channel records from an inside-out excised patch from neuroblastoma N1E 115. Channel openings are shown as downward deflections from the baseline. Holding potential was −120 mV, and the potential during the 45-ms step was −30 mV. The top trace indicates the time course of the voltage step. The bottom trace is the average of 40 consecutive single channel traces. These recordings were made 1 h after excision. Filtered at 2 KHz. Temperature was 7°C.

as well as the rate of entry into the inactivated state (Aldrich et al., 1983). For each individual patch the mean τ_h value was determined, and collected results are plotted in Fig. 4 as the percentage difference from the mean value for that patch versus time from excision. Each symbol type indicates a different patch. No recordings were made for the first 5 min after excision.

Results from different experiments ranged from two determinations separated by 17 min (open triangles in Fig. 4) to five determinations separated by 100 min (open circles). There is no obvious trend in the collected results. Channel

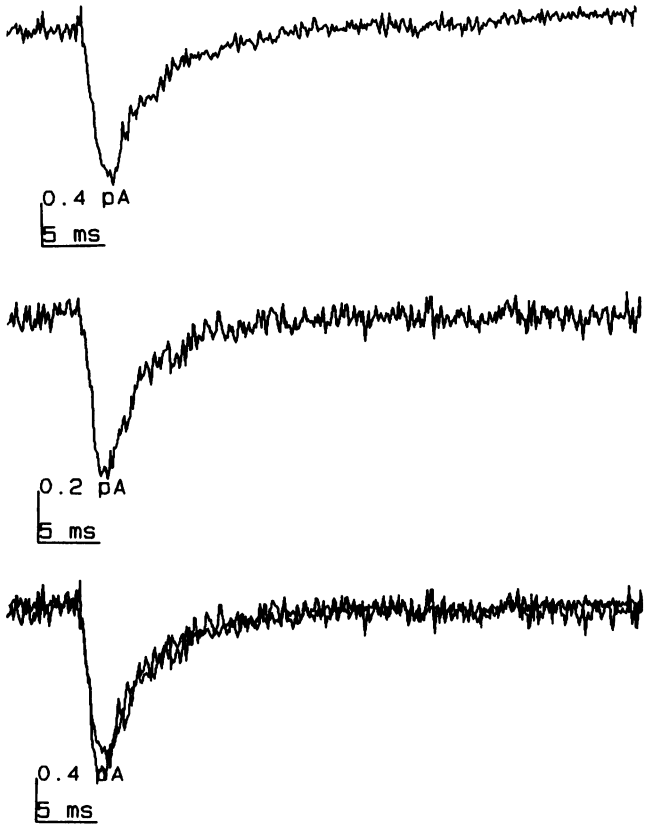

FIGURE 2 Average current at 1 h after excision (*top trace*). Average current in the same patch 30 min later (*middle trace*). Note the change in current scale. Bottom traces are the top two superimposed. There is no obvious change in kinetics over this period. Same patch as for Fig. 1.

kinetics are stationary from 5 min to at least 2 h after excising.

Na ions are an inward current carrier

Under these experimental conditions (150 mM external Na, 1.8 mM Ca) inward currents in neuroblastoma N1E 115 ought to be carried by Na ions (Moolenaar and Spector, 1977; Quandt et al., 1982; Nagy et al., 1983; Yamamato et al., 1984). To confirm that Na ions are in fact carrying inward current in these experiments, in three patches the normal internal solution bathing the cytoplasmic face of the patch membrane was replaced with one in which some CsF was replaced equivalent per equivalent with NaF. Fig. 5 presents results from one of these determinations.

The left-hand column in Fig. 5 shows four selected traces recorded in the presence of normal internal recording solution. The right-hand column shows four selected traces from this same patch with half of the 145 mmol of CsF in the internal solution replaced with NaF. Currents are clearly smaller in the presence of internal Na, indicating that these single-channel currents are Na currents.

DISCUSSION

Stationarity of kinetics

On excising, and after a 5-min waiting period, channel gating kinetics, as assayed by τ_h, show no appreciable changes over the time intervals examined. Nagy et al. (1983), also working on neuroblastoma, found that several gating parameters, including inactivation time constants, recorded in outside-out excised patches, were shifted 10–20 mV to the left along the voltage axis as compared to those recorded in whole cell configuration. These shifts must, then, be complete by the end of the 5-min waiting period.

Since the report of Fenwick et al. (1982) it has been known that Na channel gating parameters in excised patches can be shifted along the voltage axis relative to these recorded under cell attached or whole cell configurations. This shift in Na channel kinetics was reported for a number of preparations and raised the possibility that excised patches were not always well suited for kinetic observations requiring long periods of recording.

In most cases gating kinetics were observed before or at the time of excision and again sometime after, with no attempt to define the time course over which the shifts developed. However, in those studies where Na gating kinetics were examined over time after excision (Kunze et al., 1985; Vandenberg and Bezanilla, 1991; this study), Na channel gating kinetics remained stationary for long intervals in excised patches, at least after the first few minutes after excision (Kunze et al., 1985; this study). Shenkel and Sigworth (1991) even found that neither inactivation time constants nor steady-state values shifted at all on patch excision. A possibility, then, is that shifts in Na channel gating parameters on excision, when seen, typically develop rapidly with gating kinetics, remaining stationary thereafter,

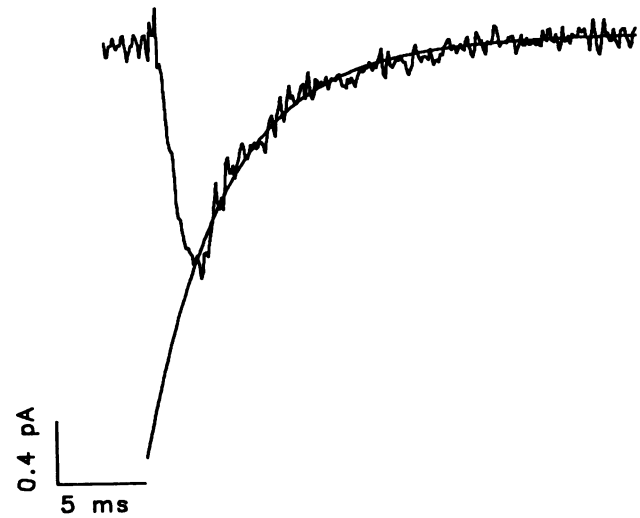

FIGURE 3 Average current record shown in Fig. 1. The smooth curve is a single exponential fitted to the time course of the current delay.

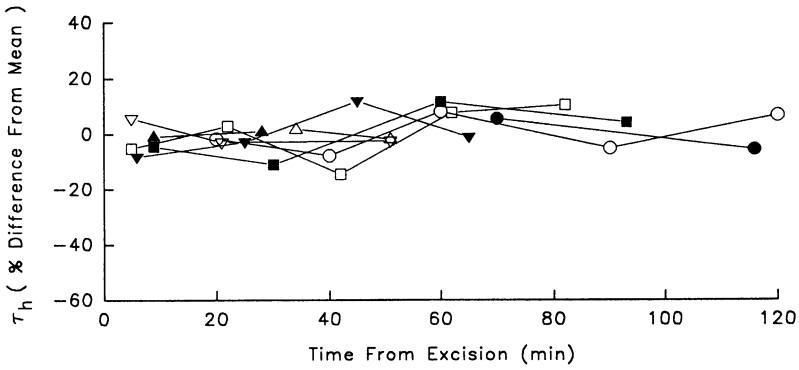

FIGURE 4 τ_h as a function of time from patch excision. Mean τ_h was determined for each patch, and values are plotted as the percentage difference from the mean for that patch. There is no obvious trend in the collected results. Each symbol indicates a different patch. Potential during the step was −40 mV for the experiments indicated by the filled circles and open triangles and −30 mV for all others. Holding potential was −120 mV throughout.

and that excised patch preparations are well suited for Na channel kinetic studies requiring long recording intervals.

Inward currents are carried by Na ions

Under experimental conditions comparable to those used here, the inward currents seen in neuroblastoma N1E 115 cells are through Na channels. Currents are blocked by tetrodotoxin (Quandt et al., 1982; Nagy et al., 1983), increased in amplitude when the external Na concentration is raised (Nagy et al., 1983; Yamamoto et al., 1984), decreased when the internal Na concentration is raised (Nagy et al., 1983), and reverse at the predicted Na equilibrium potential (Nagy et al., 1983). Consistent with those reports, raising the internal Na concentration decreased the single-channel current amplitude, suggesting that the currents studied here are through Na channels.

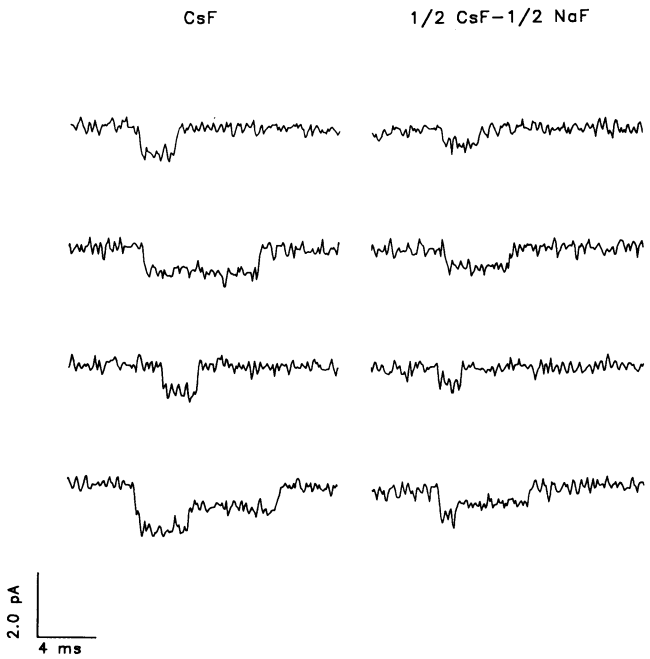

FIGURE 5 Single-channel records from a single inside-out excised patch. Solution bathing the cytoplasmic face was normal internal recording medium for the left-hand column and internal medium with half the CsF replaced with NaF for the right-hand column. Single-channel currents are clearly smaller in the presence of internal Na. Holding potential was −120 mV, and the potential during the step was −10 mV. Filtered at 2 KHz. 6°C.

I thank J. A. Michaels and C. Leffingwell for design and construction of some of the electronic equipment, W. T. Sinclair and W. G. Knapik for construction of the recording chamber and other components, and Drs. W. J. Lederer and M. F. Schneider for critical reading of the manuscript. Dr. R. D. Koos kindly allowed access to culture facilities during early stages of this project, and Dr. D. L. Gill patiently and repeatedly provided cells.

This work was supported by grant NS07734 from the National Institutes of Health, a grant-in-aid from the American Heart Association, a grant from the Bressler Research Fund, University of Maryland, and a Special Research Initiative Support award from the School of Medicine, University of Maryland.

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