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Predominance of Poorly Reopening Single Na^+ Channels and Lack of Slow Na^+ Inactivation in Neonatal Cardiocytes

M. Kohlhardt, H. Fichtner, and U. Fröbe
Physiological Institute, University of Freiburg, Freiburg/Br., West Germany

Summary. Elementary Na^+ currents through single cardiac Na^+ channels were recorded at −50 mV in cell-attached patches from neonatal rat cardiocytes kept at holding potentials between −100 and −120 mV.

Na^+ channel activity may occur as burst-like, closely-timed repetitive openings with shut times close to 0.5–0.6 msec, indicating that an individual Na^+ channel may reopen several times during step depolarization. A systematic quantitative analysis in 19 cell-attached patches showed that reopening may be quite differently pronounced. The majority, namely 16 patches, contained Na^+ channels with a low tendency to reopen. This was evidenced from the average value for the mean number of openings per sequence, 2.5. Strikingly different results were obtained in a second group of three patches. Here, a mean number of openings per sequence of 3.42, 3.72, and 5.68 was found. Ensemble averages from the latter group of patches revealed macroscopic Na^+ currents with a biexponential decay phase. Reconstructed Na^+ currents from patches with poorly reopening Na^+ channels were devoid of a slow decay component. This strongly suggests that reopening may be causally related to slow Na^+ inactivation. Poorly pronounced reopening and, consequently, the lack of slow Na^+ inactivation could be characteristic features of neonatal cardiac Na^+ channels.

Key Words slow Na^+ inactivation · Na^+ channel kinetics · reopening · neonatal cardiocytes

Introduction

Voltage-dependent inactivation represents an outstanding property of Na^+ channels in excitable membranes, including the sarcolemma of heart muscle. This process becomes rapidly dominating during depolarization and, by effectively terminating the open state of Na^+ channels, controls the Na^+ permeability of the membrane. Evidence has been accumulated from several voltage-clamped nerve preparations that the decay of Na^+ currents (I_{Na}) is more complex (for review *see* Meves, 1978) than predicted by the classical experiments of Hodgkin and Huxley (1952) in giant axons. The multiexponential I_{Na} decay disagrees with the conventional Na^+ inactivation defined as h-process in the Hodgkin-Huxley framework.

The same peculiarities were found in heart muscle. Methodologically improved, accurate I_{Na} measurements in several cardiac preparations including single myocytes (Brown, Lee & Powell, 1981; Kunze et al., 1985; Patlak & Ortiz, 1985) or Purkinje fibers (Fozzard et al., 1984), confirm earlier observations (Reuter, 1968; Dudel & Rüdel, 1970), evidenced a biexponential I_{Na} decay and indicated the coexistence of fast and slow Na^+ inactivation. The latter switches off a small portion of Na^+ conductance with time constants several-fold larger than the time constants governing fast inactivation kinetics. Chiu (1977) explained this phenomenon with two inactivated states but the differential TTX- and temperature sensitivity of fast and slow I_{Na} components in the frog node suggests two classes of channels (Benoit, Corbier & Dubois, 1985).

Slow Na^+ inactivation seems not to be obligatory in cardiac Na^+ channels. While the decay of I_{Na} in adult myocytes always follows, in a broad potential range, second order kinetics (Brown et al., 1981; Patlak & Ortiz, 1985), in embryonic heart cells, I_{Na} was reported to be devoid of a slow decay component (Ebihara et al., 1980). The present single Na^+ channel study concentrated on this discrepancy. Employing neonatal cardiocytes for the cell-attached patch-clamp experiments, slow Na^+ inactivation proved to be rare in this particular cell type and was only detectable when Na^+ channels with a high tendency to reopen (and/or with slow activation) were operating.

Materials and Methods

Elementary currents through single Na^+ channels were recorded at 19°C in cell-attached patches from cultured neonatal rat cardiocytes with an L/M-EPC 5 amplifier (List Electronic, Darm-

stadt) by the use of the standard patch-clamp technique (Hamill et al., 1981). Conventional fire-polished and Sylgard-coated patch pipettes (resistance 3–10 MΩ after filling with pipette solution, *see below*) suitable to allow seal resistances of at least 100 GΩ to develop were employed. Details of the cell culture, which combines the techniques elaborated by Mark and Strasser (1966) with the differential attachment technique of Blondel, Riojeu and Cheneval (1971) have been already described (Kohlhardt, Fröbe & Herzig, 1986). Briefly, the hearts of newborn rats were rapidly removed, minced, and subsequently disaggregated in a nominally Ca^{2+}-free, trypsin-containing saline. The myocytes were either seeded immediately or stored in CMRL medium supplemented with 10% fetal calf serum for up to 3 days before culturing for 16–20 hr. Most of the patch-clamp experiments used myocytes in an early developmental stage still having a spherical shape, which is the predominant configuration of short-time cultured cells.

After an initial equilibration in modified, Ca^{2+}-poor (0.1 mmol/liter) Tyrode solution for 5 min, the myocytes were exposed to an isotonic K^+ saline (140 mmol/liter K^+) in order to depolarize them to approximately 0 mV. Under these conditions, their tendency to beat spontaneously is abolished, and the patch stability becomes improved by eliminating cell movements. When measured in the whole-cell clamp configuration, the resting potential of K^+-depolarized cardiocytes was found to be close to 0 mV. Therefore, the holding and step potentials were related to this value and given in absolute terms.

The cell-attached patches were kept at a holding potential between −100 and −120 mV and stepped at a rate of 0.5 Hz to −50 mV for 120 msec. The command impulses were delivered from a conventional stimulator. The patch-clamp recordings were filtered (8-pole Bessel filter) at 1 kHz, digitized by a microcomputer with a sampling rate of 5 kHz, and stored on floppy discs. Na^+ channel detection required, under these recording conditions, a minimal open time of 0.2 msec.

The records were corrected for a residual capacity transient not compensated at the level of headstage and for leakage currents. The 50% unitary current method (Colquhoun & Sigworth, 1983) was applied in order to analyze the open time of and the shut time between single Na^+ channel events from samples without superpositions. Mean open time ($\bar{t}_{open}$) was calculated from $\bar{t}_{open} = \Sigma_i n_i T_i / n$ and the mean shut time ($\bar{t}_{shut}$) from $\bar{t}_{shut} = \Sigma_i n_i T_i / n$ (Fenwick, Marty & Neher, 1982), where T_i is the duration of n_i open channels and the duration of n_i shut events, respectively, and n means a count of events. The frequency distribution of the open time and of the shut time yielded τ_{open} and τ_{shut}, respectively, which resulted from the best fit of the open time and shut time histograms according to the least squares (X^2) method.

Records showing channel activity are referred to as activity sweeps and those without detectable openings as blank sweeps. No attempt was made to correct the data for unresolved events. From overlapping events, a minimum estimate (Aldrich, Corey & Stevens, 1983) of the number of Na^+ channels in an individual patch was obtained, referred to as number of functioning channels.

Some of the data are given as mean ± SEM.

Solutions (Composition in mmol/liter)

Modified Tyrode solution: NaCl 137, KCl 5.4, $CaCl_2$ 0.1, $MgCl_2$ 5, Na-pyruvate 5, glucose 20, HEPES 10, at pH 7.4. Isotonic K^+ saline: KCl 140, $CaCl_2$ 0.1, MgCl 5, Na-pyruvate 2.5, glucose 20, HEPES 10, at pH 7.4. Pipette solution: NaCl 200, $CaCl_2$ 0.2, $MgCl_2$ 5, HEPES 10, at pH 7.4. Bath temperature (controlled by a Peltier element) 19 ± 1°C.

Results

Elementary Na^+ currents could be triggered in about 50% of the cell-attached patches. A so-far unexplained, but well-documented run-down (Kunze et al., 1985; Kohlhardt & Fichtner, 1988) of the initially high Na^+ channel activity developed in about 20–30% of the patches during the first 5–10 min of their lifetime. The experimental protocol, therefore, included an initial equilibration period of 10 min so that patches with run-down could be discarded before the sweep collection began. To obtain a sufficiently large ensemble from an individual patch, only experiments with a minimum patch lifetime of 30 min were considered for analysis.

Figure 1 compares elementary Na^+ currents recorded in two cell-attached patches under identical conditions, including the tip geometry of the patch pipettes (resistance in both experiments 3 MΩ) from two neighboring cardiocytes of the same culture. The cells had been stored for two days at 4°C before seeding and were cultured for 17 hr. They showed a spherical shape with diameters of 14 and 16 μm, respectively, and, as another common morphological feature, a slightly granulated cytoplasm. The minimum estimate of functioning Na^+ channels from overlapping events revealed a number of three channels in patch 389CA and of two channels in patch 390CA. Na^+ channel activity in the two patches differed strikingly in two aspects, namely in the number of openings during depolarization and, consequently, in the pattern of opening: a considerable fraction of channel openings occurred during the later stage of membrane depolarization, ca. 50–120 msec after the onset of the command impulse, in patch 389CA with the large tendency of sequential Na^+ channel openings, but not in patch 390CA where this phenomenon was less pronounced. Taking activity sweeps without superpositions for statistical analysis, an activity sweep fraction of 89% showed sequential events in patch 389CA, but of only 36% in patch 390CA. As recently described (Kohlhardt & Fichtner, 1988), the number of events per sequence is monoexponentially distributed (*see* Fig. 1*B* and *C*). This frequency distribution yielded a mean value of openings per sequence of 5.68 in patch 389CA, but of 2.72 in patch 390CA.

Ensemble averaging of the elementary Na^+ currents in patch 389CA revealed a macroscopic I_{Na} whose decay could be best fitted by two exponentials (Fig. 2*A*). The semilogarithmic plot of I_{Na} decay against time showed an initial fast component

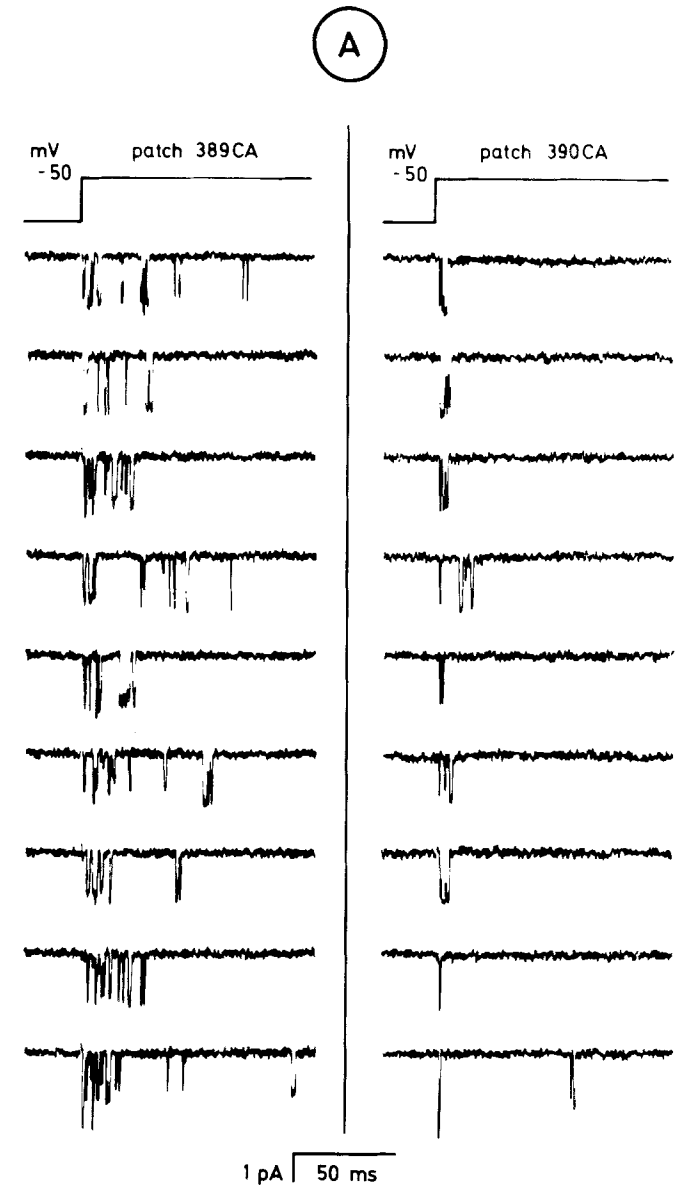
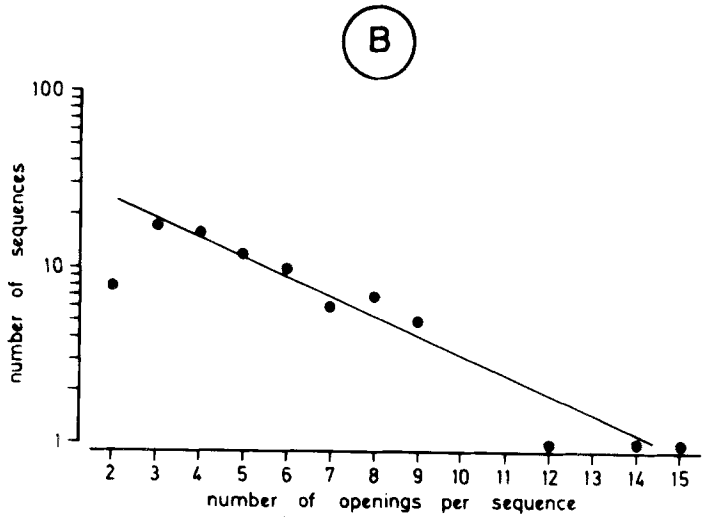
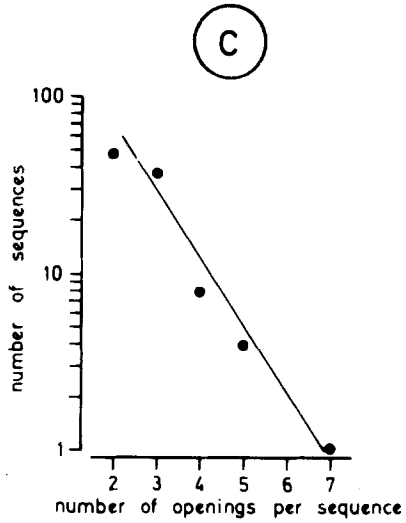


Fig. 1. (*A*) Records of elementary Na^+ currents in two individual cell-attached patches (patch 389CA, left, and patch 390CA, right) during 120 msec lasting step depolarizations from a holding potential of −100 to −50 mV. (*B* and *C*) Frequency distributions of the number of Na^+ channel openings per sequence in patch 389CA (*B*) and patch 390CA (*C*)





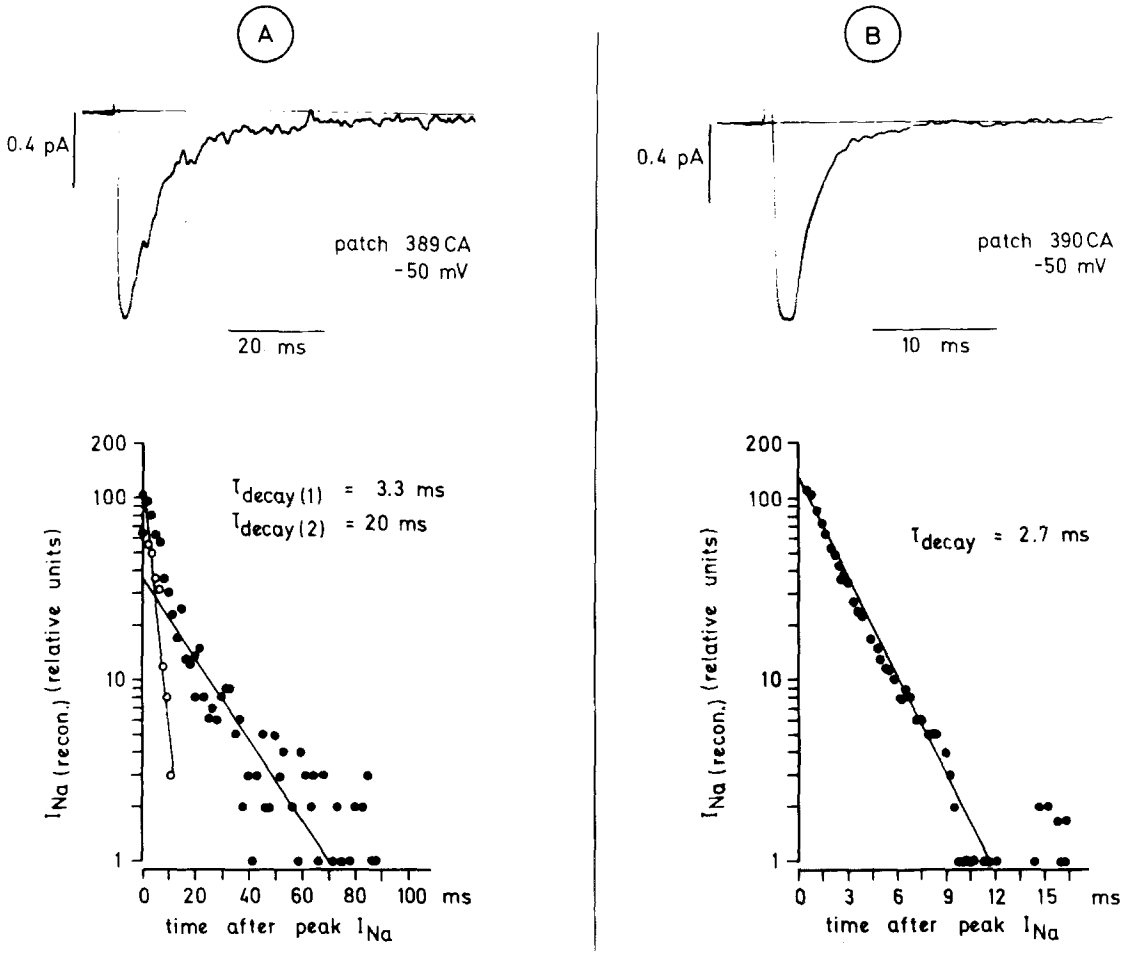


Fig. 2. Different I_{Na} decay kinetics. (*A*) Reconstructed I_{Na} from the ensemble average of 200 sweeps in patch 389CA (upper part) and a semilogarithmic plot of the I_{Na} decay against time after peak I_{Na} (lower part). (*B*) Reconstructed I_{Na} from the ensemble average of 416 sweeps in patch 390CA (upper part) and a semilogarithmic plot of the I_{Na} decay against time after peak I_{Na} (lower part). Note the difference in time scale between *A* and *B*

Table 1. Two decay components of reconstructed I_{Na} in three out of 19 cell-attached patches[a]

HP (mV)	*N*	$I_{Na(peak)}$ (pA)	$\tau_{decay(fast)}$ (msec)	$\tau_{decay(slow)}$ (msec)	$A_{(fast)}$ (pA)	$A_{(slow)}$ (pA)	$\tau_{(s)}/\tau_{(f)}$	Patch
−100	3	1.41	3.3	20	1.21	0.41	6.1	389CA
−120	2	0.46	1.7	14	0.34	0.20	8.2	398CA
−120	2	0.77	2.9	13	0.63	0.24	4.4	407CA

[a] HP means holding potential and *N* the number of functioning Na^+ channels. $A_{(fast)}$ and $A_{(slow)}$ is the current amplitude obtained by extrapolating the fast and the slow current decay to the current axis in semilogarithmic plots of the reconstructed I_{Na} against time after peak I_{Na}. $\tau_{(s)}$ and $\tau_{(f)}$ means $\tau_{decay(slow)}$ and $\tau_{decay(fast)}$. The reconstructed I_{Na} resulted from an ensemble average of 200 sweeps in patch 389CA, of 536 sweeps in patch 398CA, and of 403 sweeps in patch 407CA. In the 16 patches without detectable slow I_{Na} decay, the individual ensemble averages comprised, as a minimum, 400 sweeps.

with a time constant of 3.3 msec which was followed by a well-developed, about sixfold slower decay component. By extrapolating to the current axis, the latter was found to govern the inactivation of a peak current of approximately 0.4 pA, compared with a current of 1.4 pA which decays with fast kinetics. As expected from the pattern of channel activity, the macroscopic I_{Na} reconstructed

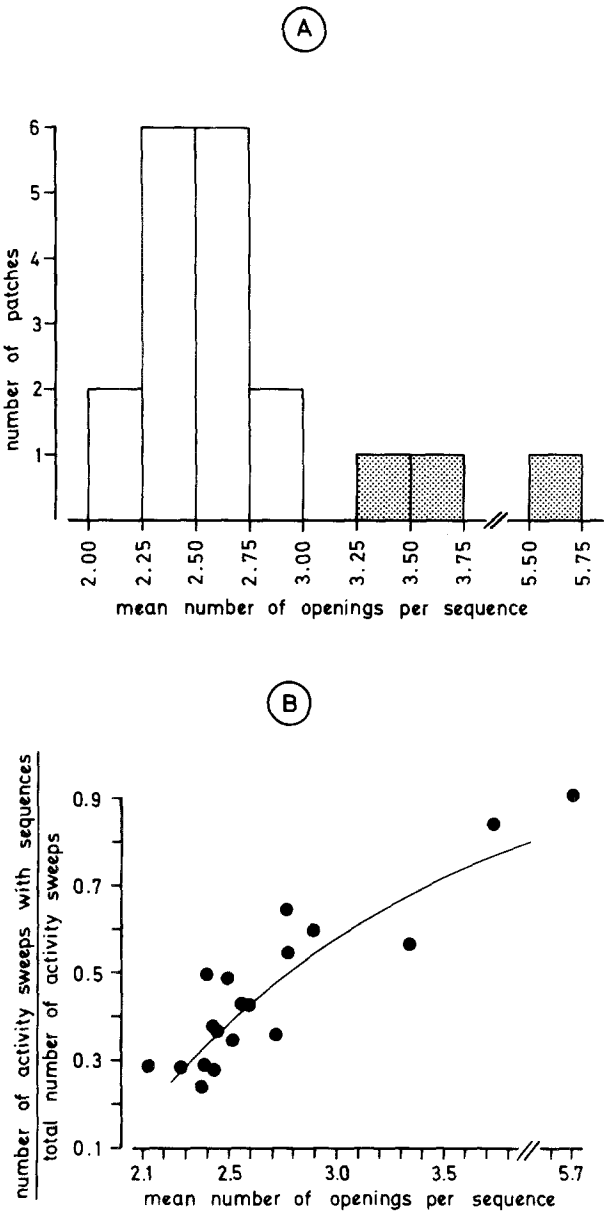


Fig. 3. (*A*) Frequency distribution of the mean number of Na^+ channel openings per sequence in 19 cell-attached patches. The shadowed columns indicate those patches where ensemble averaging showed reconstructed macroscopic Na^+ currents with a biexponential decay (patches 389CA, 398CA, and 407CA). (*B*) The dependence of the incidence of activity sweeps with sequential Na^+ channel openings on the mean number of openings per sequence. Each symbol represents an individual patch. The curve relating the ordinate values to the abscissa was drawn by eye

from the ensemble in patch 390CA was lacking a slow decay component (Fig. 2*B*). The best fit revealed single exponential inactivation kinetics, at least for the decay of 99% of peak I_{Na}.

In a total of 19 cell-attached patches analyzed under the experimental conditions just mentioned and uniformly containing 2–3 functioning Na^+ channels, the ensemble averages, each consisting of 200–700 sweeps, revealed in 16 patches a macroscopic I_{Na} whose decay could be best fitted by a single exponential. The same result was obtained from a biased ensemble average which excluded blank sweeps in order to improve the detectability of the second decay component: the decay of 99% of peak I_{Na} followed a single time constant.

A biexponential decay of macroscopic I_{Na} was detected in only three patches (Table 1). The ratio $\tau_{decay(slow)}/\tau_{decay(fast)}$ amounted to 6.2 ± 1.1 (at −50 mV). This is somewhat larger than the value (2.5) reported from conventional Na^+ current measurements in adult myocytes at the same potential (Brown et al., 1981) but well agrees with the ratio (5.7) found likewise in reconstructing I_{Na} from elementary Na^+ currents in these cells (Patlak & Ortiz, 1985). The patches 389CA, 398CA and 407CA with the detectable slow I_{Na} decay component are exceptional in yet another aspect. They showed the Na^+ channel activity with the largest tendency for sequential openings (Fig. 3*A*) when compared with the 16 patches without slow I_{Na} decay, namely 3.42, 3.72, and 5.68 mean number of openings per sequence. As an average in the 16 patches without slow I_{Na} decay obtained from the Gaussian distribution in Fig. 3*A*, the mean number of openings per sequence was 2.5. This Gaussian frequency distribution is valuable for still another reason. As the number of channel openings is voltage-dependent (Kunze et al., 1985), such a comparative analysis among individual cell-attached patches may be complicated by a systematic error in the absolute level of the step potential. The step potential given to be −50 mV is based on the assumption that the resting potential is, in 140 mmol/liter external K^+, 0 mV but ignores a possible deviation of about ±5 mV. The Gaussian distribution can be expected to largely eliminate this uncertainty and makes it possible to interpret these kinetic data with reasonable significance.

The existence of more than one channel complicates the interpretation of sequential openings. Under similar experimental conditions, evidence has been presented that an individual Na^+ channel may open more than once during step depolarization (Kunze et al., 1985; Patlak & Ortiz, 1985). The simplest argument in favor of this idea is that the number of openings in a sequence may exceed by far the number of functioning channels in the patch. Moreover, closely timed, sequential opening events separated by gaps from 0.5 to 0.6 msec are much more indicative of reopening of an individual Na^+ channel than of the opening of two different Na^+ channels. This gap time was consistently obtained from

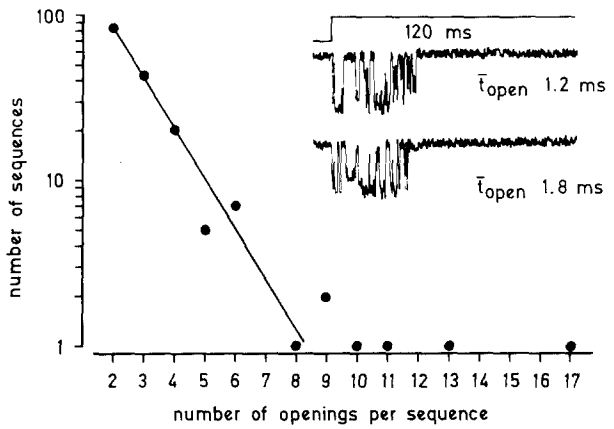


Fig. 4. Unusual frequency distribution of the number of Na⁺ channel openings per sequence. The data points obtained in patch 393CA can only be fitted by a single exponential when the sequences with the largest number of openings are disregarded. The inset shows two of the ultralong sequences, one of them with normal (1.2 msec) and the other with increased (1.8 msec) open time

a systematic histogram analysis in the 19 cell-attached patches. The shut time was monoexponentially distributed with time constants just mentioned in seven patches. The shut-time histogram consisted of the sum of two exponentials in 11 patches including the three patches with slow I_{Na} decay. The biexponential shut-time distribution yielded a $\tau_{shut(1)}$ being close to 0.6 msec while $\tau_{shut(2)}$ ranged between 1.9 and 5.9 msec. In the patches with slow I_{Na} decay, $\tau_{shut(2)}$ amounted to 4.2, 5.2, and 5.9 msec. In order to interpret sequential openings as arising mainly from reopening, it is important to see that the overwhelming number of shut events were fitted by the first exponential which yields $\tau_{shut(1)}$. This was obtained from the area under either exponential which is proportional to the number of events and expressed as a ratio calculated from

$$\int_{0.2\,\text{msec}}^{\infty} a_1 e^{\frac{-t}{\tau_{shut(1)}}} \Big/ \int_{0.2\,\text{msec}}^{\infty} a_2 e^{\frac{-t}{\tau_{shut(2)}}}$$

where a_1 means the amplitude of the first and a_2 the amplitude of the second exponential. This ratio was, in the patches with slow I_{Na} decay, 54.4, 66.5, and 71.5 but amounted to, in the patches without slow I_{Na} decay, 27.4 ± 4.8 (n = 16). As the shut time was not corrected for unresolved events, the number of short closings is underestimated so that their contribution is in reality still larger than assumed from the calculated ratio.

That the mean number of openings per sequence mainly reflects the tendency to reopen also becomes evident from Fig. 3*B*. This relationship, furthermore, is consistent with the prediction that the patches with slow I_{Na} decay are characterized by the largest incidence of sweeps with sequential Na⁺ channel activity. It should be emphasized, however, that particularly sequential openings with longer gap times are ambiguous in nature. They may arise with a considerable likelihood from the activity of two channels. This clearly invalidates the mean number of openings per sequence as a measure which could be taken to statistically define the reopen tendency.

Ultralong opening sequences that do not fit the monoexponential frequency distribution (Fig. 4) occasionally occurred in eight patches including two of the patches with slow I_{Na} decay. They consisted of up to 19 closely timed events. The contribution of this sample type to sweeps with sequential activity was also far below 1% in the patches with slow I_{Na} decay. This extremely low incidence excludes that this activity pattern is important for the appearance of slow I_{Na} decay. Open-state kinetics during such episodes are not different from those observed in the whole ensemble of activity sweeps. The only exception is illustrated in the inset of Fig. 4. One of the ultralong sequences showed a 1.5-fold larger $\bar{t}_{open}$ (1.8 msec compared with 1.2 msec in the ensemble of this patch). But even in this case, repetitive Na⁺ channel activity ceased spontaneously within some 10 milliseconds after the onset of the command impulse and did not persist during the whole duration of membrane depolarization. With respect to the 120-msec lasting step depolarization, open probability during ultralong sequential activity never exceeded a value of 25.8%, the maximum P_o observed. It is noteworthy that similar episodes were reported in adult cardiac Na⁺ channels (Patlak & Ortiz, 1985) but not in neuroblastoma Na⁺ channels (Aldrich & Stevens, 1987).

Most of the patch-clamp experiments reported here were performed with unstored neonatal cardiocytes, i.e. cells that had been cultured just after enzymatic disaggregation. Only one out of these 11 patches contained Na⁺ channels with the large tendency to reopen (mean number of openings per sequence, 3.72; 83% sweeps with sequential activity) and showed a biexponential I_{Na} decay. This is noteworthy since each set of four experiments with cardiocytes stored for 24 or 48 hr yielded a patch containing Na⁺ channels that showed this particular activity pattern.

In an attempt to elucidate still another different kinetic property, the open-state kinetics of slowly inactivating Na⁺ channels were compared with those of the dominant type, the poorly reopening Na⁺ channel. Open-time distributions were constructed from each individual patch and consisted

Table 2. Open time in patches with slow I_{Na} decay[a]

$\bar{t}_{open(ensemble)}$ (msec)	Events	$\bar{t}_{open(late\ openings)}$ (msec)	Events	Ignored period (msec)	Patch
1.06	866	1.11	250	0–25	389CA
1.10	575	1.17	106	0–15	398CA
0.90	832	0.83	190	0–15	407CA

[a] $\bar{t}_{open(ensemble)}$ means the open time obtained from an unbiased ensemble analysis of nonoverlapping single channel events. The ignored period corresponds to the duration of the fast I_{Na} decay component. It was determined from the semilogarithmic plot of reconstructed I_{Na} (*see* Fig. 2*A*), the end of the ignored period is the time when the fast I_{Na} decay has reduced the peak I_{Na} to 1% of the initial value. Consequently, events appearing after this time will be underlying the slow I_{Na} decay component and are labeled as late openings. Their separate analysis yields $\bar{t}_{open(late\ openings)}$.

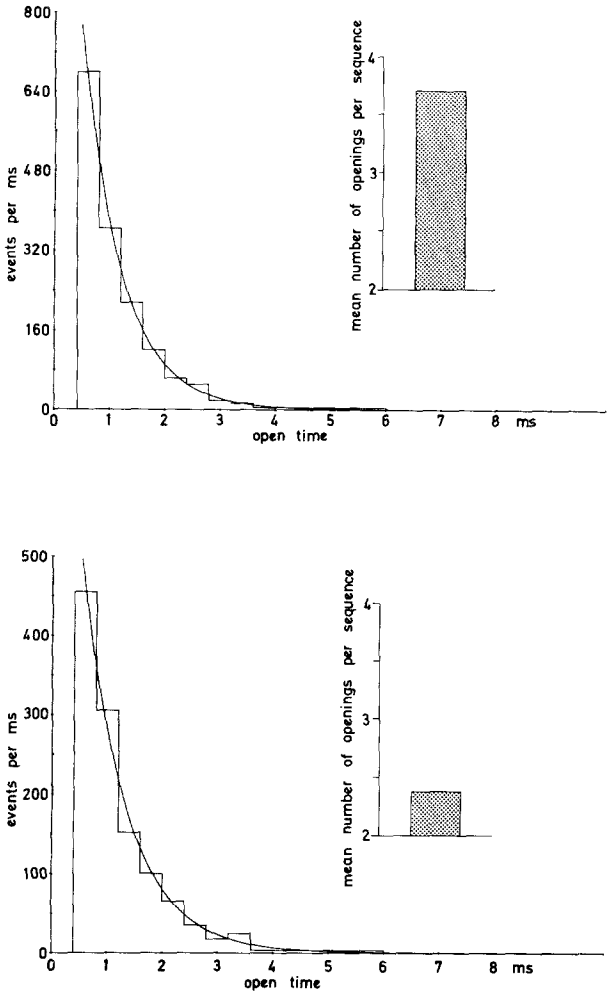


Fig. 5. Comparison of open time histograms. The upper histogram was constructed from a patch (407CA) with slow I_{Na} decay and a large number of Na^+ channel openings per sequence (*see* inset) and the lower histogram from a patch (336CA) without slow I_{Na} decay and a small number of Na^+ channel openings per sequence (*see* inset)

of 400 (as a minimum) to 800 nonoverlapping events. Consistent with earlier results in the same and other cardiac preparations (Cachelin et al., 1983; Grant, Starmer & Strauss, 1983; Kunze et al., 1985; Patlak & Ortiz, 1985; Kohlhardt, Fröbe & Herzig, 1987; Scanley & Fozzard, 1987), the open-time distribution could be best fitted by a single exponential, irrespective of the absence or presence of slowly inactivating Na^+ channels (Fig. 5). Accordingly, an open-time analysis, which only included events underlying the slow I_{Na} decay, revealed the same value for $\bar{t}_{open}$ as an unbiased analysis from the whole ensemble (Table 2).

Discussion

The present patch-clamp experiments confirm, first of all, earlier results in adult rat cardiocytes (Kunze et al., 1985; Patlak & Ortiz, 1985) that cardiac Na^+ channels may reopen during sustained depolarization, i.e., they can cycle several times between the open and a closed state until they finally attain the absorbing (Aldrich et al., 1983) inactivated configuration. The results also show that two types of Na^+ channels can be distinguished. One of them may be characterized by a low tendency to reopen. This resembles kinetic properties of neuroblastoma Na^+ channels. They were reported to reopen after having closed with a low, but voltage-dependent probability (Aldrich & Stevens, 1987). The poorly reopening Na^+ channel was dominant in 16 out of 19 cell-attached patches. The other type, an apparently high reopening Na^+ channel, was operating in only three patches. Only in the presence of the latter did a biexponential I_{Na} decay become detectable. This coincidence is noteworthy since it eliminates the possible objection that the lacking slow I_{Na} component in the 16 patches with low reopening

might be due to inaccuracies in evaluating the macroscopic I_{Na}. The interesting biological aspect is that this coincidence focuses on the genesis of slow Na^+ inactivation.

The tendency of Na^+ channels to reopen seems to be causally related to slow Na^+ inactivation. As shown by Patlak and Ortiz (1985), reopening can be explained by the model of Chiu (1977), which assumes two inactivated states, one of them governing fast I_{Na} decay and the other by allowing multiple reopening, governing slow I_{Na} decay. Support for this idea arises from the fact that late openings, as underlying the slow I_{Na} decay component, are indistinguishable from early openings during the first few milliseconds after the onset of step depolarization. They have the same open-state kinetics and, as demonstrated by Patlak and Ortiz (1985), the same reversal potential and an identical unitary conductance.

It should be emphasized that, despite its dominating character, reopening is not necessarily the only reason for slow Na^+ inactivation. Sequential Na^+ channel activity with shut times far longer than 1 msec (*see* Fig. 1*A*, patch 389CA) is ambiguous in nature and may result to a certain extent from the contribution of a late-activating Na^+ channel. The latter would represent a separate population having a latency several times longer than in the early activating Na^+ channel population. In fact, late openings were reported to possess a reduced sensitivity to tetrodotoxin (TTX) (Ten Eick, Yeh & Matsuki, 1984). This could be consistent with the differential TTX- and temperature sensitivity of fast and slowly inactivating Na^+ currents in the frog node of Ranvier (Benoit et al., 1985).

Slow Na^+ inactivation was, in the present experiments and in striking contrast to experiments with adult cardiocytes (Brown et al., 1981; Patlak & Ortiz, 1985; Clark & Giles, 1987), a rare phenomenon not existent in the majority of the cell-attached patches from neonatal cardiocytes. The detectability of slow Na^+ inactivation seems to be dependent on the choice of an appropriate test potential (Clark & Giles, 1987; Patlak & Ortiz, 1985) because, in adult ventricular myocytes, this process has been reported to disappear at a membrane potential more negative than -60 mV. Moreover, slow I_{Na} decay seems to be not prominent at very negative holding potentials (Patlak & Ortiz, 1985). This is important to note in that, in our study, the elementary Na^+ currents were analyzed at -50 mV, from holding potentials between -100 and -120 mV. This, consequently, implies that an inadequate experimental protocol cannot be the reason for this discrepancy.

It is, therefore, attractive to relate the lack of slow Na^+ inactivation to the special type of cardiocytes, cultured neonatal cells. The prevailing Na^+ channel in most of them seems to be the type with the low tendency to reopen. The lack of Na^+ channels with the apparently high reopen tendency (and/or with slow activation) is unlikely to reflect a culture artifact. This can be concluded from the observation that this activity pattern occurred with an even larger likelihood in neonatal cardiocytes that had been exposed to an extended, up to 48 hr, storage at 4°C before culturing. Although lifetime and turnover of cardiac Na^+ channels are, to our knowledge, still unknown, the *de-novo* channel protein synthesis may be suspected to require several ten hours. When injected with the Na^+ channel II-specific mRNA, *Xenopus* oocytes physiologically devoid of voltage-dependent Na^+ channels show, within 3–6 days, TTX-sensitive Na^+ currents (Noda et al., 1986). At least the neonatal cardiocytes used without storage for the present patch-clamp experiments should, therefore, still dispose of the same set of Na^+ channels as synthesized in vivo. If this holds true, it is tempting to argue that low reopening might be a characteristic kinetic property of neonatal cardiac Na^+ channels. This idea would be consistent with some other developmental aspects in Na^+ channel properties. Embryonic myoblasts were reported to have a higher portion of TTX-resistant, low conductance Na^+ channels than adult myotubes (Weiss & Horn, 1986).

Alternatively, poorly reopening Na^+ channels might be somehow due to a particular metabolic status which could dominate in cultured newborn cardiocytes. This explanation remains rather speculative unless an influence of cellular metabolism on reopening of cardiac Na^+ channels is proven. Although cAMP depresses the $^{22}Na^+$ flux through batrachotoxin-modified neuronal Na^+ channels to a certain extent (Costa & Catterall, 1984), the tendency to reopen must be not necessarily affected. In fact, in the presence of blocking antiarrhythmic drugs, reopening was recently found to be unaffected when compared with the activity pattern of cardiac Na^+ channels not treated with these drugs (Kohlhardt & Fichtner, 1988).

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