THE EFFECT OF TETRAMETHYLAMMONIUM ON SINGLE SODIUM CHANNEL CURRENTS

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ABSTRACT Voltage-dependent Na conductance of rat myotubes was studied by patch recordings of single-channels. The patches were excised from the cell with the patch electrode, and the cytoplasmic surface was bathed in either CsF or tetramethylammonium (TMA)-F. Inward currents were examined from −20 to −50 mV. In this range Cs and TMA both appeared to be nearly impermeant, but TMA blocked the channel in a voltage-dependent manner. A first-order blocking site was located a maximum of 89% of the way through the membrane field from the cytoplasmic surface.

INTRODUCTION

The Na channel of nerve and muscle is more selective for monovalent cations than for other ion species. It is also highly selective among various monovalent cations, and has a permeability sequence that is suggestive of a high field-strength interaction between the cation and the selectivity region of the channel (Hille, 1971, 1972, 1975*a*; Campbell, 1976; Pappone, 1980). The monovalent cation TMA(tetramethylammonium) is widely used as a Na substitute in studies of this channel, because it is impermeant and is believed to have no effect on the movement of other cations through the channel (see Hille, 1971, 1975*b*; Armstrong and Bezanilla, 1977; Wu et al., 1980; Oxford, 1981, for example). We have investigated the action of TMA on the Na channel of rat myotubes, using the excised patch technique (Horn and Patlak, 1980). We report here that TMA blocks Na channels from the cytoplasmic membrane surface in a voltage-dependent manner. A preliminary report has appeared (Horn et al., 1981*a*).

METHODS

We have used the patch method of recording single sodium channel currents, as introduced by Sigworth and Neher (1980) and modified by us (Horn and Patlak, 1980; Horn et al., 1981*b*). Briefly, a glass patch electrode containing Ringer's solution is pressed onto the surface of a rat myotube that has been grown under tissue-culture conditions. Suction is applied to the electrode so that the glass adheres to the membrane (Neher, 1981). When this is achieved, the electrode is withdrawn from the muscle surface, carrying with it the excised patch (Horn and Patlak, 1980). The bathing solution is changed from Ringer's solution to an "internal" (i.e. cytoplasmic-face) solution of either CsF or TMA-F. The excised patch is then voltage clamped to a holding potential of −110 mV. Na channels are activated by

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depolarizing voltage pulses to test potentials of −50 to −20 mV. Currents of amplitude >0.6 pA are easily resolved over the background noise of ~0.3 pA root mean square (rms) (filtered at a bandwidth of 1 kHz). The data were sampled every 130 μs by analog-to-digital converters with 12-bit resolution. The temperature in all experiments was maintained at ~10°C.

RESULTS

Fig. 1 shows single Na channel currents activated by voltage pulses to −40 and to −20 mV. At −40 mV the inward currents are only slightly smaller in amplitude when cytoplasmic

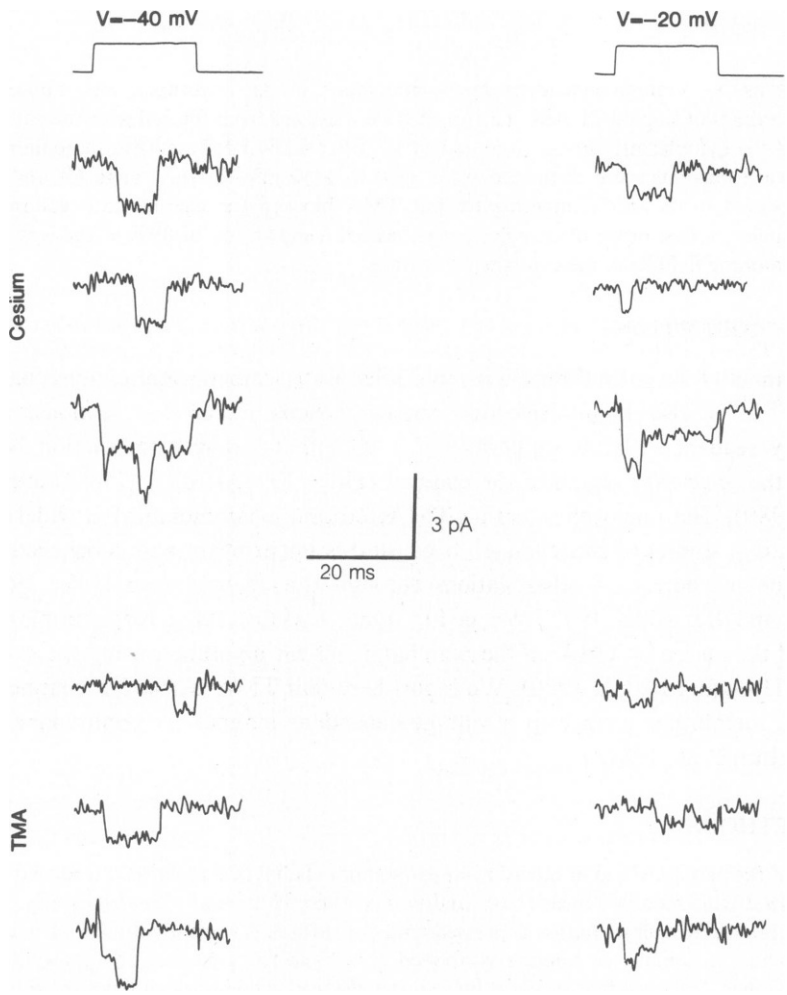

FIGURE 1 Single-channel recordings in Cs and TMA. The top and bottom halves show data from patches in Cs and TMA, respectively. The test potentials are shown above. Three individual traces are shown in each condition. Overlapping single-channel events are present in some records. Note that the currents are smaller in amplitude in the presence of TMA, especially at −20 mV. The myotubes were grown and used for experiments as described (Horn and Brodwick, 1980; Horn and Patlak, 1980). The Ringer's solution contains (in mM): 150 NaCl, 5 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 5 glucose, 5 HEPES at pH 7.4. The internal solution was either (in mM) 160 CsF + 5 Cs HEPES, or 160 TMA-F + 5 Cs HEPES, at pH 7.3. Other experimental details were described (Horn and Patlak, 1980; Horn et al., 1981*b*).

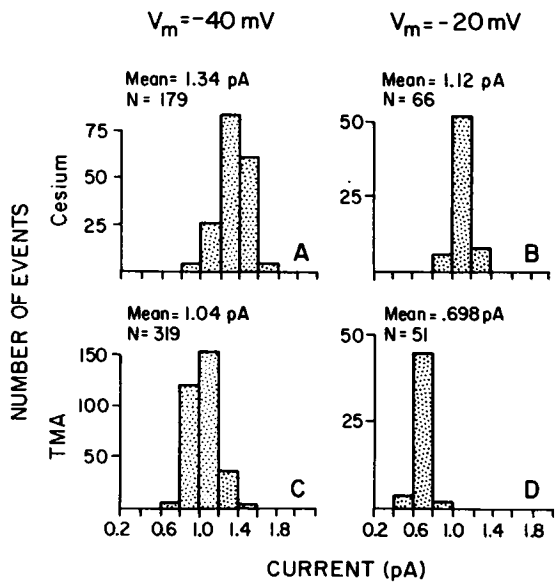

FIGURE 2 Histograms of current amplitudes. *A* and *B* were obtained from patches bathed in CsF. *C* and *D* were in TMA-F. *A* and *C* show amplitudes at −40 mV, *B* and *D* at −20 mV. Each histogram indicates the mean current in pA and the number of events (*N*). The histograms each contain data from two to four separate patches.

TMA replaces Cs. The 20-mV depolarization causes a 16% reduction in current in the presence of Cs, but a 33% decrease in the presence of TMA. In the former case the reduction could be the result of a decrease in driving force (Sigworth and Neher, 1980). The additional reduction in TMA can simply be explained as a voltage-dependent block, as discussed below.

Histograms of single current amplitudes are shown in Fig. 2. Notice that at both potentials, and with both Cs and TMA, the current histograms are unimodal, suggesting only one population of single-channel conductance under all conditions. The standard error of measurements was always <0.15 pA.

The amplitude of single-channel currents in the presence of Cs was a linear function of voltage in the range −50 to −10 mV (not shown). The conductance was 15.2 pS. This is close to the value of 18 pS obtained by Sigworth and Neher at 21°C, and suggests that Cs is inert, at least by comparison with the normal intracellular ions. In one experiment we saw no change in current amplitude when KF was substituted for CsF.

DISCUSSION

We propose the following model for the effect of TMA on the amplitude of single-channel currents. Suppose the Na channel has a TMA binding site located a fractional electrical distance of d through the membrane field from the cytoplasmic membrane surface. Suppose also that an insurmountable barrier lies just beyond this binding site. When TMA is located on the cytoplasmic membrane surface, it must hop over a smaller barrier to reach the binding site in the channel. Elementary considerations show that the dissociation constant, K_m, for TMA at this site is given by

$$K_m = \exp\left(G_s - \frac{dVF}{RT}\right) \tag{1}$$

where G_s is the energy of the site (in RT units) with respect to free solution; V is the membrane potential; and R, T, and F have their usual meanings. Then the fraction of time that the site is occupied by TMA is given by

$$\theta = \frac{[\text{TMA}]}{K_m + [\text{TMA}]} \tag{2}$$

where [TMA] is the activity of TMA. Assuming that Na current is proportional to $1 - \theta$, we can derive the following:

$$\ln\left(\frac{I_{Cs}}{I_{TMA}} - 1\right) = \frac{dVF}{RT} + A \tag{3}$$

where I_{Cs} and I_{TMA} are the currents in Cs and TMA, respectively; and $A = \ln[\text{TMA}] - G_s$.

Fig. 3 plots $\ln[(I_{Cs}/I_{TMA}) - 1]$ as a function of membrane potential. The relationship is well fitted by a straight line. The slope gives the value of $d = 0.89$ as the fractional electrical distance from the cytoplasmic membrane surface to the binding site.

After correcting for the activity coefficient for TMA (Butler, 1964), we can calculate a value for G_s of -1.33 kcal/mol. Eq. 1 shows that K_m is highly voltage dependent, decreasing threefold as the membrane is depolarized from -50 to -20 mV. The site is increasingly occupied by TMA as the membrane is depolarized. The value of θ increases accordingly from 0.16 at -50 mV to 0.37 at -20 mV.

This model is simplistic and ignores several possible complications. For example Na may compete with TMA for the binding site and relieve the block at negative potentials. This would make our apparent voltage dependence larger than the value for TMA alone. Such an error makes the value we obtained for d a maximum. It is also possible that Cs interacts with

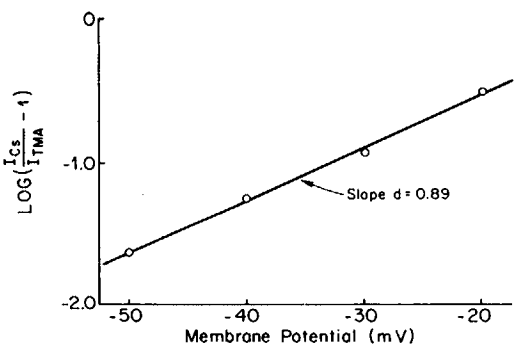

FIGURE 3 Voltage dependence of TMA block. Plot of $\ln[(I_{Cs}/I_{TMA}) - 1]$ vs. membrane potential. The data points were obtained from averages of from two to four patches, 51-398 events, under each condition. The regression line has a slope of $d = 0.89$, the fractional electrical distance from the cytoplasmic surface to the blocking site. The coefficient of determination $r^2 = 0.996$.

the same blocking site. Since we are examining relative effects of the two ions, ignoring a possible blocking action of Cs is the same as underestimating the magnitude of G_s.

We have assumed that TMA blocks the channel, and Cs is relatively inert. Logically it is possible that TMA is neutral and Cs enhances the inward Na current. We do not believe the latter possibility because the Na current in the presence of TMA rapidly approaches zero as the membrane potential approaches 0 mV. However, the expected reversal potential in the presence of a presumably inert cation is expected to be quite positive. It seems most likely to us that the rapid decrease of current with depolarization is the result of a blocking action. Another minor complication is that the value for G_s is obtained by assuming that the pre-exponential terms for the entry and exit rate constants are the same. This is unlikely to be true in general, and may depend on the "shape" of the barrier that TMA hops over (Horn and Stevens, 1980).

Our estimated value for the voltage dependence of TMA block is very steep. Although we have postulated that the value of d represents the location of the blocking site in the membrane field, it is also possible that TMA hops over multiple barriers on its way to the blocking site. In that case the value of d obtained from Fig. 3 can overestimate the electrical distance of the site (Hille and Schwarz, 1978). Such a model is supported by evidence for multiple ion occupancy of the Na channel of squid axon (Begenisich and Cahalan, 1980*a,b*). However for simplicity, and for the lack of sufficient data, we consider in the following calculations that TMA blocks by means of a simple first-order reaction.

Our data suggest that the block constitutes a rapid movement of TMA into and out of the channel. This is analagous to the proposed proton block of Na channels in nerve (Sigworth, 1980), the Ca block of the gramicidin channel (Bamberg and Lauger, 1977), and the Cs block of K channels in sarcoplasmic reticulum (Coronado and Miller, 1979). It is unlike the local anesthetic block of the acetylcholine channel, where the single-channel currents are clearly interrupted by the movement of the blocker into the channel without affecting the single-channel conductance (Neher and Steinbach, 1978). The time constant of the block must be faster than ~100 μs, or else we would have been able to resolve it. At a membrane potential of −20 mV this implies that the exit rate constant is > ~6.2 kHz, and the entry rate constant is > ~30 kHz/mol. The dwell time of TMA in its blocking site is then < ~160 μs. We might expect, in accordance with the above local anesthetic effect, that the apparent open-channel lifetime is increased by the TMA block. This is suggested by experiments in squid axon (Oxford and Yeh, 1979) and needs to be examined in detail. If TMA does interact strongly with the squid Na channel, experiments using internal TMA must be re-evaluated. It is possible that, even in the absence of Na currents, a blocker such as TMA can affect gating currents (see Cahalan and Almers, 1979*a,b*).

Although we have not studied the action of extracellular TMA, Hille (1975*b*) has shown that it has no blocking action externally. It is therefore tempting to propose that, like local anesthetics (Strichartz, 1973; Cahalan, 1978), TMA only blocks the Na channel from the cytoplasmic surface. This is entirely analagous to the situation obtained with the acetylcholine channel, which is only blocked by anesthetics from the extracellular membrane surface (Horn et al., 1980). Such an asymmetry of block suggests an asymmetrical barrier structure to ion permeation in these two types of channels. In particular we might expect a larger barrier near the extracellular membrane surface of the Na channel. Such a structure has been proposed for

the movement of Na ions through the channel (Hille, 1975*b*; Begenisich and Cahalan, 1980*a,b*). It is possible that this larger barrier is rate limiting at some potentials and also the "selectivity filter" of the channel.

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