Expressed Na channel clones differ in their sensitivity to external calcium concentration

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INTRODUCTION

Ca^{2+} has long been known to suppress the excitability of nerve and muscle cells, largely by reduction of Na^+ influx through Na channels. Two effects of raising extracellular [Ca^{2+}] have been described, a decrease of the open probability and a reduction of the single channel conductance, γ. The former action of Ca^{2+} is often ascribed to a shift of the voltage dependence of activation gating by screening of negative charges located either on the Na channel itself or on the surrounding lipid. The reduction of γ is thought to be caused primarily by a rapid block of open channels by Ca^{2+} ions in the pore. A recent report suggests that both actions might be due to Ca^{2+} binding to a common site (1).

Ca^{2+} binding is likely to involve an acidic amino acid, because trimethyloxonium (TMO), which O-methylates carboxyl oxygens, reduces the Ca^{2+} block of open Na channels (2–4). TMO treatment, which removes a negative charge, has two other related effects. It abolishes the extracellular block of the cationic toxins tetrodotoxin (TTX) and saxitoxin (STX), and it reduces γ (2–4). It has also been shown that a point mutation (Glu-to-Gln) in an extracellular region of the Na channel abolishes TTX sensitivity (5). These data suggest that negative charges near the extracellular mouth of the Na channel play a role in producing high affinities for all cations, including Ca^{2+}, TTX^+, STX^{2+}, as well as extracellular Na^+ (thus enhancing γ).

A search for all the relevant acidic amino acids by point mutagenesis is a herculean task. Fortunately nature has provided a TTX-insensitive (TTX-I) variant of a Na channel in mammalian striated muscle. Single channel recording from neonatal rat skeletal muscle cells showed that TTX-I channels had a lower γ and a reduced sensitivity to Ca^{2+} block than did TTX-S channels in the same cells (6, 7), consistent with the above scenario. These studies were hampered by the presence of a heterogeneous population (TTX-S and TTX-I) of channels in the same patch, and by the absence of a molecular identity of the functional channels. Recently the TTX-S and TTX-I channels of rat skeletal muscle cells were cloned and expressed in *Xenopus* oocytes (8–10), allowing measurements to be made on each channel isoform independently. We report here the effect of [Ca^{2+}] on outside-out patches from oocytes injected with cRNA transcribed from these two clones.

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MATERIALS AND METHODS

Full length cDNA constructs were constructed in the *Xenopus* expression vector pSP64T, and synthetic sense cRNA was transcribed in vitro and injected into *Xenopus* oocytes, as described previously (8, 10, 11). The clones we used correspond to TTX-S channels (rSkM1) or TTX-I channels (rSkM2).

Macroscopic and single-channel currents from cRNA-injected oocytes were measured in outside-out patches. The bath solution was (in millimolar): 150 NaCl, 2 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 Hepes (pH 7.4). The pipet solution was 130 CsF, 10 CsCl, 5 EGTA, and 10 Cs-Hepes (pH 7.3). Variations in [Ca^{2+}] were effected without correction for changes in ionic strength or osmolarity. Temperature, 21°C. Technical details of recording and data analysis are given in reference 11.

RESULTS AND DISCUSSION

Outside-out macropatches were used to measure macroscopic Na^+ currents. Increasing [Ca^{2+}] from 1.5 to 10 mM reduced the currents for both the TTX-S clone rSkM1 and the TTX-I clone rSkM2, and caused a shift of the peak current-voltage relationship in a depolarizing direction (Fig. 1). The effects of [Ca^{2+}] were qualitatively similar for both types of currents. The reduction of Na^+ current could be due to actions of Ca^{2+} on the open probability (e.g. due to screening of negative surface charges) and/or the single-channel current amplitudes. To distinguish these possibilities we resorted to single channel recording in outside-out patches.

Fig. 2 shows examples of single-channel currents from an oocyte expressing either rSkM1 (above) or rSkM2 (below) at two test potentials. The amplitudes of the TTX-S singles are larger than those of the TTX-I

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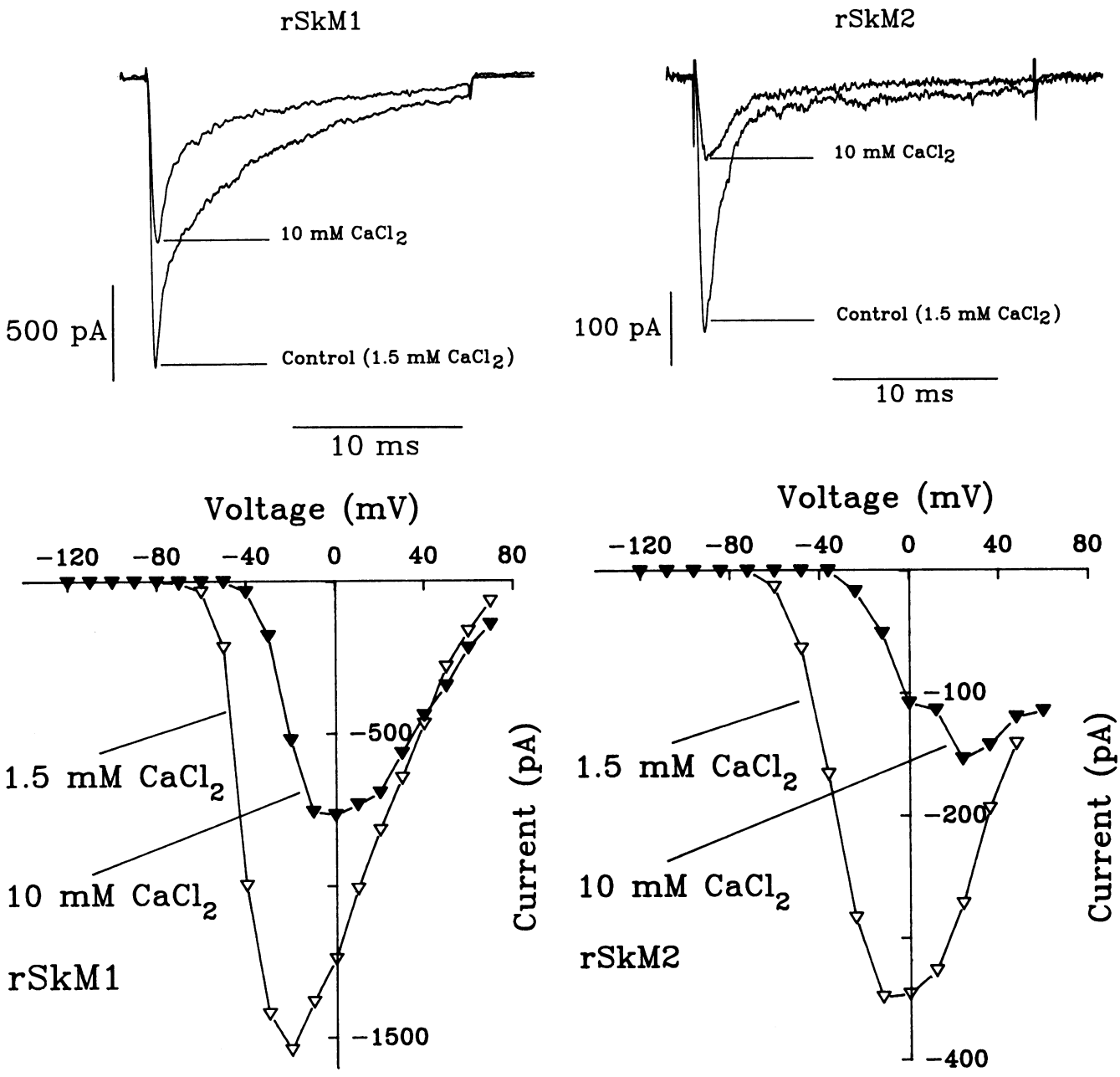

FIGURE 1 Ca^{2+} block of macroscopic currents in macropatches. *A* and *B* show currents elicited in rSkM1 and rSkM2 by depolarizations from −100 mV to a test potential of −20 mV and the reduction of current by increasing $[Ca^{2+}]$ from 1.5 to 10 mM. Note the slow inactivation typical for rSkM1 expressed in oocytes. *C* and *D* show the peak *I-V* relationships for the currents from the same patches.

singles, in agreement with patch recordings from rat muscle cells (6). The current-voltage (i-V) relationships for these amplitudes are approximately linear for both types of channel, with conductances of 32 pS for rSkM1 and 10 pS for rSkM2 (11). The conductance of rSkM2 is similar to that seen for TTX-I channels in patches from neonatal rat muscle at 9.3°C, whereas that of rSkM1 is almost three times larger in oocytes than in muscle.

Fig. 3 shows the *i-V* curves for these two channel types (squares indicate 10 mM Ca^{2+} and circles 0 Ca^{2+}). In the absence of Ca^{2+} the conductances were 42 pS for rSkM1 and 13 pS for rSkM2. The TTX-S channel was much more sensitive than the TTX-I channel to $[Ca^{2+}]$. At $[Ca^{2+}]$ = 10 mM the *i-V* relationship was outwardly rectifying in rSkM1, consistent with a rapid open-channel block, the blocking site located 19% of the way

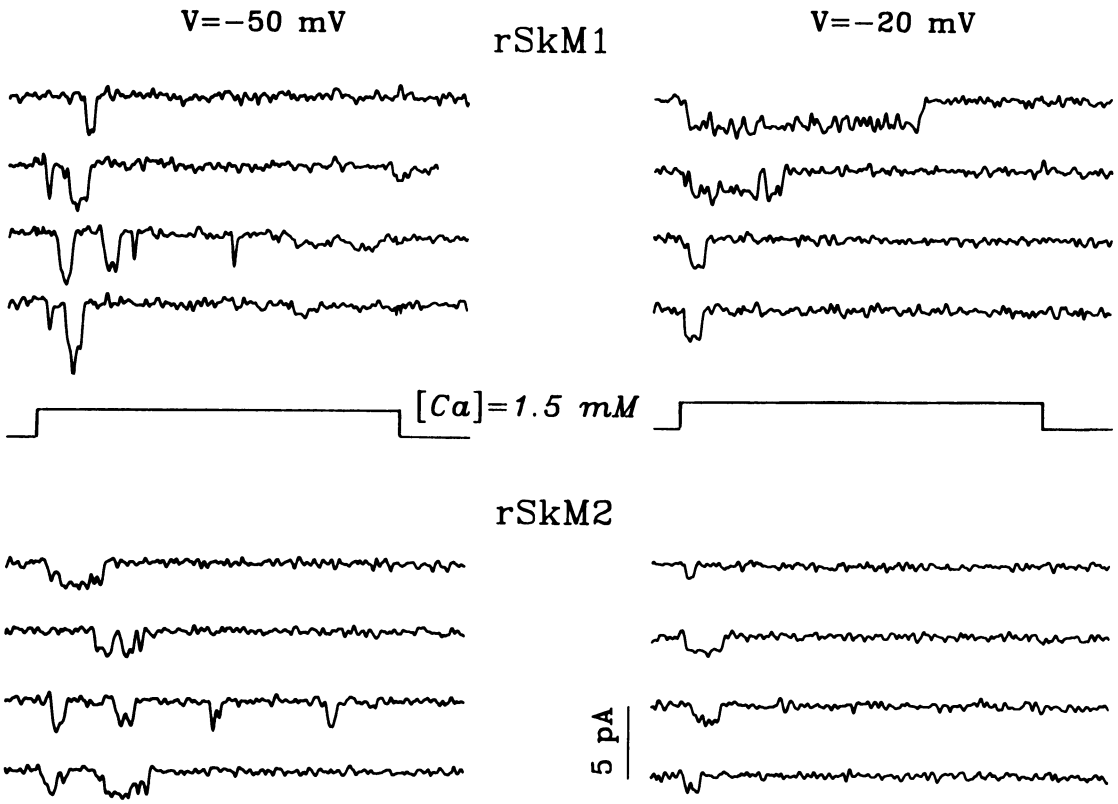

FIGURE 2 Selected records of single-channel currents in outside-out patches from rSkM1 (*above*) and rSkM2 (*below*). The 20-ms test pulse to −50 mV (*left*) or −20 mV (*right*) is indicated. Holding potential, −110 mV. Low-pass filter, 2 kHz, $[Ca^{2+}]$ = 1.5 mM.

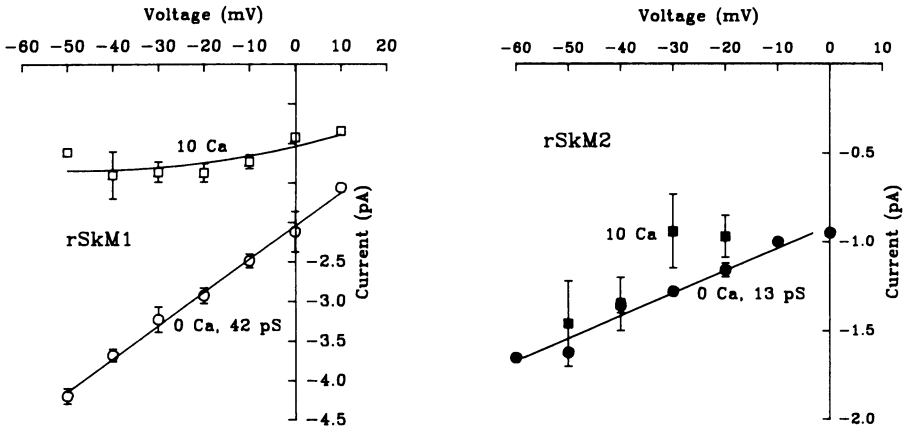

FIGURE 3 Effect of $[Ca^{2+}]$ on the single-channel *i-V* relationship for rSkM1 (*left*) and rSkM2 (*right*). Symbols show mean ± SEM for data from 3–5 patches under each condition. Straight lines are regressions fits for the data in 0 mM Ca^{2+}. The curved line for the rSkM1 *i-V* relationship in 10 mM Ca^{2+} was obtained from a fit of the data to a model in which the affinity for block is an exponential function of voltage (4, 12, 13). The dissociation constant for Ca^{2+} block was 10.2 mM at 0 mV, and the location of the blocking site was 19.1% into the membrane field from the extracellular surface.

into the electrical field from the extracellular surface (see legend of Fig. 3). Similar voltage dependence has been found for Ca^{2+} block of TTX-S Na channels in other studies (4, 12, 13).

Our data are consistent with those obtained on what are presumed to be the same channel isoforms *in situ* (9, 14). However, our data do not agree with a number of studies of mammalian TTX-I Na channels in cardiac tissue, where the Ca^{2+} sensitivity is similar to that found in TTX-S channels (13, 15, 16). Because rSkM2 mRNA is prominently expressed in rat cardiac muscle (17), this discrepancy is even more puzzling. However, the possibility remains that the Na channels studied *in situ* in cardiac cells are either the product of a different gene or are functionally altered by their local environment, or by posttranslational modification specific to cardiac cells. Our data for TTX-I channels are also inconsistent with the notion that the Ca^{2+} effects on open probability and single channel currents are mediated by a common site (1), because Ca block of the macroscopic current of rSkM2 is not accompanied by equivalent effects on the amplitudes of single-channel currents.

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