Short communication

Two Types of Potassium Channels From the Internal Membrane System of the Crayfish Muscle Incorporated into Planar Lipid Bilayers

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Potassium channels constitute the most diverse and widespread class of ion channels known. K^+ channels have been found in all cells which display action potentials mediated by sodium or calcium ions. The different types of K^+ channels are very often present in the same cells which shows, that their diversity is not associated with the cell type (for review see Latorre and Miller 1983; Rudy 1988).

A recent advance has been the incorporation of ion channels into planar lipid bilayers, which enables the study of channels not accessible to patch-clamp pipette. In skeletal muscle, Ca^{2+}-activated K^+ channels (Latorre et al. 1982; Moczydlowski and Latorre 1983; Eisenman et al. 1986; Oberhauser et al. 1988) and K^+ selective SR channels (Miller 1978; Labarca and Miller 1981) have been described using this technique. These channels were favorite objects to study in planar lipid bilayers, because of their high conductance (150—250 pS, depending on concentration of K^+ ions). In the case of Ca^{2+}-activated K^+ channel its kinetic behavior (McManus and Magleby 1988) and pharmacology (Strong 1990; Oberhauser et al. 1988) are well described.

We report here the results of a study into the composition of channels of the internal membrane system of crayfish muscle fibres, which are known to spike on the calcium principle (for review see Zachar 1971). They thus differ qualitatively from the muscle fibres, which generate sodium action potentials, both on the surface and in the tubular membranes. Three kinds of K^+ channels have been described in these fibres in voltage clamp conditions (Henček et al. 1978; Mounier and Vassort 1975).

The results presented concern two types of K^+ channels in the internal

ABBREVIATIONS: EGTA — ethyleneglycol-bis-(aminoethylether)-N,N,N′N′-tetraacetic acid; HEPES — N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, PE — phosphatidylethanolamine, PS — phosphatidylserine, SR — sarcoplasmic reticulum, TEA — tetraethylammonium.

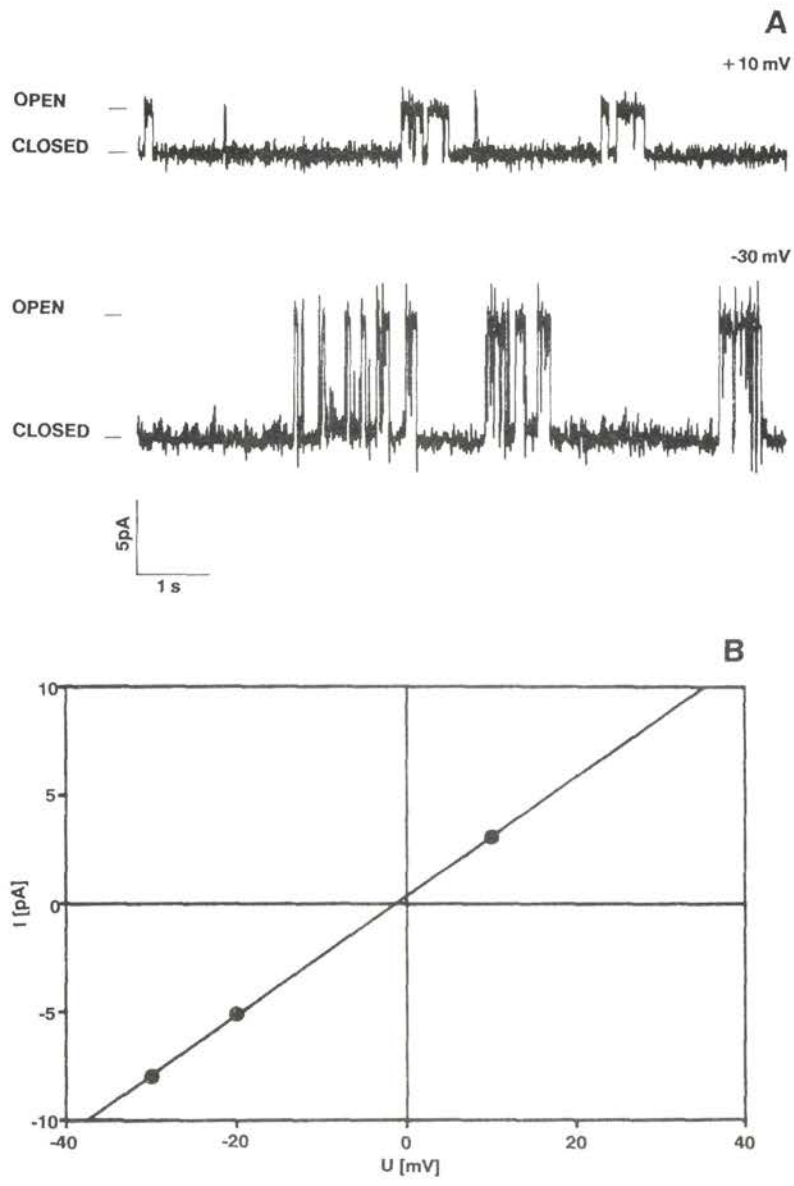

Fig. 1. (*A*) Comparison of recordings from crayfish muscle at two different voltages (+10 mV and −30 mV). For details see Materials and Methods. (*B*) Voltage dependence of the Ca^{2+}-activated K^+ channel in the measured range.

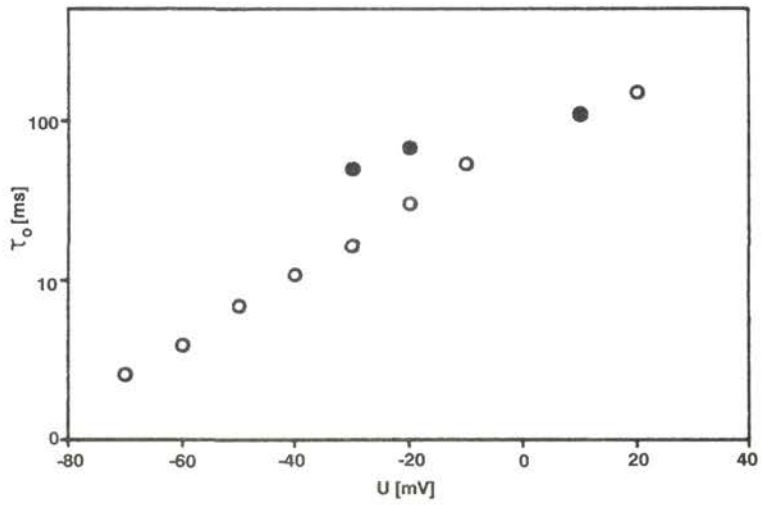

Fig. 2. Effect of potential on the mean durations of the channel open times for rabbit (open symbols) and crayfish (filled symbols).

membrane system of crayfish skeletal muscle, which are compared with K^+ channels in the internal membrane system of rabbit skeletal muscle.

Internal membranes were isolated on discontinuous sucrose gradient as described by other authors (Tomková et al. 1984). Aliquots of 100 μl of the vesicles were stored at the temperature of liquid nitrogen.

All experiments were carried out on painted membranes formed from a solution of PE:PS = 7:3 or from azolectine (35 mg/ml) in *n*-decane. The phospholipids were prepared in our institute, azolectin and *n*-decane were from Fluka, EGTA and HEPES from Sigma, other chemicals were of analytical grade. Membranes were painted on holes (0.4—0.6 mm in diameter) in Teflon partitions separating aqueous solutions in two compartments. All experiments were carried out in solutions of 250 or 500 mmol/l KCl, 5 mmol/l HEPES, pH = 8.0 at both sides of the membrane. In some measurements a 0.1 mmol/l EGTA was added to both sides. The side, to which the vesicles were added contained 1 mmol/l $CaCl_2$. The membrane system was connected to a current-to-voltage transducer via agar salt bridges in series with silver chloride electrodes. Data were sampled at a frequency of 800 Hz and stored in a SM 4-20 computer. The analysis of the data was done automatically by the computer.

Two types of K^+ channels were found in the internal membrane system of the crayfish. One, usually present in the membrane fractions corresponding to

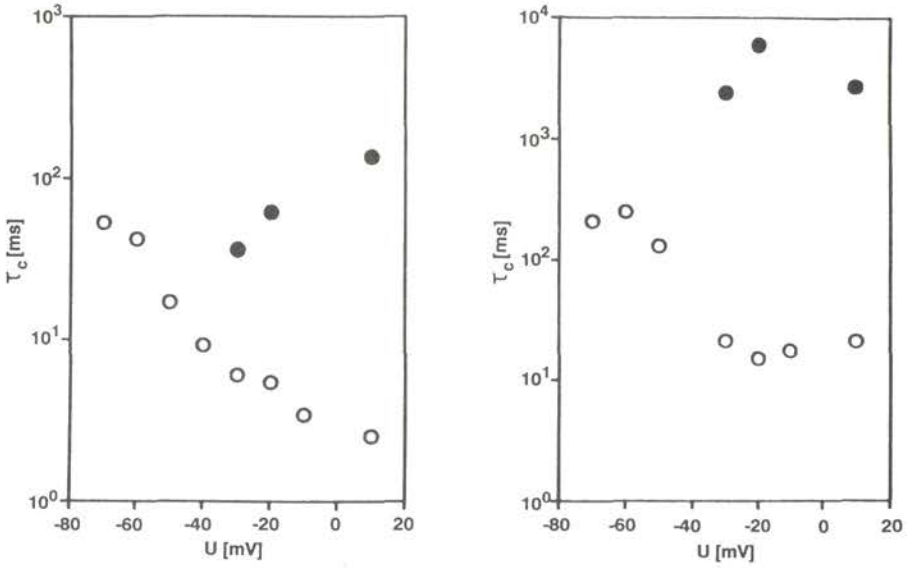

Fig. 3. Effect of voltage on both components of the two exponential distribution of closed time histograms for the rabbit (open symbols) and crayfish (filled symbols).

the T-tubule system, had properties similar to that of the Ca^{2+}-activated K^+ channel from the T-system of the rabbit muscle (Latorre et al. 1982; Moczydlowski et al. 1983; Eisenman et al. 1986). The selectivity for K^+ over Cl^- was tested in 100 mmol/l K_2SO_4 (results not shown). For comparison, some experiments were performed with T-tubules fraction from rabbit skeletal muscle. Both channels open and close within the range of milliseconds. The activity occurs in bursts, interrupted by silent periods that may last for several hundred milliseconds (Fig. 1*A*).

As follows from the recordings at different voltages, the channel is voltage dependent with a conductance of 277 pS in 500 mmol/l KCl solution (Fig. 1*B*). The amplitude as well as the open and closed times, change with the imposed membrane voltage. The depolarizing membrane potential prolongs the interval, during which the channel remains in the conducting state. The distribution can be fitted with one exponential, which gives values for mean open times (τ_0). The voltage dependence of τ_0 (Fig. 2) is much smaller for the channel from crayfish skeletal muscle than for rabbit skeletal muscle.

As could be expected, the closed times distribution shows an inverse pattern. With the increasing membrane depolarization, the fraction of time,

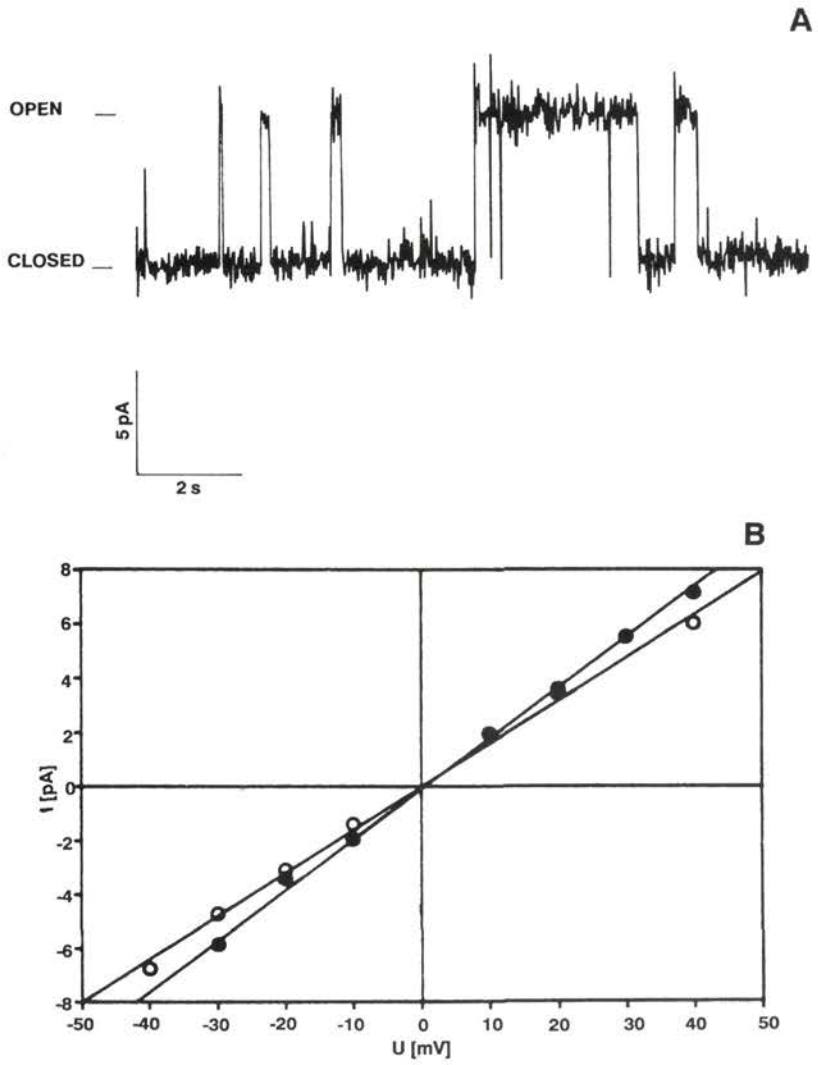

Fig. 4. (*A*) Current fluctuations of K^+ channel from crayfish SR fraction recorded at a voltage of −30 mV. The recording was carried out in 250 mmol/l KCl, 5 mmol/l HEPES, 0.1 mmol/l EGTA (*cis*/*trans*), pH = 8.0. (*B*) Voltage dependences of K^+ channel at 250 mmol/l KCl (open symbols) and 500 mmol/l KCl (filled symbols).

during which the channel remains in the nonconducting state decreases (Fig. 3). These two components (τ_{C1} and τ_{C2}) of the exponential distribution have much larger values (about one order in magnitude) for the channel from crayfish

skeletal muscle than for those obtained from rabbit skeletal muscle. This is probably caused by a relatively low sampling frequency and thus missed brief closings; however, no higher frequencies have been applied in our experiments. This might also explain the ascending character of the voltage dependences of closed times. A major problem we were facing was the inactivating character of this type of channel in the crayfish skeletal muscle. It inactivates within about 5 minutes at a constant holding potential, and thus differs from a similar channel in the rabbit skeletal muscle.

We found another type of K^+ channel in the membrane fraction from the sarcoplasmic reticulum of the crayfish muscle with conductances of 160 pS in 250 mmol/l KCl and 180 pS in 500 mmol/l KCl (Fig. 4). These properties are comparable to those obtained by Miller (1978) on rabbit skeletal muscle SR membranes. Also in this case control measurements were performed in 100 mmol/l K_2SO_4. The effect of TEA was tested by addition of 50 mmol/l TEA into the electrolyte at the *cis* side of the membrane. TEA lowers the amplitude of the opening by only $\approx 5\%$ (not shown). We were not able to characterize, from technical reasons, the kinetics of this channel, because of the long lasting openings and closings.

It can be stated that the internal membrane system of the crayfish skeletal muscle contains two types of K^+ channels. One is similar to the Ca^{2+}-activated K^+ channel in the internal membrane system (most probably in the T-tubules) of the rabbit skeletal muscle. The second type of K^+ channel is a long lasting one with open and closed times in the range of several hundred milliseconds to seconds, and with a smaller conductance; it is probably located in the sarcoplasmic reticulum itself.

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