The Effects of the Replacement of K^+ by Tl^+, Rb^+, and NH_4^+ on the Muscle Membrane Potential

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Abstract. The resting membrane potential (RMP) of mouse diaphragm muscle was measured in solutions containing several concentrations of K^+ (0.4 to 5 mmol/l) or one of the following cations: Tl^+ (0.4, 1 or 2 mmol/l), Rb^+ (1, 2 or 5 mmol/l), or NH_4^+ (4, 8 or 16 mmol/l). In terms of controlling the RMP, the ratios of the efficacies were $Tl^+:K^+:Rb^+:NH_4^+ = 2.5:1.0:1.0:0.12$. These ratios are similar to those of the selectivities of the voltage dependent K^+ channel (delayed rectifier) in frog nerve and muscle, and this similarity suggests that the resting membrane potential may be controlled by this channel.

Key words: Membrane potential — Diaphragm muscle — Potassium channel

Introduction

The membrane potential of muscle is normally dependent on the potassium gradient across the muscle membrane (Ling and Gerard 1950). There are several K^+ conductances in muscle membrane (Adrian et al. 1970b). The two more important ones seem to be: the delayed rectifier channel, which is responsible for the falling phase of the action potential and the inward rectifier (Katz 1949). To determine which of these channels is responsible for the resting potential, the effects of the replacement of K^+ by Tl^+, Rb^+ and NH_4^+ on this potential have been examined. The data suggest that the resting potential may be controlled by the delayed rectifier.

Materials and Methods

The experiments were performed on diaphragms dissected from female white specific pathogen free mice. The thoracic side of the muscle was carefully cleaned of pleura, which often covers the endplate zone of the muscle. The diaphragms were washed several times, and then pinned to small discs of

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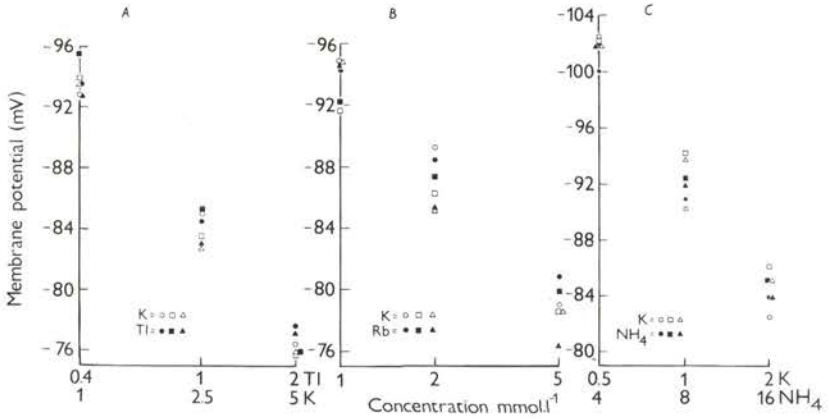

Fig. 1. Relationship between membrane potential and ion concentration (logarithmic scale). Each graph shows the potentials in K^+ and in one test cation, Tl^+ (A), Rb^+ (B) and NH_4^+ (C) measured on one muscle. The different symbols denote different muscles. The concentrations were scaled, as indicated, to make the potentials similar. The potentials are the means of 50 readings. The s.e.'s were 0.5 mV or less for about 2/3rd's of the readings.

Sylgard. The preparations were then placed in an oxygenated (95% O_2, 5% CO_2) solution containing (mmol/l): NaCl 137, $MgCl_2$ 1.0, $NaHCO_3$ 11.0, NaH_2PO_4 1.0, glucose 11.0 and KCl or a substitute cation with the stated concentration. The Tl^+ experiments and the K^+ controls therefore were done in solutions containing $NaNO_3$, $TlNO_3$ and KNO_3 because of the limited solubility of TlCl.

In the experiments in which the resting membrane potentials (RMPs) were measured, the muscles were first washed in solutions without KCl for 5—10 minutes and then the test cation added in the lowest concentration. After 5 min, 50 fibers were impaled with a 3 mol/l KCl filled micropipette ($R = 15$—$30\ M\Omega$) and the RMPs were measured with a conventional cathode follower. The ion concentration was increased and the procedure repeated. After the RMPs were measured at three concentrations of one ion, the muscles were washed again in the K^+-free solution, and the procedure repeated with another cation. In all experiments, K^+ was one of the two ions used, and the sequence of the exposure to the two ions was varied. No differences were noted between the two sequences but only three sets of measurements were made with each test cation.

Results

Effects on resting membrane potential

The relationships between the resting membrane potential (RMP) and the logarithm of the concentrations of the test cations are shown in Fig. 1. For Tl^+, the concentrations on the abscissa are 0.4 times that of K^+. The Rb^+ and K^+ concentrations are equal and the NH_4^+ concentrations are 8 times that of K^+. For the three ions the scaling factors gave potentials quite similar to those in K^+. Three muscles were examined for each ion and the results were about the same in all

cases. Therefore in terms of controlling the RMP, the ratios of the efficacies found were $Tl^+:K^+:Rb^+:NH_4^+ = 2.5:1.0:1.0:0.12$.

Discussion

The results provide some insight into the properties of the channel responsible for the K^+ sensitivity of the resting potential. Consider the Goldman—Hodgkin—Katz equation in the form

$$E = \frac{RT}{F} \ln \frac{P_C[C]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}$$

where C represents either K^+ or one of the substitute cations, and the other terms have their usual meanings. If the potential in the presence of a concentration [C] of the substitute cation is equal to that in the presence of a concentration [K] of K^+ and if the internal levels of the ions and the permeabilities of the membrane are unaffected by the substitution, then

$$P_C[C] = P_K[K]$$

or

$$P_C/P_K = [K]/[C]$$

This equation gives the selectivity ratio in terms of the ratio of the concentrations necessary to give identical membrane potentials. The data above gave $P_{Tl}:P_K:P_{Rb}:P_{NH} = 2.5:1.0:1.0:0.12$. The relative permeabilities for the voltage dependent K^+ channel in frog nerve are 2.3:1.0:0.91:0.13 (Hille 1973). For frog muscle $P_K:P_{Rb} = 1.0:0.95$ (Gay and Stanfield 1978). The selectivities of the channels in muscle for Cs^+, Na^+ and Li^+ are also quite similar to those in nerve, and the data suggest that the selectivities of the two channels may be the same (see review by Edwards 1982). In muscle the similarity between the selectivities of the voltage dependent K^+ channel and that responsible for the resting potential suggests that the two may be the same, i.e. that the K^+ fluxes across the membrane of inactive muscle may pass through the voltage dependent K^+ channels.

There are at least three potassium currents in the membrane of frog muscle (Adrian et al. 1970b). The delayed rectifier channel, which is responsible for the falling phase of the action potential, inactivates more quickly and more completely during a depolarization than does the same channel in squid nerve (Nakajima et al. 1962; Adrian et al. 1970a). There are also an inward rectifying K^+ channel (Katz 1949) and a slower component. It has been suggested that the principal K^+ conductance channel in the resting membrane is the inward rectifier; since the delayed rectifier channels are activated by depolarization to -40 mV (Adrian et

al. 1970b), they are usually considered to be closed at the normal resting potential. For the inward rectifier, the value of P_{Rb}/P_K can be estimated from the data in Standen and Stanfield (1980) to be about 0.67 (membrane potential = −14 mV in 80 mmol/l K_2SO_4 and −24 mV in 80 mmol/l Rb_2SO_4). P_{Tl}/P_K has been found to be about 1.66 (Ashcroft and Stanfield 1983). Thus the selectivity sequence for this channel is the same as that for the voltage dependent K^+ channel, although the numbers are somewhat different ($P_{Tl}:P_K:P_{Rb} = 1.66:1.0:0.67$). In the membrane of the starfish egg, the selectivities of the inward rectifier are similar to those in frog muscle: $P_{Tl}:P_K:P_{Rb}:P_{NH_4} = 1.5:1.0:0.3-0.4:0.03-0.04$ (Hagiwara and Takahashi 1974). Thus the selectivities of the delayed rectifier and the inward rectifier are somewhat different, and the selectivities for the channel controlling the resting membrane potential are closer to those of the delayed rectifier.

Additional insight into what conductances are operating in the resting muscle of frog muscle may be found by examining the magnitudes of the conductances of the inward and delayed rectifiers. The maximum value of the latter has been estimated to be 8.5 to 20 mS cm^{-2} (Adrian et al. 1970a); if as few as 1% of these channels are open at rest, the conductance would be 0.085 to 0.2 mS cm^{-2}. In 40 mmol/l K^+, the conductance due to the inward rectifier at −85 mV is about 0.6 mS cm^{-2} (Leech and Stanfield 1981). The inward rectifier in the membrane of muscle seems to be quite similar to that in the starfish egg, and for the latter, the relationship between this conductance, g_K, the membrane potential, V, and the Nernst potential for K^+, V_K, is (Hagiwara and Takahashi 1974):

$$g_K = A[1 + \exp\left(\frac{V - E_K - V_h}{v}\right)]^{-1}([K^+]_o)^{1/2}.$$

where V_h represents the value of $V - E_K$ at which the value of g_K is 1/2 the maximum value, and v is a constant which characterizes the shape of the relationship. Reducing K^+ from 40 to 2.5 mmol/l will reduce g_K by $(2.5/40)^{1/2}$ or 1/4 because of the concentration term and will also reduce it by making E_K more negative. Therefore the conductance due to the inward rectifier is likely < 0.1 mS cm^{-2}; thus it is comparable in size to, if not less than, the conductance due to 1% of the delayed rectifier channels.

In experiments on frog muscle, the membrane potentials of freshly dissected muscles were found to be quite similar in Rb^+ and K^+ at 2.5 and 5 mmol/l; at 1 mmol/l, the potential in K^+ was about 7 mV more negative than in Rb^+ (Adrian and Slayman 1966). The measurements were performed in solutions in which Cl^- was replaced by SO_4^{2-}. Adrian (1964) has estimated P_{Rb}/P_K to be about 0.55 in frog muscles in 100 mmol/l SO_4^{2-} solutions. P_{Tl}/P_K of the frog muscle membrane has been found to be 1.14 in solutions in which Cl^- was replaced by SO_4^- or acetate (Gay 1981).

The relative permeabilities of the membranes of several other cells to the

cations used herein have been measured from the relationships between the membrane potential and the ion concentration. The data for the giant axon of the squid (internal perfusion, Baker et al. 1962; external solution, Hagiwara et al. 1972), neurons in *Navanax inermis* (Levitan and Barker 1972) and muscle of the barnacle, *Balanus*, (Hagiwara et al. 1971) are quite similar to those reported here. In frog muscle, the slope of the membrane potential-log concentration relationship is similar for Tl^+ and K^+ (Mullins and Moore 1960) and Tl^+ was reported to be about twice as effective as K^+ in eliciting contractures in rat diaphragm (Hughes et al. 1976). However these cells have voltage dependent K^+ channels; in at least some of the experimental conditions the membrane was depolarized and so more of the voltage dependent channels were open than at rest. Therefore the measured potential changes reflect a summation of unknown contributions from the K^+ channels which contribute to the resting potential and the voltage dependent K^+ channels.

Similar experiments have been performed on two tissues whose membranes lack voltage dependent K^+ channels. In *Necturus*, the relative permeabilities of the membrane of the glia in the optic nerve are $P_{Tl}:P_K:P_{Rb}:P_{NH_4}=2.3:1.0:0.55:0.16$ (Bracho et al. 1975). The permeabilities of Na^+, Li^+, guanidine and tris are too small to be measured. In the barnacle, the permeabilities of the membrane of the dark adapted photoreceptor measured from the membrane potential-ion concentration relationship are $P_K:P_{Rb}:P_{Na}:P_{Li}=1.0:0.87:0.1:0.1$ (Brown and Saunders 1977). In both cases the sequences are the same as that found for the voltage dependent K^+ channel in frog nerve (Hille 1973), and the numbers are similar, although not the same.

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