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Investigation of ion selectivity in membranes of muscle cells

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Abstract

The sequences of conductance ratios (G_X/G_K) and permeability ratios (P_X/P_K) for monovalent cations (X = Rb^+, Cs^+, Na^+, Li^+, NH_4^+) were studied in frog skeletal muscle before and after addition of the channel-forming antibiotic gramicidin A. The experiments were carried out under current clamp conditions using a double sucrose gap technique. For inwardly rectifying potassium channels the selectivity measured by membrane conductance ratios before gramicidin treatment was $K^+ > Rb^+ > Cs^+ > NH_4^+ > Na^+ > Li^+$. In gramicidin channels of the same muscle fibre after addition of 5×10^{-7} M or 1×10^{-6} M gramicidin both the permeability and the conductance ratios had the sequence $NH_4^+ > Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$.

Key words: frog muscle fibre, gramicidin channel, inward rectifier potassium channel, ion conductance, ion selectivity, permeability.

Introduction

The main function of skeletal muscle, contractility, depends on specific membrane ion channel proteins, among other proteins. The two key properties of ion channels are selective ion conduction and gating. Selective conductance reflects a channels ability to select one ionic species among those present in the cellular environment and to catalyze its rapid flow through the pore. The characteristics of selectivity for a monovalent cation in an ion channel can be represented by sequences of conductance ratios (G_X/G_K) and permeability ratios (P_X/P_K), where X = Rb^+, Cs^+, Na^+, Li^+, NH_4^+. Permeability (P) is expressed as the amount of ions transported through the channel in a time unit. Ion conductance (G) is reversely proportional to the membrane resistance (R).

In lipid layers the antibiotic gramicidin A forms selective ionic channels with well-characterized molecular structure (Woolley et al. 1997; Chadwick et al. 2000). Since the gramicidin channel has a number of properties in common with ionic channels of muscle, nerve, and synapse, its study provides useful information about the fundamentals of ion permeation through biological membranes. While selectivity sequences for alkali cations of gramicidin channels in lipid bilayers are well known (Haydon, Hladky 1972; Myers, Haydon 1972; Anderson 1983), corresponding data for biological membranes are scarce.

The aim of our experiments was to measure selectivity for cations in two different kinds of ion channels in the muscle fibre membrane under the same experimental conditions: a natural potassium channel of inward rectification (IRK) and an induced gramicidin channel, a widely used model for cation channels.

Materials and methods

The experiments were performed on single phasic fibres from *ileofibularis* and *semitendinosus* muscles of the frog *Rana esculenta*. After preparation the fibres were incubated in isotonic potassium sulphate solution containing (in mM) 160 K^+, 8 Ca^{2+}, 88 SO_4^{2-}, 2 Tris-malate, pH 7.2. This solution contained only K^+ to carry a substantial current through the membrane, and after 30 min of equilibration the K^+ concentrations inside and outside the cell were nearly identical. In test solutions, K^+ was successively replaced by equimolar amounts of Rb^+, Cs^+, NH_4^+, Na^+ or Li^+. A gramicidin-containing solution was prepared from K_2SO_4 solution by addition of gramicidin A (Serva, Heidelberg; 70 - 85 % gramicidin A) to a final concentration of 5×10^{-7} M and 1×10^{-6} M gramicidin and 0.1 % (v/v) ethanol.

Conductance measurements were performed by means of a double sucrose gap technique described in detail previously (Isenberg, Küchler 1970; Caffier et al. 1980). Hyperpolarizing square wave pulses (0.02 - 0.06 mA) of 300 ms duration were applied to the membrane and the corresponding voltage responses (V_0) recorded (see Fig. 1A, regular vertical lines, hyperpolarization downward). The steady state cord conductance G was obtained as $1/R_m$ where R_m is the membrane resistance (kΩ cm^{-2}) of 1 cm^2 of the outer surface of the muscle fibre membrane, calculated by

$$R_m = V_0 \times S / I \times f^2. \quad (1)$$

V_0 is the voltage measured, I – amplitude of the current pulse, S – the membrane area under investigation, f – the short circuit factor given by the relation V_0/V_i, where V_i is the potential change recorded simultaneously with an intracellular microelectrode. To calculate the membrane surface area, S, both the width of the test compartment and the diameter of the preparation were measured under a microscope.

Besides V_0, the changes of membrane resting potential E were continuously measured. In isotonic K_2SO_4 solution E remained at a near-zero resting level within the intervals between the test pulses (Fig. 1A, horizontal straight line). In sulphate solutions containing other cations the resting potential changed to a new value (ΔE). The permeability ratios P_X/P_K were determined from ΔE by the Goldman-Hodgkin-Katz (GHK) equation. The conductance ratio was expressed as G_X/G_K. P_X and G_X are permeability and conductance measured at the same time under asymmetric conditions (K^+ inside, X outside the fibre, where X corresponds to Li^+, Na^+, Rb^+, Cs^+ or NH_4^+). P_K and G_K are permeability and conductance values of potassium.

Results

At the beginning of the experiment the selectivity sequences for the potassium channel of inward rectification were tested. The resting potential in experimental solution (160 mM K^+) was found to be 0.1 ± 0.3 mV and $[K^+]_{in}$ – 159.3 mM (Leech, Stanfield 1981). By substituting cation X for K^+ the resting potential in our experiments reached a new more

Table 1. Conductance ratios (G_X/G_K) and permeability ratios (P_X/P_K) for monovalent cations in potassium and gramicidin channels of muscle fibre membrane

Before gramicidin treatment (potassium channels)

Fibre	K^+	Rb^+	Cs^+	NH_4^+	Na^+	Li^+
No.	G_X/G_K	G_X/G_K	G_X/G_K	G_X/G_K	G_X/G_K	G_X/G_K
1	1				0.27	0.23
2	1	0.21	0.16	0.17	0.15	0.15
3	1	0.21	0.15	0.26	0.14	0.13
4	1	0.47	0.41	0.34	0.30	0.28
5	1	0.48	0.41	0.29	0.21	0.18
6	1	0.28	0.26	0.27	0.21	0.18
Mean	1	0.33	0.28	0.27	0.21	0.19

After gramicidin treatment (gramicidin channels)

Fibre	NH_4^+		Cs^+		Rb^+		K^+		Na^+		Li^+	
No.	G_X/G_K	P_X/P_K	G_X/G_K	P_X/P_K	G_X/G_K	P_X/P_K	G_X/G_K	P_X/P_K	G_X/G_K	P_X/P_K	G_X/G_K	P_X/P_K
3	1.10	1.82	1.15	1.35	1.05	1.19	1	1	0.62	0.40	0.28	0.16
4	1.48	1.79	1.19	1.25	1.10	1.13	1	1	0.54	0.33	0.23	0.17
5							1	1			0.22	0.19
6	1.16	1.43	1.05	1.15	1.03	1.09	1	1	0.69	0.53	0.38	0.22
7	1.23		1.08	1.74	1.07	1.36	1	1	0.72	0.18	0.54	0.04
8	1.03	1.90	1.09	1.41	1.14	1.21	1	1	0.37	0.40	0.24	0.18
Mean	1.20	1.74	1.10	1.38	1.08	1.20	1	1	0.59	0.37	0.32	0.16

hyperpolarized level (ΔE). The hyperpolarization tended to increase in the sequence $Rb^+ < Cs^+ < NH_4^+ < Na^+ < Li^+$ (Fig. 1C). Permeability ratios (relative to K^+) defined by the expression for bi-ionic potentials using the GHK equation

$$E = (R \times T / z \times F) \ln (P_X / P_K) \quad \text{(Myers, Hayden 1972)}, \quad (2)$$

gave a sequence corresponding to $K^+ > Rb^+ > Cs^+ > NH_4^+ > Na^+ > Li^+$. The same sequence was obtained by membrane conductance ratios G_X/G_K, measured in the same experiment before gramicidin treatment (see Table 1). This sequence is commonly referred to as the Eisenman sequence IV (Eisenman, Horn 1983).

The application of gramicidin (1×10^{-6} M) into K_2SO_4 solution resulted in an increase of conductance due to the formation of new gramicidin channels in the membrane. The steady state gramicidin-induced conductance constituted about 80 % of the entire conductance *G*. Since the gramicidin-induced conductance of the muscle cell membrane was irreversible (i.e. after removal of gramicidin from the solution G remained unchanged or decreased only slightly; Shvinka et al. 1979), *G*, ΔE and V_0 measured after gramicidin treatment were characteristics of the gramicidin channel. Under these experimental conditions the replacement of external K^+ by equimolar Rb^+, Cs^+ or NH_4^+ caused a deviation of *E* toward depolarization whereas Na^+ and Li^+ shifted *E* in the hyperpolarizing direction (Fig. 1A, B). Using Equ. (2) the following sequence of permeability ratios P_X/P_K for the gramicidin channel was obtained (see also Table 1): NH_4^+ (1.74) > Cs^+ (1.38) > Rb^+

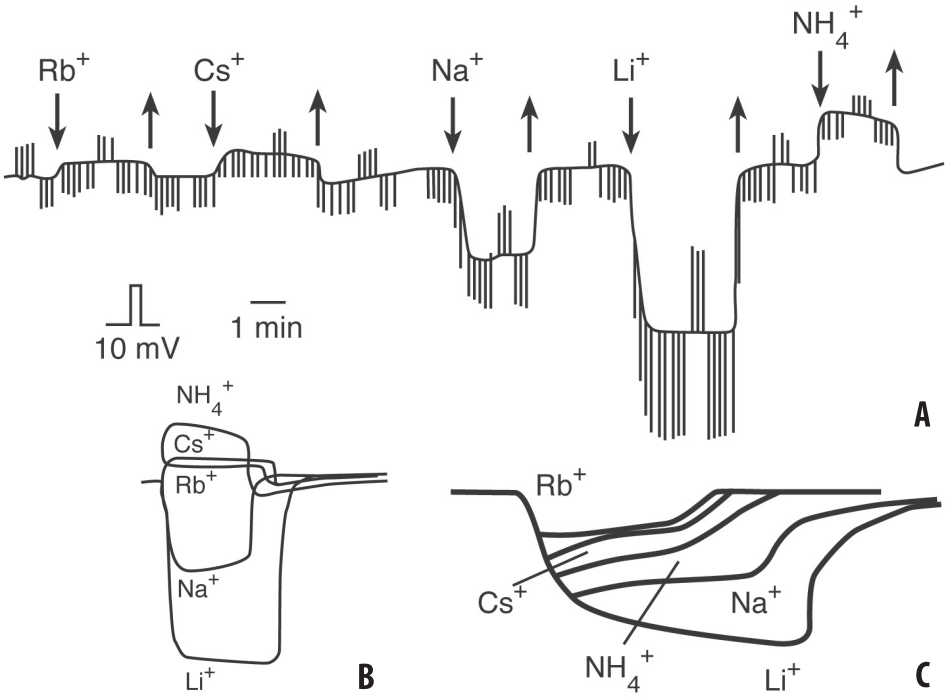

Fig. 1. Effect of monovalent cations on membrane voltage recorded during periodic application of constant current pulses. Measurements with double sucrose gap technique. A typical experiment made on one muscle fibre is shown. A, fibre in isotonic sulfate solution after application of 10^{-6} M gramicidin. Periodic vertical lines are voltage responses to pulses applied once every 10 s at alternate polarities (depolarization upward, hyperpolarization downward). Pulse duration 300 ms, intensity 0.03 μA. Horizontal line, membrane potential (E) in isotonic K_2SO_4 solution. Arrows indicate change from isotonic K_2SO_4 to solutions with different cations and vica versa. B, superimposed changes of resting potential (ΔE) shown in Fig. 1A. C, superimposed ΔE measured before gramicidin treatment on the same fibre.

(1.20) > K^+ (1) > Na^+ (0.37) > Li^+ (0.16). This type of selectivity corresponds to sequence I given by Eisenman and Horn (1983). The same selectivity sequence was obtained from the membrane conductance ratios: NH_4^+ (1.20) > Cs^+ (1.10) > Rb^+ (1.08) > K^+ (1) > Na^+ (0.59) > Li^+ (0.32). It should be mentioned, however, that the numerical values of conductance ratios were not equal to the permeability ratios (see also Table 1).

Discussion

In our experimental conditions (membrane potential near null) hyperpolarizing pulses initiate K^+ outfluxes through the potassium channels of inward rectification (IRK). Rectifier is a term that comes from electronics, referring to devices that conduct electrons only in one direction. In biology, rectification in IRK channels is important because it is used to control the cell resting membrane voltage (Doupnik et al. 1995; Nishida, Mac Kinnon 2002). At voltages favoring the outward flow of K^+ ions the pore becomes blocked

by intracellular Mg^{2+} and polyamines (Aidley, Stanfield 1996). The measurements at the beginning of our experiments prior to gramicidin treatment estimate the activity of this type of K^+ channel. The second measurement on the same muscle fibre after gramicidin treatment demonstrates the selectivity of the gramicidin channels.

The selectivity sequences of gramicidin channels in our experiments are close to those observed for lipid bilayer membranes (Myers, Haydon 1972). The relation of conductance (*G*) to permeability (*P*) ratios in the gramicidin channel has been widely discussed (Myers, Haydon 1972; Anderson 1983). In a limiting low ion concentration (10 mM) the ratio of conductance is equal to the permeability ratio when they are measured at the same voltage. Our results are obtained at higher ion concentrations (160 mM) and under asymmetric conditions (i.e. K^+ inside and ion *X* outside the cell). In this situation the conductance ratio can be computed from the GHK equation by

$$G_X / G_K = \frac{\Delta E + V_0}{V_0 P_K} \left(\frac{P_K e^{(\Delta E + V0)F / RT} - P_X}{e^{(\Delta E + V0)F / RT} - 1} \right), \qquad (3)$$

where ΔE is the polarization which occurs immediately on replacement of the external K^+ by another cation, and V_0 is the voltage recorded at the same time in response to periodic current pulses. After insertion of the experimental values both parts of Equ. (3) became identical. Thus, the inequality of P_X/P_K and G_X/G_K presented in the Table 1 may result from asymmetric conditions of conductance ratio measurements.

Taken together, our results demonstrate the similarity between selectivity sequences of gramicidin channels in muscle cell membrane and in lipid bilayers. In contrast, there is markable difference in the cation transport of natural IRK channels and gramicidin channels induced in the same membrane.

Our study using muscle cell membrane as a model of animal cell cholesterol-containing membranes should aid in elucidating the complex relationship between the structure, biophysics and physiology of ion channels.

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Jonu selektivitātes pētījumi muskuļšķiedru membrānās

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Kopsavilkums

Pētīta monovalento katjonu selektivitāte vardes skeleta muskuļu izolētās šķiedrās pirms un pēc kanālveidojošās antibiotikas gramicidīna A pielietošanas. Selektivitāte raksturota gan kā jonu vadītspējas attiecība G_X/G_K, gan arī kā jonu caurlaidības attiecība P_X/P_K ($X = Rb^+, Cs^+, Na^+, Li^+, NH_4^+$). Eksperimenti veikti strāvas fiksācijas režīmā, izmantojot divkāršu saharozes tiltiņa izolāciju. Eksperimenta sākumā, pirms gramicidīna iedarbības, selektivitātes rinda $K^+ > Rb^+ > Cs^+ > NH_4^+ > Na^+ > Li^+$ raksturoja anomālās iztaisnošanas K^+ kanālu (IRK). Pēc 5×10^{-7} M vai 1×10^{-6} M gramicidīna pielietošanas tajā pat muskuļšķiedrā, katjonu caurlaidības un vadītspējas selektivitāte veidoja rindu $NH_4^+ > Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$, kas raksturīga gramicidīna kanālam mākslīgajos lipīdu dubultslāņos.