Effect of Temperature on the Inward Rectifier and Gramicidin A-Induced Channels in the Membrane of Frog Skeletal Muscle Fibres

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Abstract. The conductance of frog skeletal muscle fibres in isotonic K_2SO_4 solution has been measured. Experiments were carried out under current-clamp conditions using a double sucrose-gap technique. The potassium conductances of the inward rectifier and the gramicidin channel in the same muscle fibre were compared. Potassium conductance of the inward rectifier increased with the temperature, with a value of Q_{10} 1.55 ± 0.09 ($n = 8$) under hyperpolarization, and Q_{10} 2.38 ± 0.23 ($n = 6$) for the depolarizing stimulus, the difference between Q_{10} of potassium and gramicidin channels being statisticaly insignificant.

Key words: Frog muscle — Inward rectifier — Gramicidin A-induced conductance — Effect of temperature

Introduction

The antibiotic gramicidin A increased cation conductance in frog muscle fibres (Leung and Eisenberg 1973; Caffier and Shvinka 1979) which is believed to result from the formation of gramicidin channels in the muscle cell membrane (Shvinka et al. 1979; Shvinka and Caffier 1981). It is of interest whether properties of gramicidin channels in biological and artificial membranes are similar. As the temperature dependence of the gramicidin channel, incorporated in a biological membrane, has so far not been studied, we tried to investigate the effect of temperature on the gramicidin channel in muscle fibre membrane, and to compare Q_{10} of gramicidin-induced and natural potassium channels.

Materials and Methods

Experiments were performed on single fibres from ileofibular muscles of the frog *Rana esculenta*. The double sucrose gap technique used in our experiments has been described in detail elsewhere (Isenberg

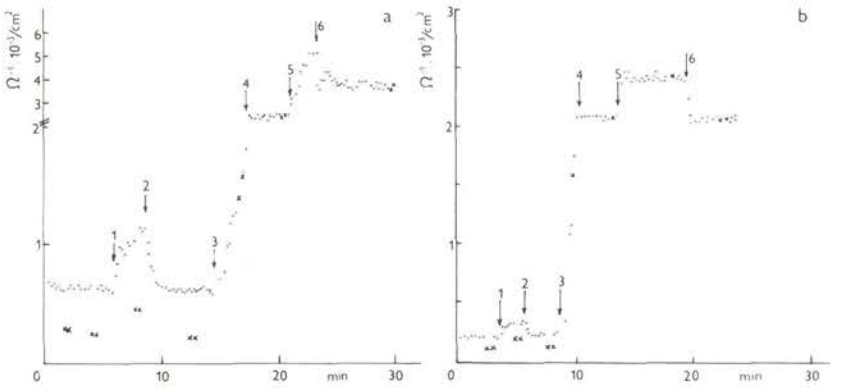

Fig. 1. Effects of temperature and gramicidin A on the membrane conductance of isolated frog muscle fibres. Two typical experiments are shown. Records of conductance in 160 mmol/l K^+ isotonic solution. Abscissa: time (min). Ordinate: conductance, $G(\Omega^{-1} \cdot 10^{-3}/cm^2)$. The intensity of constant current pulses was 0.02 μA. Symbols: Points represent values obtained under hyperpolarizing pulses, crosses represent values obtained under depolarizing pulses. Intervals between arrows: 1—2, temperature increase by 9 °C; 3—4, addition of 5×10^{-7} mol/l gramicidin to the solution; 5—6, temperature increase by 8 °C. The conductance recovery after the second temperature change was complete in *b* and incomplete in *a*.

and Küchler 1970; Caffier et al. 1980). The membrane resistance was tested using hyperpolarizing square wave pulses (0.02—0.06 μA) of 300 ms duration applied once every 9 s. The amplitude of the pulses was selected to produce a voltage drop of the order of 20 mV. To characterize inward rectification the direction of current pulses was changed. The test compartment in our experiments was 400 μm wide, enabling the assumption of potential uniformity in the test gap (Isenberg and Küchler 1970). Membrane resistance ($k\Omega \cdot cm^2$) was referred to 1 cm^2 of the outer surface of muscle fibres and calculated by $R_m = V \cdot S/I$, where V is the voltage measured, I is the amplitude of the current pulse, and S is the membrane area of the preparation in the test compartment. Since current leakage occurs in both the current passing and the voltage measuring sites, the values of V/I measured were divided by the short-circuiting factor $(kf)^2$ (Isenberg and Küchler 1970). The value of kf was equal to V/Vi, where Vi is the potential change recorded with an intracellular microelectrode under conditions of double sucrose gap. The short-circuiting factor in our experiments was 0.87. 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. All experiments were carried out first at room temperature (22 ± 1 °C) and subsequently at a temperature raised by about 10 °C. Temperature changes were measured with a thermistor placed in the test gap of the chamber. Thermistor was used to record difference between room temperature and the temperature in the test gap. The sensitivity of the thermistor was about 0.4 mV/°C. The solution used contained (in mmol/l): 160 K^+; 8 Ca_4^{2+}; 88 SO^{2-}; 2 Tris-maleate (pH 7.2). In this solution the resting potential was +0.1 ±0.3 mV, and $[K]_{in} = 159.3$ mmol/l (Leech and Stanfield 1981). The gramicidin-containing solution was prepared from the above solution by adding gramicidin A (Serva Heidelberg; 70—85% gramicidin A) to a final concentration of 5×10^{-7} mol/l gramicidin and 0.1 (v/v) ethanol. Both solutions contained only K^+ to carry current through the membrane, and the concentrations of K^+ after equilibration for 30 min were nearly identical both inside and outside the cell. The steady-state K^+ chord conductance, G, was obtained as $1/R_m$. In intervals between test pulses the fibres remained at a near zero resting potential.

Table 1. Q_{10} of inward rectifier and gramicidin-induced channels

Fibre N	Current amplitude (μA)	Q_{10} of inward rectifier		Q_{10} of gramicidin channels	
		hyperpolarization	depolarization	hypoerpolarization	depolarization
1	0.02	1.291			
2	0.04	1.481	2.837	1.440	1.212
3	0.02	2.012	2.992	1.319	
4	0.06	1.269	1.870		
5	0.02	1.543		1.648	
6	0.02	1.464	1.567	1.374	
7	0.01	1.784	2.678	1.619	
8	0.01	1.578	2.358	2.310	1.532
Mean ± S.E.M.		1.553 ± 0.09	2.384 ± 0.23	1.619 ± 0.15	

Results

In 160 mmol/l–K^+ isotonic solution fibres showed a property of inward rectification which allows K^+ to move in more easily than out across the cell membrane (Stanfield et al. 1981). Inward rectification is shown in Fig. 1*a*, *b*; prior to gramicidin-treatment the potassium conductance (G_1) under hyperpolarization was remarkably higher (points) than under depolarizing current pulses (crosses). Thus, G_1 measured before the addition of gramicidin characterized the K^+ movement across the inward rectifier. G_1 increased reversibly with the temperature as illustrated in Fig. 1*a*, *b* (arrows 1 and 2). Q_{10} for G_1 was expressed as the relation of G_1 at a temperature raised by 10 °C to G_1 at room temperature. The mean Q_{10} for inward rectification was 1.55 ± 0.09 ($n = 8$) under hyperpolarization, and 2.38 ± 0.23 ($n = 6$) under depolarization (see Table 1). This difference was statisticaly significant ($p < 0.01$). The corresponding activation energy was estimated to be about 0.033 kJ/mol for hyperpolarization, and 0.065 kJ/mol for depolarization. As illustrated in Fig. 1, the conductance was about the same before and after temperature changes. The channel-forming antibiotic, gramicidin A, was then added to the isotonic 160 mmol/l K^+ solution. An increase in conductance was observed due to the formation in the membrane of new gramicidin channels. The difference (ΔG) between the potassium conductance after the addition of gramicidin (G_2) and G_1 was the characteristic of the K^+ current in gramicidin channels. It should be mentioned that the gramicidin-induced conductance was irreversible, i. e. G_2 did not change after the removal of gramicidin from the solution. The irreversibility of gramicidin-induced conductance in the muscle cell membrane has

been reported earlier (Shvinka et al. 1979; Shvinka and Caffier 1983). The temperature dependence of the gramicidin channel was examined after the removal of gramicidin from the external solution. The constant background conductance after gramicidin removal (G_2 max) showed that the number of gramicidin channels did not change. The temperature-dependent increase in $G_{2\,max}$ is shown in Fig. 1 (arrow 5). In some experiments the values of $G_{2\,max}$ were identical both before and after temperature changes (Fig. 1*b*), while incomplete recovery was observed in others (Fig. 1*a*). The incomplete recovery may be associated with the formation of some additional gramicidin channels during the temperature increase. Q_{10} for gramicidin channels was estimated as a relation of conductances in gramicidin channels ($G_{2\,max}$—G_1) at temperature raised by 10 °C and at room temperature. At room temperature $G_{2\,max}$ was usually recorded after the temperature change. The amplitude of the potassium conductance in gramicidin channels increased with the temperature, with a value of Q_{10} 1.62 ± 0.15 ($n = 6$) for hyperpolarizing current pulses (see Table 1). The difference between Q_{10} for the gramicidin channel and the inward rectifier was statisticaly insignificant ($p > 0.1$). The value of Q_{10} 1.62 corresponds to activation energy of 0.036 kJ/mol. The value of Q_{10} for the gramicidin channel was smaller under depolarization than under hyperpolarization (see Table 1, fibres 2 and 8). The quality of inward rectification was thus lacking in the gramicidin channel.

Discussion

Values of Q_{10} measured for inward rectification in our experiments are in agreement with those found by Almers (1971) (1.65—1.69) and by Leech and Stanfield (1981) (1.66) for frog skeletal muscle. A similar value of Q_{10} has been reported for inward rectification in starfish egg at temperatures greater than 10 °C (Hagiwara and Yoshii 1980). Ohmori (1978) observed in the tunicate egg an almost identical Q_{10} for the conductance of a single channel as well as for the maximum chord conductance. This implies that the number of open potassium channels at a given $V—V_k$ is relatively temperature independent. It is well known that the depolarizing current passes not only through the inward rectifier, but also through leakage channels. However, the higher value of the activation energy for the outward K^+ current as compared with the inward current under the same conditions in our experiments, seems to support models of the inward rectifier with a high energy barrier near the inside of the membrane, and a low external barrier (Leech and Stanfield 1981). The activation energy of 0.036 kJ/mol for the gramicidin channel in our experiments is comparable with the activation energy for the macroscopic membrane conductance of 0.041 kJ/mol reported for lipid bilayers by Goodall (1973). The calculated activation energy of ion migration through the single channel induced by gramicidin in dioleoyllecithin membranes

was 0.031 kJ/mol (Bamberg and Läuger 1974). It should be emphasized that the Q_{10} values of 1.55 and 1.62 for the inward rectifier and gramicidin channels in muscle membrane, respectively, are close to those reported for simple electrolyte solution (1.4~1.6). The similarity of Q_{10} values for hyperpolarizing pulses in gramicidin channels and in inward rectifier allows the suggestion that energy barriers for K^+-inward movement in both types of channels are nearly the same.

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