Biochimica et Biophysica Acta, 986 (1989) 146–150
Elsevier

BBAMEM 74616

Single K^+ currents during differentiation of embryonic muscle cells in vitro

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(Received 3 March 1989)
(Revised manuscript received 4 July 1989)

Key words: Potassium ion channel; Single-channel conductance; (Chicken myoblast); (Chicken myotube)

After 3–7 days in culture, chicken myotubes possess five types of K^+ channel: two high-conductance channels of 195 and 105 pS which are sensitive to tetraethylammonium (TEA), an ATP-sensitive channel of 64 pS and two low-conductance channels of 40 and 15 pS which are insensitive to TEA and ATP. The same population of channels is to be found in EGTA-treated muscle cells with blocked fusion and, with the exception of the ATP-sensitive channel, also in 1-day-old myoblasts. There are differences between myoblasts and myotubes in the percentage of incidence of individual channel types. High-conductance K^+ channels are most frequently to be observed in myotubes, but they are rare in myoblasts and EGTA-treated cells where low-conductance K^+ channels predominate.

Introduction

The fusion of undifferentiated myoblasts into myotubes which occurs between 24 and 72 h in culture [1] is accompanied by an increase in the resting membrane potential [2–5], accumulation of acetylcholine receptors [6], Na^+/K^+-ATPase molecules [1,5,7] and voltage-dependent Na^+ channels [4,8] in the plasma membrane. Little is known about the development of K^+ channels in skeletal muscle cells. Using the patch-clamp technique, we compared the population of K^+ channels in 2 to 7-day-old myotubes with that found in undifferentiated myoblasts maintained for less than 24 h in culture and with myoblasts the fusion of which had been blocked by cultivation in a calcium-free medium.

Material and Methods

Cell cultures

Pectoral muscles were taken from 10-day-old chick embryos, mechanically dissociated in a Ca^{2+}-free and Mg^{2+}-free isotonic phosphate-buffered solution and plated on Petri dishes precoated with rat-tail reconstituted collagen. The cells were grown in a modified Eagle's medium (MEM) [9].

In experimental cultures the myoblast fusion was prevented by adding 1.7 mM EGTA to the medium [5,6].

Single channel recordings

Single channel currents from inside-out membrane patches were recorded using a standard patch clamp technique [10]. A patch-clamp device constructed in our laboratory was used (see Fig. 1). In all experiments, the pipettes were filled with a solution containing 120 mM KCl, 1 mM $CaCl_2$ and 10 mM Hepes adjusted to pH 7.4 with KOH. The basic solution in the bath contained 140 mM KCl, Ca^{2+} buffered by EGTA to 0.1 μM and 10 mM Hepes adjusted to pH 7.4. To study the selectivity properties of the channels, the effect of substituting NaCl for 140 mM KCl in the bath was tested. Currents were digitized from magnetic tape recordings and stored into 5 120-point data blocks at a sampling rate of 6.7 kHz. The digital data were analyzed manually under visual control and by an interactive program on a DECLAB 11/03 computer. The program stored amplitude information and evaluated reverse potential and current-voltage relationships.

Results

Types of channel

In 1-day-old myoblasts, we found four types of K^+ channel which significantly differed in their conductiv-

Abbreviations: TEA, tetraethylammonium; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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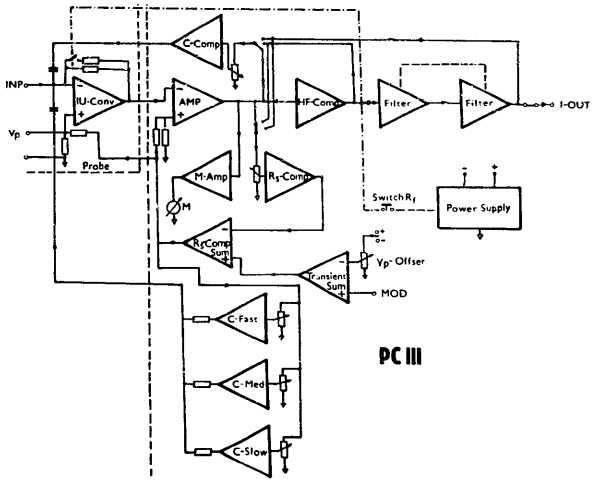

Fig. 1. Block scheme of the patch clamp technique has two parts. The IU-Converter (IU-Conv) serves as the input unit connected to the recording electrodes. The main part of the equipment consists of a voltage amplifier for compensation of the input capacity (C-Comp). An increase of frequency range is ensured by a high frequency compensator (HF-Comp) and the frequency is limited by a two-step eight-pole filter. Compensation of the capacitance discharge artefacts at the rise and decay sites of the polarization pulse is performed by three compensatory circuits (C-Fast, C-Med and C-Slow). For compensation of the in-series resistor (R_s), including the electrode impedance, the R_s-Comp circuit is used. R_s-Comp, Sum and Transient Sum in the figure indicate the circuits enabling the superposition of polarization and R_s compensatory voltages. A monitoring device (M) is coupled to the system by an additional amplifying circuit M-Amp.

ity and ion selectivity (Table I). The first two high-conductance channels (with $g = 195 \pm 55$ and 105 ± 20 pS) were inhibited by 5 mM TEA applied to the bath but their selectivity for K^+ ions differed. The two low-conductance channels (with $g = 40 \pm 5$ and 15 ± 3 pS) were insensitive to 5 mM TEA, 3 mM ATP + 1 mM Mg^{2+} and their K^+ selectivity was low. We did not find channels with conductances higher than 300 pS in 1-day-old myoblasts. Of 23 patches, six possessed no channels and five contained more than one, usually two or three channels.

In control experiments, 36 patches from mature myotubes grown for 3–7 days in cell culture were examined. In these cells, as well as in 40 patches from fusion-blocked cells grown for 2–7 days in the presence of EGTA, we identified five types of K^+ channel with mean conductances of 195, 105, 64, 40 and 15 pS, and channels of extremely high conductances of 300–500 pS (Table I and Figs. 2 and 3). The conductances of the latter channels remained unchanged when KCl in the bath was exchanged for NaCl. This means that these were most probably Cl^- channels, which have been seen in many cell types including rat myotubes [11] and neuroblastoma cells [12]. Both 195 and 105 pS channels in myotubes were identical to those found in 1-day-old myoblasts as they were highly K^+ selective and sensitive to TEA. The channel with a g value of 64 ± 17 pS was insensitive to TEA, but it could be inhibited by 3 mM ATP + 1 mM Mg^{2+} in the bath. The channels with conductances of 40 and 15 pS were also identical to those found in myoblasts: they were relatively non-selective and insensitive to TEA or ATP (Table I). In control myotubes, six patches were without channels and nine patches contained 2–3 types of channel. In EGTA-treated cells 12 patches were silent and in 12 there were more than one type of channel.

Frequency of channel occurrence

Although the population of K^+ channels found in 1-day-old myoblasts was similar to that found in

TABLE I

Conductance, selectivity and incidence of channels found in inside-out patches from 1-day-old myoblasts (A), myotubes (B) and EGTA-treated cells (C)

The values of the conductance and selectivity ratio are mean values ± S.D. of all channels of the same type found in A, B, and C. The ratio of permeability for K^+ (P_K) to permeability for Na^+ (P_{Na}) was calculated from the reversal potential (V_R) estimated with 120 mM K^+ in the pipette and 140 mM Na^+ in the bath. According to the Student's *t*-test, the differences between channel conductances and selectivities are significant at * $P < 0.01$. Relative frequencies of channel occurrence were tested using the χ^2-test. Differences between myoblasts (A and C) and myotubes (B) were significant at ** $P < 0.05$. *n* indicates the total number of channels found in 23 patches from A, 36 patches from B and 40 patches form C and includes zero channel incidence in the silent patches.

Conductance (pS)	V_R (mV)	P_K/P_{Na}	Incidence		
			A ($n = 28$)	B ($n = 45$)	C ($n = 52$)
195 ± 55	58	11.6 ± 2.7	6	13	5 **
105 ± 20 *	45	7.0 ± 1.5 *	3	11	5
64 ± 17 *	30	3.8 ± 1.5 *	0	5	6
40 ± 5 *	25	3.1 ± 1.8	7	4	10
15 ± 3 *	10	1.7 ± 2.0	6 **	1	9 **
300–500	0	1	0	5	5
No channels	–	–	6	6	12

myotubes and EGTA-treated cells, there were differences in the frequency of the occurrence of individual channel types. In myotubes, both kinds of TEA-sensitive high-conductance channels were observed more often than in 1-day-old myoblasts and EGTA-treated cells. Comparing myotubes and EGTA-treated cells the difference in occurrence frequency of a 195 pS channel was statistically significant (Table I). ATP-sensitive channels have been found in myotubes and EGTA-treated cells, but not in myoblasts. The 40 pS channels

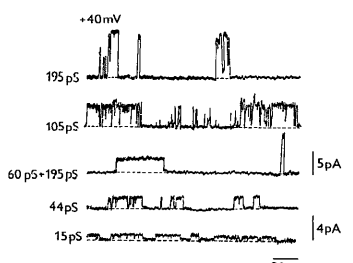

Fig. 2. Typical patch current recordings showing the activity of five K^- channel types found at +40 mV (with respect to the pipette) in cultured chicken muscle cells. The pipette contained 120 mM K^+ and the bath contained 140 mM K^+. A low-pass filter at 3 kHz was applied (temperature 22° C).

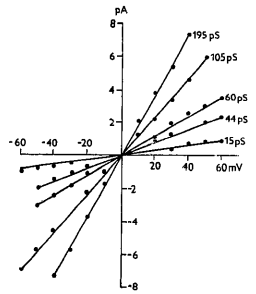

Fig. 3. Current-voltage relationships of five types of channel shown in Fig. 2. The conductance of all the channels appears to be linear between −60 and +60 mV. The reversal of currents in all cases was about −2 to −7 mV due to slight asymmetry of the patch pipette and bath concentration of KCl (120 mM and 140 mM, respectively) and all curves were therefore normalized so that the reversal point is at zero.

seem to occur less frequently in myotubes than in myoblasts or EGTA-treated cells. The 15 pS channels had a significantly lower incidence in myotubes as compared to both myoblasts and EGTA-treated cells.

The probability of finding a channel in the open state (p_o)

Recordings lasting for several seconds (5–10) were analyzed for the time during which a channel was in the open (t_{open}) or closed (t_{closed}) state. The probability of finding a channel in the open state was then calculated as [13]

$$p_o = \frac{t_{open}}{t_{open} + t_{closed}}$$

Fig. 4 demonstrates that p_o was highest for the 15 pS

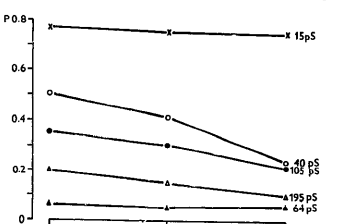

Fig. 4. Probability of finding the channels in an open state (p_o) and its voltage-dependence in the range of positive potentials applied to the pipette.

channels, which occurred in regular bursts (Fig. 2) and independently of the membrane potential. The activity of the ATP-sensitive channel was very low and was not dependent on the membrane potential. The 40 pS channels and the high-conductance K^+ channels of 195 pS and 105 pS were partially inactivated by positive potentials applied to the pipette. The mean open time of the 195 pS channels was shorter than that of the 105 pS channels.

Discussion

In the present study we have demonstrated that myoblasts which are already undifferentiated possess several K^+ channel types similar to those present in mature myotubes, namely the TEA-sensitive and highly K^+-selective channels with conductances of 105 and 195 pS which are similar to values of 100–200 pS reported for Ca^{2+}-activated K^+ channels in the rat [14,15] and chicken myotubes [16] and pharmacologically non-characteristic channels with 40 pS conductance and low K^+ selectivity, which resembled the 30 pS channel reported by Guharray and Sachs [16].

There are also differences between myoblasts and myotubes. Channels with a conductance of 64 pS, inhibited by ATP, have been found in myotubes but not in 1-day-old myoblasts. Such ATP-sensitive channels have been observed in many cells including cardiac [17,18] and frog skeletal muscle cells [19]. In 1-day-old myoblasts, we found a high percentage of pharmacologically non-characteristic and relatively non-selective channels with conductance values of 15 pS which were almost absent in myotubes. High-conductance K^+ channels of 105 and 195 pS in myotubes occurred about twice as frequently as in myoblasts. In EGTA-treated cells with blocked fusion, all channel types which had been found in myotubes were observed, including ATP-sensitive K^+ channels. However, the distribution of low- and high-conductance channels remained at the level observed in 1-day-old myoblasts (Table I).

This agrees with studies which indicate that blockade of myoblast fusion does not prevent the differentiation process, similar to what has been observed in studies on the developmental changes of acetylcholine receptors [6], voltage-dependent Na^+ channel [8] and Na^+/K^+ pump [1].

The question arises of whether the changes in the incidence of channel occurrence after cell fusion could be responsible for increases in K^+ permeability and, thus, for an increase in the resting membrane potential. The activity of ATP-sensitive channels in myotubes was very low. It is supposed that these channels play a role in the restoration of the decreased resting membrane potential in metabolically fatigued cells, because they become open after ATP exhaustion [17]. There is, probably, no such need in myoblasts with a naturally low membrane potential and limited excitability. Provided that the patch area of our pipettes was about 2 μm^2, we could estimate the mean membrane conductance for myoblasts ($G_{myoblasts}$), myotubes ($G_{myotubes}$) and EGTA-treated cells ($G_{EGTA\,cells}$) if we summarize the conductance contributions of individual channel types. This can be calculated according to the equation

$$G = g \cdot p \cdot N$$

where g is single-channel conductance, p is the probability of finding a channel in the open state and N is the number of channels per cm^2 [20]. Assuming that the resting membrane potential of 1-day-old myoblasts and of EGTA-treated cells is approx. -20 mV [2,5], and that of myotubes is about -60 mV [2], the calculated values of mean membrane conductance for potassium were $G_{myoblasts} = 1.2\ mS \cdot cm^{-2}$, $G_{myotubes} = 0.8\ mS \cdot cm^{-2}$, and $G_{EGTA\,cells} = 1.0\ mS \cdot cm^{-2}$ which is, by order of magnitude, comparable to the K^+ conductance of mature skeletal muscle cells [21]. According to this estimate, the mean membrane permeability for potassium does not increase, but rather decreases during the differentiation of skeletal muscle cells, so that it cannot be responsible for the increase of resting membrane potential. It is well known that chloride permeability is very high in mature skeletal muscles [22] and represents up to 85% of total resting membrane permeability [23].

We did not find any chloride channels in 1-day-old myoblasts, but these channels were detectable in EGTA-treated cells with a low resting potential [5]. The low P_K/P_{Na} ratio of low-conductance channels indicates that the resting sodium permeability mediated by these channels might essentially depolarize the membrane of 1-day-old myoblasts and EGTA-treated cells. Alternative electrogenic mechanisms, such as decreased resting permeability for sodium [3] or increased activity of the electrogenic Na^+/K^+ pump [5] should, therefore, be borne in mind.

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