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THE EFFECTS OF THE ANTIBIOTICS GRAMICIDIN A, AMPHOTERICIN B, AND NYSTATIN ON THE ELECTRICAL PROPERTIES OF FROG SKELETAL MUSCLE

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SUMMARY

The antibiotics gramicidin A, amphotericin B, and nystatin drastically decrease the membrane resistance of frog skeletal muscle fibers without changing the total capacitance. The resting potential of muscle fibers treated with these antibiotics is essentially normal if the Ringer solution does not contain Na^+.

INTRODUCTION

The antibiotics nystatin, amphotericin B, and gramicidin A are known to increase the permeability of biological and artificial membranes[1]; considerable progress has been made in determining the mechanisms by which these antibiotics increase the permeability of thin lipid bilayers[2,3]. The polyene antibiotics nystatin and amphotericin increase the permeability of thin lipid membranes containing sterols by interacting with the sterols to form nonstatic aqueous channels which are permeable to small anions. These antibiotics also increase the permeability to Na^+ and K^+ but to a much lesser extent, the relative permeability to anions and cations depending on the surface potential of the lipid which forms the bilayer. The linear pentadecapeptide antibiotic gramicidin forms channels in lipid bilayers as well; however, they are cation selective and in neutral bilayers have roughly equal permeability to Na^+ and K^+. We took advantage of the action of these antibiotics to alter the membrane conductance of skeletal muscle fibers.

MATERIALS AND METHODS

Electrical properties were measured on the sartorius muscle of the frog *Rana pipiens* at 23 °C using the method described by Fatt and Katz[4]. The experimental procedure and the analysis of the electrical constants was similar to that of Gage and Eisenberg[5] except that the membrane time constant was computed as 4 (instead of 4.398) times the extrapolated half time at zero electrode separation. The internal resistivity was taken to be 169 $\Omega \cdot cm^2$ (ref. 6). Ringer solutions containing nystatin or amphotericin were made by adding an aliquot from a saturated ethanol solution

TABLE I

ELECTRICAL PROPERTIES OF MUSCLE FIBERS IN ANTIBIOTIC SOLUTIONS

V_m is the resting potential; R_m is the membrane resistance; C_m is the membrane capacitance; R_0 is the input resistance; λ is the length constant; τ is the time constant, equal to $R_m C_m$. The numbers in parentheses are the S.E. of the number given immediately above.

	Solution	$V_m(mV)$	$R_m(\Omega \cdot cm^2)$	$C_m\left(\frac{\mu F}{cm^2}\right)$	$R_0(k\Omega)$	$\lambda(mm)$	$\tau_m(ms)$	*N*
A	Normal Ringer	92.2	2774	7.8	439	1.6	20.7	10
		(1.8)	(519)	(1.4)	(68)	(0.3)	(2.5)	
	1% ethanol Ringer	90.4	2272	7.7	440	1.4	16.9	11
		(1.0)	(335)	(2.1)	(145)	(0.2)	(2.1)	
B	Initial choline Ringer	93.8	6690	6.7	541	2.7	35.5	10
		(5.8)	(1155)	(1.9)	(191)	(0.5)	(3.4)	
	Gramicidin–choline Ringer	93.4	701	—	288	0.7	—	8
		(2.9)	(205)		(84)	(0.1)		
	Returned to choline Ringer	93.6	3023	9.0	450	1.7	26.3	7
		(4.3)	(1048)	(1.4)	(61)	(0.5)	(4.2)	
C	Normal Ringer	88.8	3524	5.8	314	2.1	20.4	10
		(1.9)	(355)	(0.5)	(88)	(0.2)	(2.2)	
	Amphotericin (supernatant)	88.8	2463	5.6	281	1.8	13.4	23
		(1.9)	(512)	(1.1)	(117)	(0.3)	(2.0)	
D	Normal Ringer	90.3	3484	5.9	534	1.8	19.6	7
		(0.5)	(745)	(1.4)	(270)	(0.2)	(2.1)	
	Nystatin (supernatant)	88.2	2516	6.5	343	1.7	15.9	18
		(2.0)	(527)	(1.9)	(75)	(0.3)	(2.6)	
E	Nystatin (saturated)	55.0	411	—	116	0.6	—	24
		(13.6)	(192)		(49)	(0.1)		

stored at 4 °C. The solutions made this way contain 1% (v/v) ethanol and approximately $1 \cdot 10^{-5}$ M of the antibiotics. Ringer containing 1% (v/v) ethanol had no effect on the electrical constants of the muscle (Table I: Row A). Ringer solution containing gramicidin was made from a stock solution composed of $1 \cdot 10^{-4}$ M antibiotic dissolved in ethanol. The extent and speed of action of the antibiotics depends on stirring in an ill-defined way; we followed a standard procedure of stirring the bath immediately before each measurement.

RESULTS

Ringer solution containing $1 \cdot 10^{-7}$ M gramicidin and 0.1% (v/v) ethanol drastically reduced the membrane resistance (to less than 20 $\Omega \cdot cm^2$) and changed the resting potential to a value between -5 and -20 mV. Under these conditions it is difficult to determine the electrical properties of the fibers, especially since the low resting potentials were probably associated with drastic swelling of the cell. To circumvent the problem of low resting potentials and membrane resistance,

we replaced all of the Na^+ with choline and removed half of the K^+ from the Ringer solution. Table I: Row B and Fig. 1A show the electrical constants of muscle fibers in such a Ringer solution. The electrical parameters were measured with the muscle immersed in choline Ringer without gramicidin (labelled "initial choline Ringer").

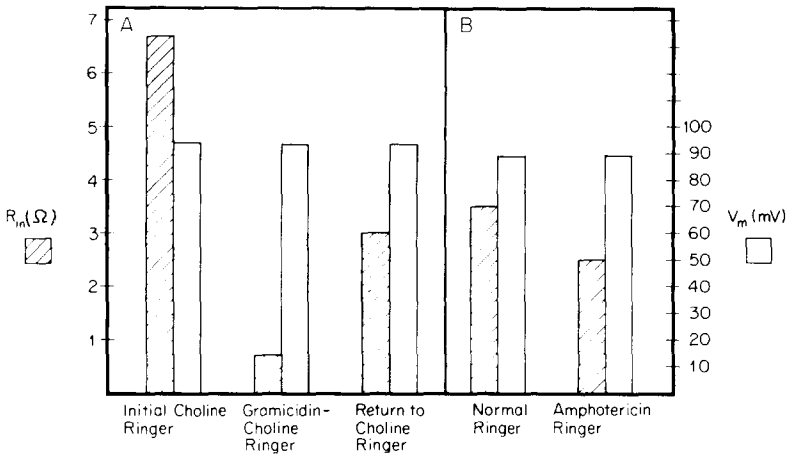

Fig. 1. A bar graph showing the resting potential and membrane resistance of muscle fibers. (A) The left-hand side of the figure illustrates an experiment in which the muscle was bathed in choline Ringer, then placed in a similar choline Ringer to which gramicidin had been added (see text for details of concentration and stirring) and then returned to choline Ringer. (B) The right-hand side of the figure illustrates an experiment in which a muscle was bathed in normal Ringer, and then placed in a similar Ringer containing amphotericin. In both experiments note the large decrease in membrane resistance accompanied by little change in resting potential.

The muscle was then immersed in choline Ringer containing $1 \cdot 10^{-7}$ M gramicidin and measurements were made from 30 min to 6 h after immersion in the gramicidin Ringer. The effect of the antibiotic was progressive, continuously increasing in time and not reaching steady state even in 6 h. The results given (labelled "Gramicidin–Choline Ringer") are the average of results over the whole time period. Most noteworthy is the finding that the membrane resistance is reduced by a factor of up to 100 (average factor is 10) although the resting potential is unchanged. After immersion in the gramicidin Ringer the muscle was placed back in the control choline Ringer and electrical measurements were again made (labelled "Returned to Choline Ringer"). The membrane resistance immediately increased to 0.5 of its original value before gramicidin treatment, probably because some of the antibiotic which had partitioned into the membrane was removed. The unaltered resting potential and the low membrane resistance of the fibers in the gramicidin–choline Ringer suggest that the antibiotic increases the permeability to K^+. It is possible that the antibiotic produces a large increase in Cl^- conductance but this seems less likely since gramicidin is known to induce selective permeability to cations in artificial membranes made from neutral lipids.

The insolubility of these antibiotics in polar solvents makes it difficult to establish a well defined equilibrium between the antibiotics and the membrane of the fiber. The amount of antibiotic partitioned into the membrane will depend on the concentration and the time the muscle is bathed in the antibiotic Ringer solution.

These problems were particularly pronounced for the less effective antibiotics nystatin and amphotericin. Either nystatin or amphotericin added to a Ringer solution from the supernatant of the saturated ethanol stock solution took 3 h to reduce the membrane resistance by 1/3, again with no change in resting potential (Table 1: Rows C and D; Fig. 1B; data measured between 3 and 6 h). This change in membrane conductance did not alter the shape of the action potential (Fig. 2) and a vigorous twitch was present. Since the resting potential was not altered, the conductance change must be due to an increase in the K^+ or Cl^- conductance.

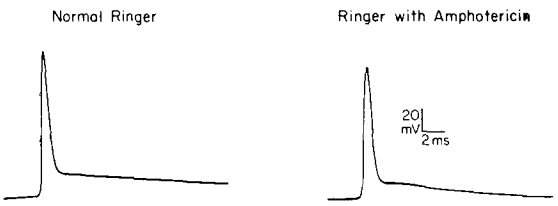

Fig. 2. Action potentials recorded in normal Ringer and in Ringer containing amphotericin.

However, in artificial membranes made from neutral lipids and cholesterol, both antibiotics have been found to increase the Cl^- conductance 10 times more than the cation conductance, suggesting that most of the conductance change we observe is due to an increase in chloride conductance. When large amounts of the nystatin stock solution were added to Ringer so that the Ringer solution itself also became saturated with the antibiotic, the membrane resistance immediately fell to less than 20 $\Omega \cdot cm^2$ and the resting potential changed to -5 to -20 mV. The change in the resting potential might be explained if nystatin increases Na^+ conductance in the muscle membrane as it does in artificial membranes. However, the depolarization was not completely blocked by the replacement of the Na^+ with choline; the membrane potential still quickly changed to -70 to -75 mV and then slowly depolarized further. Perhaps Ca^{2+} can permeate through the channels formed by nystatin in muscle membrane, as it seems to in squid[7] and bilayers[8], and hence depolarize the muscle fiber. The data in Table I: Row E was taken from muscles bathed in a saturated nystatin Ringer in which all of the Na^+ had been replaced with choline.

DISCUSSION

Skeletal muscle fibers have an intricate structure with two membrane systems (the surface membrane and the tubular membrane) limiting the flow of current from the cell interior to the cell exterior. We have no direct evidence which demonstrates the site of action of these antibiotics. It seems unlikely, however, that they act exclusively on the tubular membrane and indeed it is possible to interpret the measurements of capacitance to indicate that the antibiotics do not act on the tubular membrane to a large extent. If the antibiotic acted on the tubular membrane one would expect in some cases to have a drastic increase in the conductance of the wall of the tubules (in the cases described by Row B and C of Table I). A drastic increase in conductance would decrease the depth to which current could penetrate radially

into the tubular system and so would decrease the apparent tubular capacitance as measured by our technique[9,10]. We have not observed such changes.

There is still another system of membranes in muscle fibers which could conceivably be a site of action for the antibiotics: the membranes which comprise the sarcoplasmic reticulum. In muscle fibers bathed in normal Ringer, potential changes applied to the surface membrane do not spread into the sarcoplasmic reticulum at least under resting conditions. Current cannot spread from the tubular lumen to the interior of the reticulum because the junction between the reticulum and the tubule is an impermeant barrier[11]. The electrical properties of the reticulum cannot therefore be measured by recording potential changes across the surface membrane. If the antibiotics were to act at the junction between the sarcoplasmic reticulum and the tubular system so as to make this junction highly permeable, then a potential change across the surface membrane could spread into the sarcoplasmic reticulum and we would measure the electrical properties of the reticulum as well as those of the tubular and surface membrane. The large membrane area of the sarcoplasmic reticulum would be reflected in a large value of the apparent capacitance, a value some 10–20 times larger than normal. We have not measured a significant increase in capacitance and so conclude that these antibiotics do not make the junction between the tubular system and the sarcoplasmic reticulum highly permeable to ions.

The antibiotics gramicidin, nystatin and amphotericin can increase the conductance of frog skeletal muscle fibers by a factor of at least 10, yet the resting potential of the fibers remain quite reasonable if the batching solution does not contain Na^+. Such a preparation of muscle with high membrane conductance but quite normal in other respects may prove useful: in such a preparation there is a prominent variation in potential around the circumference of the fiber and so measurements from the preparation can be used to test the theories of the three dimensional spread of current[12]. The high permeability of the surface membrane produced by the antibiotics might also be exploited to study the properties of the internal membrane systems and the contractile protein of muscle fibers without mechanically removing the surface membrane.

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