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Nystatin-, mycoheptin- and levorin-induced conductance in the membrane of frog skeletal muscle fibres

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Abstract. The effects of the polyene antibiotics nystatin (2×10^{-5}–10^{-4} mol/l), mycoheptin (1.3×10^{-6}–10^{-5} mol/l) and levorin (10^{-8}–5×10^{-5} mol/l) on isolated frog skeletal muscle fibres and whole sartorius muscles of the frog have been investigated. Cation conductance was measured under current clamp conditions using a double sucrose-gap technique. Cation effluxes were studied by means of flame emission photometry. All three antibiotics increased the cation conductance and efflux rates; however, differences between the polyenes were found in the steady state values of induced cation transport at a given concentration. The values of both induced conductance g_{A_∞} and efflux rate constants K_A formed the following sequence: levorin > mycoheptin > nystatin, demonstrating a correlation with the order of antifungal activities. The dose-response curves of lg polyene-induced cation transport against lg of antibiotic concentration in our experiments had slope values which were much lower than those in bilayers: 1.7 and 1.3 for nystatin and mycoheptin, respectively, whereas the aromatic heptaene levorin had an even smaller concentration dependence. The decline in the equilibrium conductance caused by nystatin- and mycoheptin removal was very fast (during the first minute $\tau = 0.74$ and 2.39 min, respectively). In contrast, levorin-induced conductance was irreversible. It is proposed that the processes which limit the rate of channel formation are different in biological and model membranes.

Key words: Polyene antibiotics – Cation conductance – Cation effluxes – Muscle fibre membrane

Introduction

It is well known that the anti-fungal polyene antibiotics nystatin, mycoheptin and levorin form ion selective channels in lipid bilayers (Marty and Finkelstein 1975; Ermishkin et al. 1976; Kasumov et al. 1981; Borisova et al. 1988). One-sided nystatin application at high concentrations leads to cation selectivity whereas two-sided action results in formation of anion selective channels (Marty and Finkelstein 1975). In the channels formed by two-sided application anion-cation selectivity decreases in the following order: nystatin > mycoheptin > levorin (Kasumov et al. 1981). Levorin exhibits only cation selectivity, probably due to a reduction in the number of hydroxyl groups in the hydrophilic chain.

There has been considerable work done with nystatin on biological membranes. Nystatin applied extracellularly induces potassium loss from erythrocytes (Cass and Dalmark 1973; Chen et al. 1977) Candida albicans (Chen et al. 1977; Johnson et al. 1978; Hammond et al. 1974) and Acholeplasma laidlawii (De Kruijff et al. 1974). Nystatin has been shown to cause a rapid and reversible increase of conductance in molluscan neurons (Russell et al. 1977; Tillotson and Horn 1978). In addition, nystatin increases the cation conductance in frog skeletal muscle fibres and cation effluxes in frog whole muscles (Shvinka and Caffier 1989; Caffier and Shvinka 1989). Recently, some workers have succeeded in incorporating nystatin into a cell-attached patch, to increase its conductance (Horn and Marty 1988; Horn 1991). Nevertheless, experimental evidence for a complete similarity of the action of nystatin on artificial and cell membranes is still lacking. Very little is known about mycoheptin and levorin effects on biological membranes. The aromatic heptaene candicidin D, with a molecular structure similar to that of levorin, has been shown to induce efflux of potassium ions from Candida albicans (Hammond et al. 1974). Most of the experiments with aromatic polyenes have been done on yeasts and human red blood cells (Liras and Lampen 1974; Cybulska et al. 1983), whereas on cholesterol-containing animal cells no information is available on the effect of levorin. No attempt has been made to investigate the mycoheptin- and levorin-induced conductance on single isolated cells. Thus, the molecular organization of the nystatin, mycoheptin and levorin complexes with biological membranes remains obscure. The aim of this study was to investigate the cation conductances and ionic fluxes in-

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duced by nystatin, mycoheptin and levorin in muscle cell membrane to characterize the concentration dependence and reversibility of steady state conductance, and to compare the results with those reported for artificial lipid bilayer membranes.

Materials and methods

All conductance experiments were performed on single fibres from m. ileofibularis and m. semitendinosus of the Rana esculenta by the double sucrose gap method (Isenberg and Küchler 1970; Caffier et al. 1980). The membrane conductance was tested using hyperpolarizing square wave pulses (0.02–0.06 μA) of 300 ms duration applied once every 10 s. The test compartment in our experiments was 400 μm wide. Membrane conductance was calculated by $g = If^2/V_0S$, where V_0 is the voltage measured, I is the amplitude of the current pulse, S is the membrane area of the preparation in the test compartment and f is the short-circuiting factor. The value of f was equal to V_0/V_i, where V_0 is the potential change recorded with an extracellular electrode and V_i is the potential change recorded with an intracellular microelectrode under conditions of double sucrose gap. The short-circuiting factor in our experiments was 0.76. 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. The antibiotic-induced conductance g_A equals $g\text{-}g_0$, where g and g_0 are potassium conductances in the presence of antibiotic and prior to the antibiotic-treatment, respectively. $g_{A\infty}$ is the steady-state value of induced conductance, measured at the end of every incubation period with antibiotic. The decline in the equilibrium conductance after removal of antibiotics from the solution was analyzed by assuming that the conductance decreases exponentially with time, that is $g = g_{A\infty 1}\exp(-\rho_1 t) + g_{A\infty 2}\exp(-\rho_2 t) + g_0$ (1).

The solution used contained (in mmol/l): 160 K^+, 8 Ca^{2+}, 88 SO_4^{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). Thus, the K^+ concentration was nearly the same inside and outside the cell, and there was only K^+ for carrying a substantial current through the membrane. In some experiments K^+ was changed for an equimolar amount of Rb^+.

The effluxes of K^+, Rb^+ and Na^+ were studied on whole sartorius muscles isolated from the frog Rana temporaria. The muscles were enriched in sodium and rubidium by leaving them overnight at 3 °C in a solution containing (in mmol/l): 120 NaCl; 2.5 RbCl; 1.8 $Ca(NO_3)_2$; Tris-HCl, buffer, pH 7.2. The muscles were thereafter immersed in Na,K-free magnesium Ringer solution (in mmol/l: 76 $MgCl_2$; 1.8 $Ca(NO_3)_2$; Tris-HCl, buffer, pH 7.2) for 70–80 min at room temperature. It has been demonstrated that the time required to remove all extracellular sodium in a sodium-free solution is about 1 h (Vereninov et al. 1980). Then, the effluxes of K^+, Rb^+ and Na^+ were measured in Na,K-free magnesium Ringer solution. It has recently been found that $MgCl_2$ is the best substitute for sodium and potassium and in magnesium Ringer solution the

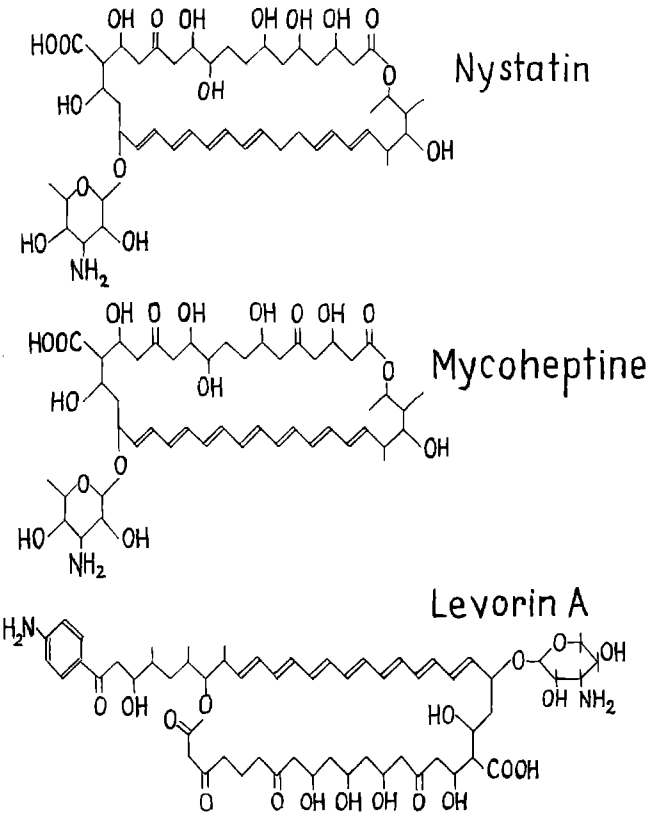

Fig. 1 Structural formulae of nystatin, mycoheptin and levorin molecules

muscles remained close to the physiological situation: the value of the resting potential was close to normal (80–90 mV) and muscles exhibited stable ion fluxes and rate constants for long periods of time (Vereninov et al. 1980; Vereninov and Marakhova 1981). The rate constants of potassium and sodium loss in magnesium Ringer solution did not differ much from that in Ringer solution. The muscles were incubated one at a time in a series of Pyrex tubes containing 4 ml of magnesium Ringer solution and were kept for 10 min in each tube. Thus, the cation content in 4 ml of the respective solution was collected within 10 min. The cation content in the solution was determined using a Perkin-Elmer flame photometer. At the end of the experiments the muscles were dried, weighed and the ion content in each muscle was determined. The cation contents were expressed as μmol/g dry weight. Efflux (μmol/g dry weight × min) was expressed as a concentration of the cation lost per minute during each collection interval. Efflux rate constants K (min^{-1}) were estimated by dividing efflux by ion concentration in muscle, corresponding to the mean time for each collecting period. The antibiotic-induced efflux rate K_A equals $K\text{-}K_0$, where K_0 and K are efflux rate constants measured without antibiotic at the start of experiment and after about 60 min incubation in the presence of antibiotics. The cation concentration in muscle for each time point was calculated as the end concentration plus the amounts lost by muscle and measured for this particular point and for the various incubation times after this particular point.

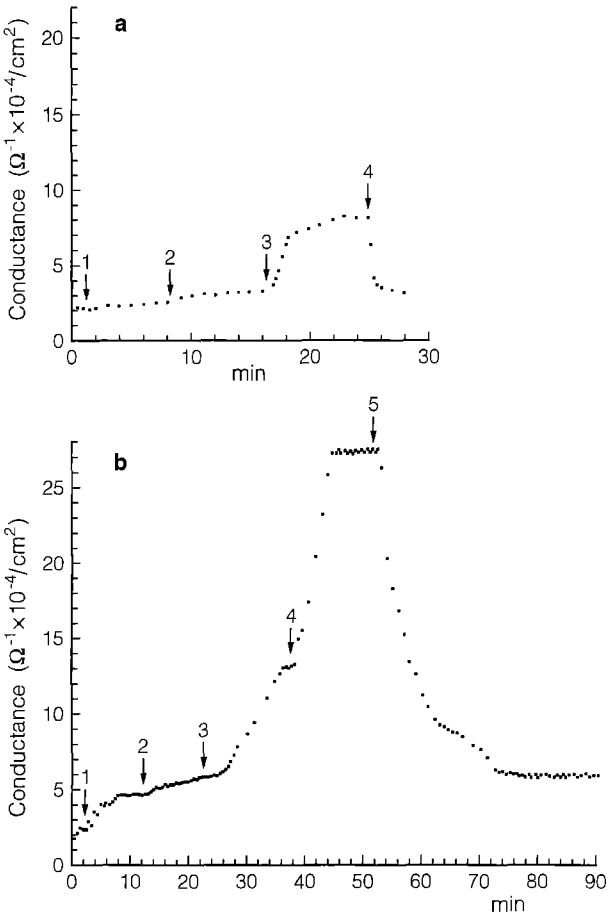

Fig. 2 Effects of nystatin *a* and mycoheptin *b* on the membrane conductance of isolated frog muscle fibre. Records of conductance in 160 mmol/l K^+ isotonic solution. Points represent values obtained under hyperpolarizing constant current pulses with intensity 0.02 μA *a* and 0.06 μA *b*. *a* $g_0=2.15\ \Omega^{-1}\times10^{-4}$/cm^2; arrows indicate application of nystatin to the solution at the following concentrations (in mol/l): I: 2×10^{-5}; 2: 4×10^{-5}; 3: 8×10^{-5}. Arrow 4 shows the removal of nystatin from the solution. *b* $g_0=2.04\ \Omega^{-1}\times10^{-4}$/cm^2, arrows indicate application of mycoheptin to the solution at the following concentrations (in mol/l): I: 1.3×10^{-6}; 2: 2.5×10^{-6}; 3: 5×10^{-6}; 4: 10^{-5}. Arrow 5 shows the removal of mycoheptin from the solution

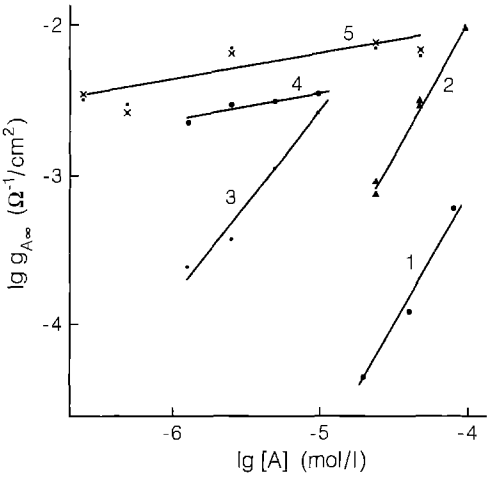

Fig. 3 The concentration-dependence of induced conductance and efflux rates. Abscissa: lg [A] (mol/l) where [A] is the antibiotic concentration. Ordinate: lg $g_{A\infty}$(Ω^{-1}/cm^2): (1) – nystatin, (3) – mycoheptin, (4) – levorin; lg K_A (min^{-1}): (2) – nystatin, (5) – levorin. $g_{A\infty}$ and K_A are the antibiotic-induced conductance and efflux rate coefficients, respectively. The meaning of the two sets of symbols of curve 5: points – K^+, crosses – Rb^+. The data of the experiments demonstrated in Figs. 2, 5, 6 and 7 are used

Polyene antibiotics, a kind gift from Dr. Vainshtein of the Research Institute of Antibiotics and Med. Enzymes (St. Petersburg), were dissolved in dimethylsulphoxide (DMSO) to the concentration of 10^{-2} mol/l. From the DMSO stock solution all antibiotics were added to experimental solutions to give a final concentration (in mol/l) of 2×10^{-5}–10^{-4} nystatin, 1.3×10^{-6}–10^{-5} mycoheptin, 10^{-8}–5×10^{-5} levorin. The final concentration of DMSO never exceeded 0.8% in conductance experiments and 0.5% in efflux experiments. Control experiments showed that this does not have any significant effect on membrane conductance or cation effluxes. Fresh solutions were prepared daily.

Results

When added to the external medium, nystatin and mycoheptin cause a concentration-dependent increase of potassium conductance in muscle fibre (Fig. 2a, b). In the absence of the drug the conductance is stable during the experiment. Different concentrations of the antibiotics are required for equal effects. Nystatin is only slightly effective at concentrations at which mycoheptin greatly enhances membrane conductance. The following values of polyene-induced conductance g_A were obtained (mean ± S.E.): nystatin (2×10^{-5} mol/l), $0.78\pm0.12\ \Omega^{-1}\times10^{-4}$/cm^2 ($n=6$); mycoheptine ($10^{-5}$ mol/l), $25.29\pm1.10\ \Omega^{-1}\times10^{-4}$/cm^2 ($n=6$). On a logarithmic scale, the dependence of induced conductance on the antibiotic concentration gives a straight line with a slope of about 1.7 for the nystatin-induced potassium conductance (Fig. 3, line 1) and of about 1.3 for the mycoheptin-induced conductance (Fig. 3, line 3). Our data show that both nystatin- and mycoheptin-induced conductances were reversible within minutes (Fig. 2a, arrow 4; Fig. 2b, arrow 5). From the slopes of 1 g $[(g-g_0)/g_{A\infty}]$ vs. time plots, according to formula (I), we calculated the values ρ (the relaxation rate constants) for nystatin- and mycoheptin removal (see Fig. 4). Curve 1 in Fig. 4 illustrates the kinetics of nystatin removal whereas curve 2 shows that of mycoheptin. From the reciprocals of the constants ρ we obtained two pairs of time constants τ as 0.74 and 1.67 min for nystatin and 2.39 and 4.64 min for mycoheptin removal.

The aromatic heptaene levorin, which apart from an amino sugar (mycosamine) contains a positively charged aromatic ketone *p*-amino-acetophenone, in our experiments gives values of induced-conductance higher than those of mycoheptin and nystatin (see Fig. 5). At a concentration of 10^{-5} mol/l levorin-induced conductance was $42.26\pm2.10\ \Omega^{-1}\times10^{-4}$/cm^2 (mean ± S.E; $n=6$). Thus, the values of conductances induced by polyenes at a given concentration follow the order levorin > mycoheptin > nystatin. The levorin concentrations used are close to satura-

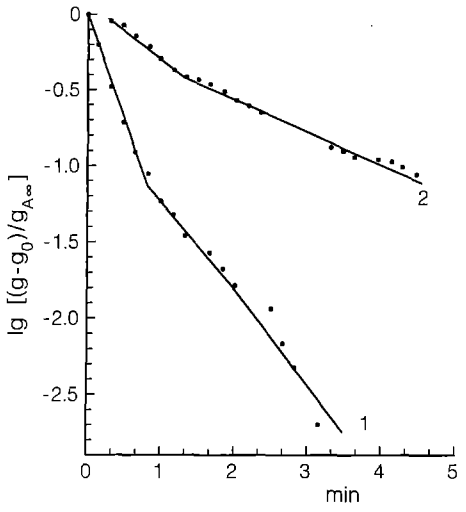

Fig. 4 Decline of induced conductance after removal of nystatin (1) and mycoheptin (2) from the solution. Records of conductance in 160 mmol/l Rb^+ (1) and 160 mmol/l K^+ (2) isotonic solutions. Abscissa: time (min). Ordinate: lg $[(g-g_0)/g_{A\infty}]$, were g_0, g and $g_{A\infty}$ are the conductance values prior the antibiotic-treatment, after the treatment and at the steady-state, respectively. Note that the decline of conductance caused by both antibiotics is a double-exponential process

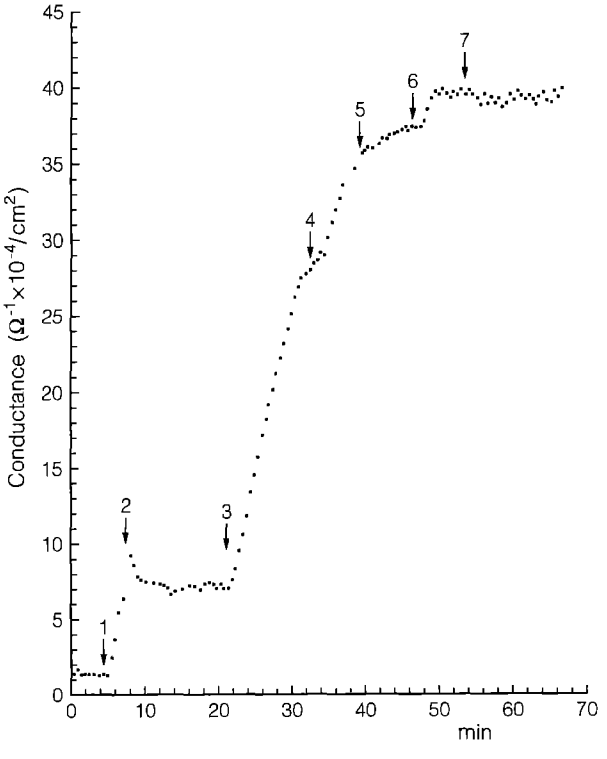

Fig. 5 Effect of levorin on the membrane conductance of isolated frog muscle fibre. Records of conductance in 160 mmol/l K^+ isotonic solution. Points represent values obtained under hyperpolarizing constant current pulses (0.03 μA). $g_0 = 1.35\ \Omega^{-1}\times10^{-4}/cm^2$. Arrows indicate application of levorin to the solution at the following concentrations (in mol/l): 1 and 3: 1.3×10^{-6}; 4: 2.5×10^{-6}; 5: 5×10^{-6}; 6: 10^{-5}. Arrows 2 and 7 indicate the removal of levorin from the solution

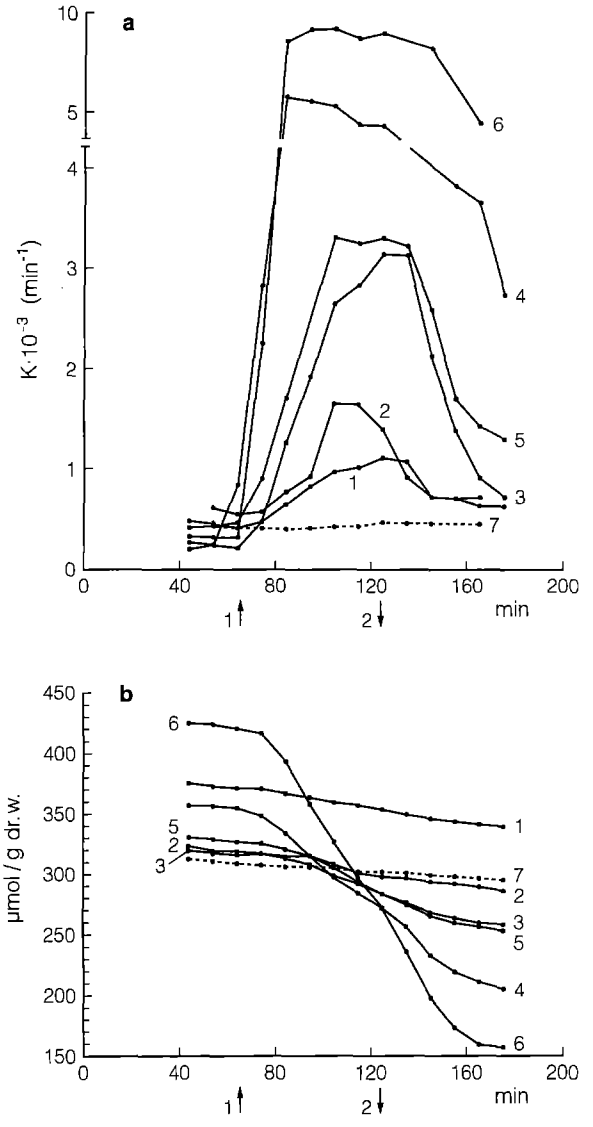

Fig. 6 Effects of nystatin on the cation efflux rate coefficient (K) and cation concentration in frog sartorius muscle. The muscles were initially incubated for 75 min in Na,K-free magnesium Ringer solution. Abscissa: time (min). Ordinate: *a* efflux rate coefficients, K ($min^{-1}\times10^{-3}$) for potassium; *b* potassium concentration (μmol/g dr.v.) in the same experiments. Here and in Fig. 7 b, d the concentration for each time point was calculated as the end concentration plus the amounts measured for this particular point and for the various incubation times after this particular point. Arrows 1 and 2 indicate application and removal of nystatin respectively. Interval between arrows – addition of nystatin in concentration (mol/l): (1) and (2) 2.5×10^{-5}; (3) and (5) 5×10^{-5}; (4) and (6) 10^{-4}. (7) – control muscle in Na,K-free magnesium Ringer solution with 0.5% DMSO present in the interval between arrows

tion. In a logarithmic plot of $g_{A\infty}$ vs. concentration for levorin (Fig. 3, line 4), in which the conductance data are the same as in Fig. 5, the slope is very low, indicating a weak concentration dependence in the range of concentrations used. It should be emphasized that the levorin effect on the muscle fibre membrane is irreversible (Fig. 5, arrows 2 and 7).

The data obtained by measuring the potassium and rubidium effluxes, the efflux constants and cation content in

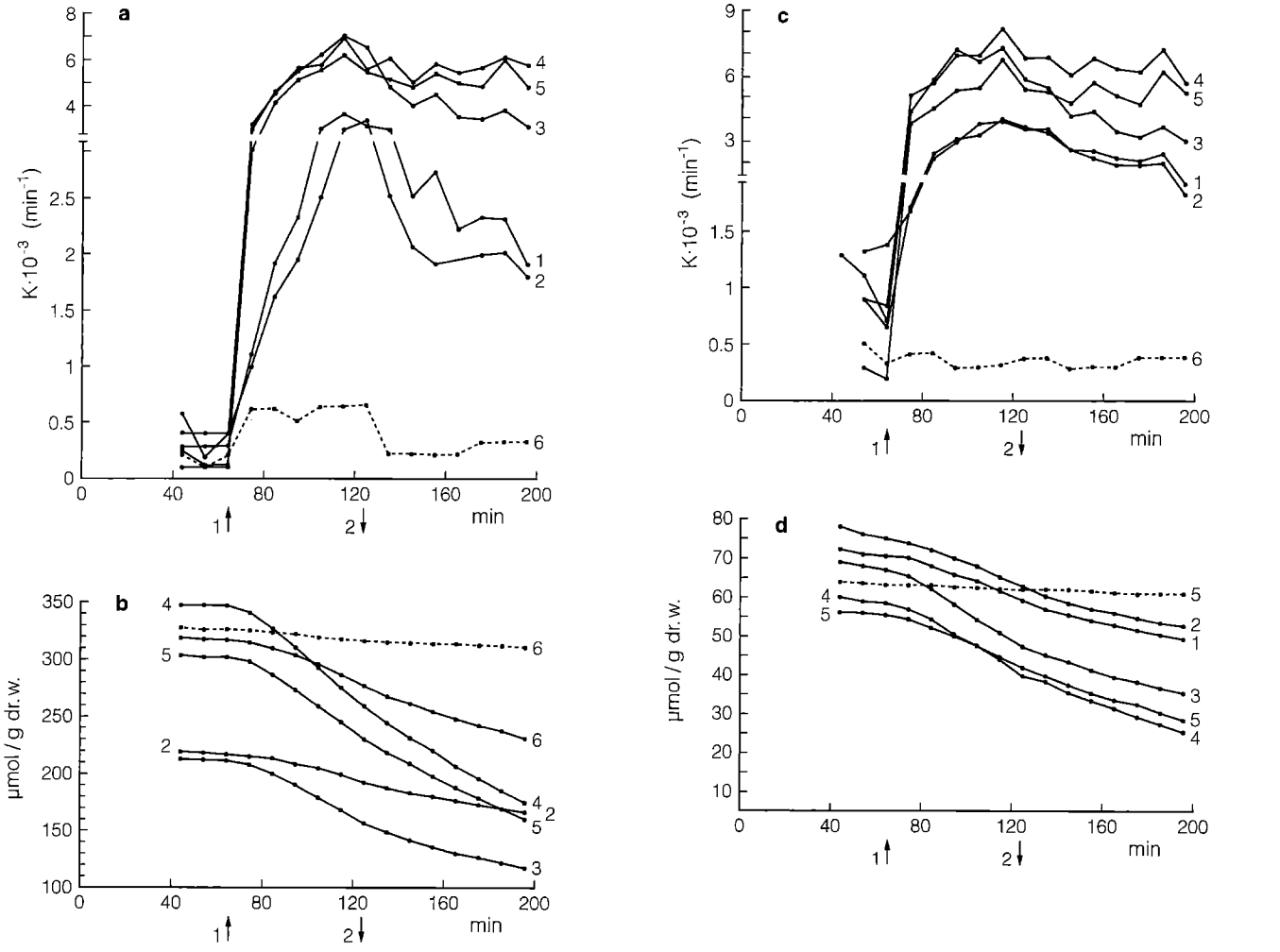

Fig. 7 Effects of levorin on the cation efflux rate coefficient (K) and cation concentration in frog sartorius muscle. The muscles were initially incubated for 75 min in Na,K-free magnesium Ringer solution. Abscissa: time (min.) Ordinate: *a* and *c* – efflux rate coefficients, K ($min^{-1} \times 10^{-3}$) for potassium and rubidium, respectively, *b* and *d* – potassium and rubidium concentration (µmol/g dr.w.) in the same experiment. Arrows 1 and 2 indicate application and removal of levorin correspondingly. Interval between arrows – addition of levorin in concentration (mol/l: (1) 2.5×10^{-7}; (2) 5×10^{-7}; (3) 2.5×10^{-6}; (4) 2.5×10^{-5}. (6) – control muscle in Na,K-free magnesium Ringer solution with 0.5 DMSO present in the interval between arrows

sartorius muscle support the results of conductance experiments reported above. Figure 6 a shows an increase in the potassium efflux rate upon the addition of 2.5×10^{-5}, 5×10^{-5} and 10^{-4} mol/l nystatin (solid lines between the arrows), and no effect in control muscle (dotted line) in the absence of this antibiotic. Figure 6 b illustrates the corresponding changes of potassium content. The induced potassium transport is reversible (Fig. 6 a, arrow 2). The logarithmic plot of induced efflux rate K_A vs. nystatin concentration for this experiment gives the same slope of about 1.7 (Fig. 3, line 2) as in conductance measurements.

In Fig. 7 the levorin-induced efflux rate constants (Fig. 7 a, c) and corresponding changes of potassium and rubidium content (Fig. 7 b, d) in sartorius muscle are demonstrated. The effect is found to be irreversible (Fig. 7 a, arrow 2; Fig. 7 c, arrow 2). As seen from Fig. 3, there is a very low concentration dependence of efflux rates for K^+ (line 5, dots) and Rb^+ (line 5, crosses) within a rather large range of levorin concentrations (from 2.5×10^{-7} to 5×10^{-5} mol/l). More detailed analysis at still lower levorin concentrations reaching 10^{-8} mol/l also demonstrate a slight dependence of lg K_A for K^+ and Rb^+ effluxes on lg levorin concentration, however it is less steep than that in model membranes (Kasumov and Liberman 1973).

The potassium efflux rates K_A, induced by the three polyenes at a concentration of 2.5×10^{-5} mol/l, are (mean ± S.E.): levorin, $5.19 \pm 0.34 \times 10^{-3}$ min^{-1} ($n = 6$); mycoheptin, $2.49 \pm 0.11 \times 10^{-3}$ min^{-1} ($n = 7$); nystatin, $0.68 \pm 0.07 \times 10^{-3}$ min^{-1} ($n = 7$). Thus, the order of rate constants both for K^+ and Rb^+ is the same as for conductances: levorin > mycoheptin > nystatin. As to the sodium efflux rate and content they are not affected by polyene-treatment in this situation. In additional experiments the muscles were enriched with lithium by leaving them at 3 °C overnight in a solution containing (in mmol/l): 80 NaCl; 40 LiCl; 1.8 $Ca(NO_3)_2$; Tris-HCl, buffer, pH 7.2. Under these con-

ditions polyene antibiotics were found to increase the sodium efflux as well, following the order: levorin > mycoheptin > nystatin.

Discussion

The question arises of whether saturation due to unstirred layer limitation can occur in our conductance experiments.

The conductance was measured in an isotonic solution of potassium sulfate, there was no diffusion gradient in the system without current and it can be considered that the current was transferred across the membrane only by potassium ions. The experiments were carried out under current clamp.

One can estimate the possible role of K^+ diffusion from the bulk solution via the unstirred membrane-adjacent layer to the membrane. The diffusion flow via the membrane-adjacent layer is described by the equation

$$\frac{dm}{dt} = \frac{D}{1} - (C_1 - C_2),$$

where D is the K^+ diffusion coefficient, 1 is the thickness of the unstirred layer (assumed to be equal to 0.05 mm, which is the radius of the fibre), c_1 and c_2 are the concentrations of K^+ ions in, respectively, the bulk of solution and the membrane-adjacent layer.

At $D = 1.8 \times 10^{-5}$ cm^2/s, $1 = 5 \times 10^{-3}$ cm and the maximally feasible gradient of potasssium concentration (Δc_{max}) of 1.6×10^{-4} mol/cm^3, the limiting diffusion flux shall be equal to

$$I_{lim} = \frac{D \Delta c_{max}}{1} = 5.76 \times 10^{-7} \text{ mol/cm}^2 \cdot \text{s}.$$

At the clamped current of 40 μA the experimental flux of potassium is

$$I_{exp} = \frac{I}{F \cdot S} = 3.30 \times 10^{-10} \text{ mol/cm}^2 \cdot \text{s}$$

Thus, $I_{lim} \gg I_{exp}$, and diffusion will not play any significant role in our studies of induced ion conductance.

Another possibility is that levorin concentrations used can lead to saturation because of filling-up of the space available. However, it is not the case with mycoheptin and nystatin in our experiments where enhancement at concentrations near 10^{-5} mol/l show no signs of saturation.

It has been shown that at concentrations above 10^{-6} mol/l polyenes in aqueous media are considerably aggregated (Mazerski et al. 1982; Cheron et al. 1988; Mazerski et al. 1990; Bolard et al. 1991). Our studies focus almost exclusively on concentrations within the range 10^{-6}–10^{-4} mol/l, except for levorin which is applied at lower concentration. It is proposed that in this range of concentrations polyenes are incorporated into the membranes mainly in self-associated form as small oligomers (Bolard et al. 1991), most probably dimers (Mazerski et al. 1990). If both monomers and dimers are active in channel formation, then depending on the mole fraction of monomeric and dimeric forms responsible for the induced cation conductance the slope values of dose-response curves may be in the range obtained in our experiments. The dose-response curves of lg conductance against lg antibiotic concentration in our experiments have slope values which are close to those obtained for amphotericin B-induced ion transport in mammalian erythrocytes (Deuticke et al. 1973), muscle fibre membrane (Shvinka and Caffier 1989; Shvinka et al. 1991), for the nystatin-induced conductance in Aplysia neurons (Russell et al. 1977) and rabbit bladder (Lewis et al. 1977) as well as for the exchange of K^+ against Li^+ in nystatin-treated red cells (Cass and Dalmark 1973). On the other hand, the power dependence of induced conductance on antibiotic concentration in our experiments is lower than that in artificial membranes, in thin lipid membranes it is proportional to about the 6–12 (Cass et al. 1970), 10 (Kasumov and Liberman 1972), 4–5 (Kleinberg and Finkelstein 1984) power of nystatin concentration and to the 2–5 power of levorin concentration (Kasumov and Liberman 1973).

Our results of almost complete reversibility of nystatin- and mycoheptin-induced conductance after an ~1 min wash in antibiotic-free solution show that these polyene antibiotics can readily exchange between the aqueous phase and the biological membrane. Our data support those obtained with nystatin on Aplysia neurons (Russell et al. 1977), rat lacrimal gland cells (Horn and Marty 1988) and red cells (Cass and Dalmark 1973). The results obtained on lipid bilayers when polyenes are applied from two sides show much slower relaxation kinetics. Thus, on thin lipid membranes nystatin-induced conductance decreases in antibiotic-free medium with a half-time of about 20 min (Cass et al. 1970). The relatively fast conductance decline by antibiotic removal in our experiments suggests that the structure of the antibiotic-induced channels and/or the channel assembly-disassembly reactions may be quite different in biological membranes and in bilayers with symmetric application of antibiotics. The kinetics of conductance decrease consisting of fast and slow components has been reported by us earlier for amphotericin B removal (Shvinka and Caffier 1991). The time constant of the fast component of conductance decline by antibiotic removal probably reflects the kinetics of the channel disassembly reactions. The appearance of the slow component seems to result from nonconductive forms of antibiotics, such as nonconducting "half pores" (Marty and Finkelstein 1975) and/or micelles within the membrane (O'Neill et al. 1986), which slow down the relaxation kinetics.

All the polyenes used in our experiments are drugs frequently used to treat systemic fungal infections. Therefore, a question arises as to what extent the observed effects on muscle cell membrane are correlated with antifungal activities of polyenes. Minimal fungistatic concentration (μg/ml), determined on Candida albicans, Candida tropicalis and Cryptococcus neoformans, for the polyenes used in our experiments, followed the same sequence, as in the conductance and efflux measurements: levorin > mycoheptin ≥ nystatin.

Thus, the acitivity of levorin is much higher than that of nystatin and mycoheptin both in cholesterol-containing muscle membranes and in ergosterol-containing fungal cells. The aromatic heptaene macrolide antibiotics such as levorin are found to be more potent that nonaromatic polyenes also in inducing yeast growth inhibition, red blood

cell lysis, and increase in the ionic permeability of large unilamellar lipid vesicles (Cybulska et al. 1983; Mazerski et al. 1983). It appears that the activity of aromatic heptaenes mainly depend upon the structure of their polar head groups (carboxyl in position C_{18} and amino sugar). However, the influence of the hydrophilic part of the ring cannot be disregarded. Unlike nystatin, the aromatic antibiotics are thought to be adsorbed on the surface of the lipid bilayer in monomeric from (Mazerski et al. 1983). It may be that the greater length of levorin, due to the presence of the aromatic group, enables the antibiotic to form a transmembrane channel, even in monomeric form. The irreversible nature of levorin-induced conductance, first stated in our experiments, is to be expected due to the extremely low solubility of levorin in water. Since the toxic side effects of polyene antibiotics probably result from interaction with cell membranes, dissociation of polyene-membrane complexes can be expected to play a significant role in antibiotic-caused toxicity. From this point of view the irreversibility of the levorin effect in the muscle membrane suggests some toxic side effects of this antibiotic and, as a consequence, the preference of nystatin and mycoheptin in antifungal therapy.

In experiments reported here the antibiotic concentration inducing conductance increases the rate constant of K^+ and Rb^+ efflux and decreases the cation content in intact muscle. Since the nystatin-induced pathway is essentially impermeable for divalent ions (Cass and Dalmark 1973; Russell et al. 1977), in our efflux studies when Mg^{2+} and Ca^{2+} are the only external cations, passive K^+, Rb^+ and Na^+ loss could occur only with simultaneous loss of Cl^-. It is well known that nystatin channels only weakly discriminate between cations (Russell et al. 1977). In accordance with this, it is logical to expect an increase in passive fluxes of K^+ and Rb^+ as well as Na^+ in polyene-treated muscles. While polyene-induced K^+ and Rb^+ effluxes in our experiments seem to be of a passive nature, the exact mechanism of Na^+ efflux is more complicated. It has been shown that Na^+ efflux in magnesium Ringer solution consists of ouabain sensitive active efflux via the Na^+/K^+ pump (40%), of passive Goldman type efflux (<2%) and of a remaining component (Vereninov et al. 1980). The latter may be active ouabain insensitive transport via the Na^+/K^+ pump and/or Na^+ efflux by Na^+-Ca^{2+} exchange. As to the lack of polyene-effect on Na^+ efflux in our experiments, it may be suggested that (i) polyenes do not influence the active sodium transport; (ii) the polyene-induced passive Na^+ efflux cannot be detected on the fluctuating background of the relatively high Na^+ efflux values. Preincubation of muscles in Li^+-containing solution probably slows down active sodium transport and thus passive polyene-induced Na^+ efflux becomes detectable.

In general, our results seem to suggest that the steady-state characteristics of polyene complexes responsible for the permeability induction in biological membranes, and especially the kinetics of channel formation, might differ from those in model membranes for symmetric addition of antibiotics. It is quite probable that the polyene channels in biological membranes are mobile "half pores" spanning the entire membrane which has been postulated earlier for lipid bilayers with one-sided nystatin effects (Marty and Finkelstein 1975; Kleinberg and Finkelstein 1984). The "half pore" must be a highly dynamic structure with a short life time compared to the complete pore formed in lipid bilayers by two-sided polyene application. Moreover, the antibiotics can induce membrane permeability by forming other molecular structures such as amphotericin B dimers (Bolard et al. 1991) and two kinds of conformers detected at high amphotericin B concentrations (Vertut-Croquin et al. 1983).

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