Short communication

Effects of Amphotericin B Methyl Ester on Potassium Conductance and Effluxes in Frog Skeletal Muscle

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The polyene antibiotic amphotericin B is known to form anion selective channels in lipid bilayers when applied from two sides (Ermishkin et al. 1977; Borisova et al. 1986), whereas one-sided application produces cation selective channels in bilayers (Brutyan 1982) and biological membranes (Bolard 1986; Shvinka and Caffier 1989). When acting from two sides, the amphotericin B channels would be constituted of two "half-pores", hydrogen bonded end to end (Silberstein 1989). The molecular organization of the amphotericin channels in biological membranes remains unknown. It seems quite probable that a "half pore" spanning the entire biological membrane is responsible for the amphotericin effect (Marty and Finkelstein 1975). The question is how to explain the cation selectivity of such a "half pore". One possible explanation is that a negative charge of carboxyl groups of C_{16} imparts a negative electrostatic potential to the pore entrance, thus favoring the entry of cations into the channel. Metamphocin, an alkyl derivative of amphotericin B, has a $-CH_3$ group which replaces the hydrogen in the carboxyl group. Assuming that the above selectivity hypothesis is correct, the values of cation conductances induced by metamphocin and amphotericin B should be different. The aim of this study was to investigate the potassium conductances in frog muscle fibres and potassium effluxes from the sartorius muscle caused by application of metamphocin and amphotericin B. Both antibiotics were dissolved in dimethylsulphoxide and then added to both solutions to give final concentrations (in mol/l) of 10^{-5} amphotericin B, $5.10^{-6} - 4.10^{-5}$ metamphocin, and no more than 0.4% (v/v) dimethylsulphoxide.

All conductance experiments were performed on single fibres from m. ileofibularis and m. semitendinosus of the frog *Rana esculenta* by the double sucrose gap method (Caffier et al. 1980). Measurements were made in isotonic K_2SO_4 solution containing (in mmol/l): 160 K^+; 8 Ca^{2+}; 88 SO_4^{2-}; 2 TRIS-maleate, pH 7.2. Potas-

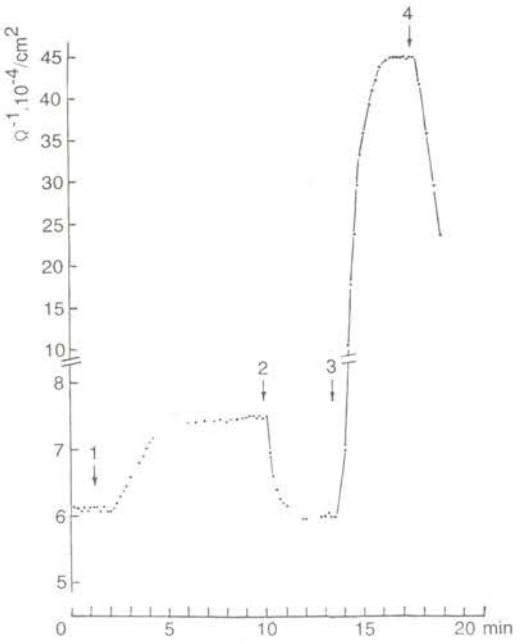

Figure 1. *A*: Effects of amphotericin B methyl ester and amphotericin B on membrane conductance of isolated frog muscle fibre. Records in 160 mmol/l K^+ isotonic solution. Abscissa: time (min). Ordinate: conductance ($\Omega^{-1}.10^{-4}/cm^2$). The intensity of hyperpolarizing constant current pulses was 0.04 μA. Intervals between arrows: 1–2, addition of 10^{-5}mol/l metamphocin; 3–4, addition of 10^{-5}mol/l amphotericin B.

sium efflux was studied on whole sartorius muscles isolated from the frog it Rana temporaria. The muscles were incubated overnight at 3 °C in a solution containing (in mmol/1): 40 NaCl; 80 LiCl; 2.5 KCl; 1.8 $Ca(NO_3)_2$; Tris-HCl buffer, pH 7. Then, the muscles were incubated one at a time in a series of Pyrex tubes containing 1 ml of magnesium Ringer solution each (in mmol/l: 76 $MgCl_2$; 1.8 $Ca(NO_3)_2$; TRIS-HCl buffer, pH 7) for 10 min in each tube. The potassium content in the solution was determined using a Perkin-Elmer flame photometer. The efflux rate constant (min^{-1}) was calculated from efflux per minute divided by ion concentration in muscle.

Minimal fungistatic concentrations, determined on *Candida albicans*, for metamphocin and amphotericin were correspondingly 0.39 and 0.79 μg/ml.

Addition of 10^{-5} mmol/l metamphocin to muscle fibre induces an increase of potassium conductance (Fig. 1, interval between arrows 1–2) which is much smaller than that induced by 10^{-5} mmol/l amphotericin B in the same experiment. (Fig. 1, interval between arrows 3–4). The potassium efflux rate constant K (Fig. 2) also

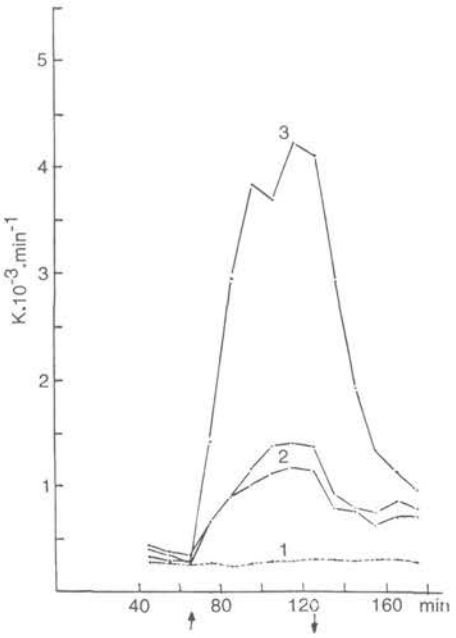

Figure 2. *A*: Effects of amphotericin B methyl ester and amphotericin B on potassium efflux rate coefficient K for frog sartorius muscle. The muscle were initially incubated for 75 min in Na^+, K^+-free magnesium Ringer solution. Abscissa: time (min). Ordinate: efflux rate coefficient ($K.10^{-3}.min.^{-1}$). Interval between arrows: (1), addition of dimethylsulphoxide to a final concentration of 0.1% (control muscle); (2) addition of 10^{-5} mol/l metamphocin; concentration of dimethylsulphoxide 0.1%; (3), addition of 10^{-5} mol/l amphotericin B; concentration of dimethylsulphoxide 0.1%.

reflects the difference between the effects of metamphocin and amphotericin, the first one being remarkably smaller. For comparison, potassium loss from human erythrocytes produced by amphotericin B methyl ester hydrochloride was also less pronounced than that after amphotericin B (Chen et al. 1977), whereas no change of activity was observed with the methyl ester on thin lipid membranes as compared to amphotericin (Cass et al. 1970). It should be mentioned that both amphotericin- and metam-phocin-induced conductances were completely reversible. There was no remarkable difference in relaxation kinetics between both antibiotics after their washout. In contrast, results obtained on thin lipid membranes (Cass et al. 1970) showed a conductance decrease with a half-time of about 120 min for amphotericin and 1 min for methyl ester derivatives, whereas with bilayers from total bovine brain phospholipids the time constant decreased about 4 times per CH_2 group (Kasumov and Malafriev 1984).

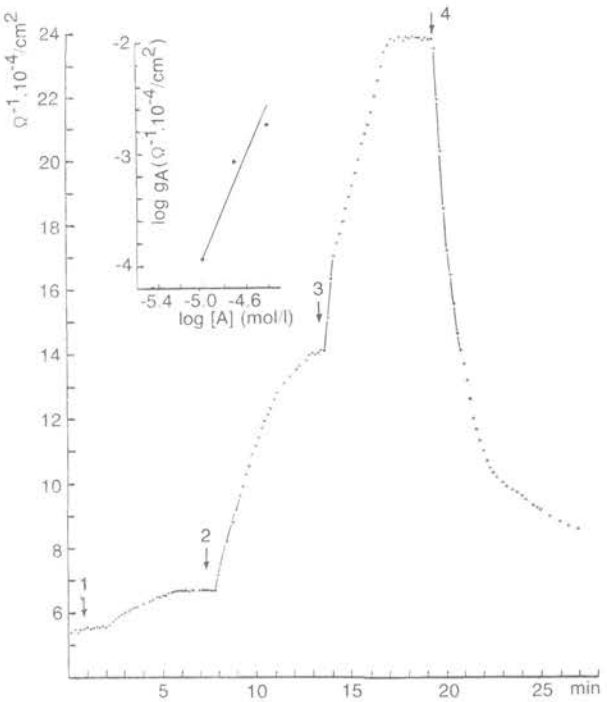

Figure 3. *A*: Conductance dependence on metamphocin concentration. Records in 160 mmol/l K^+ isotonic solution. Abscissa: time (min). Ordinate: conductance ($\Omega^{-1}.10^{-4}/cm^2$). The intensity of hyperpolarizing constant current pulses was 0.02 μA. Intervals between arrows indicate application of metamphocin (in mol/l): 1–2, 10^{-5}, 2–3, 2.10^{-5}; 3–4, 4.10^{-5}. Inset: the dependence of steady state conductance on metamphocin concentration, logarithmic scale. Abscissa: log [A](mol/l); [A] is the antibiotic concentration. Ordinate: log g_A ($\Omega^{-1}.10^{-4}/cm^2$), g_A is metamphocin-induced conductance expressed as the difference between conductance values after and prior to antibiotic treatment.

Fig. 3 illustrates the metamphocin concentration dependence of conductance. On a logarithmic scale, the dependence of the induced steady state conductance on the antibiotic concentration (see Fig. 3, inset) gives a slope of about 2, which is close to the values for the amphotericin-induced potassium conductance (1.8) and efflux (1.6) in muscle membrane (Shvinka and Caffier 1989), and lies within the slope range of 1.5–2.5 obtained for the amphotericin-induced enhancement of erythrocyte permeability (Deuticke et al. 1973).

Thus, our results seem to support the hypothesis of carboxyl groups playing a role in the determination of cation selectivity of polyene antibiotic-induced conductance in biological membranes. The higher activity of amphotericin com-

pared to metamphocin in muscle membrane and the reverse relation between them in *Candida albicans* allows a suggestion that metamphocin may be a perspective anti-fungal agent.

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