Antagonism by Sterols of the Action of Amphotericin and Filipin on the Release of Potassium Ions from *Candida albicans* and *Mycoplasma mycoides* subsp. *capri*

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Polyene antibiotics alter the permeability of the plasma membranes of eukaryotic cells by interacting with sterols. The change in permeability can be monitored by measuring the rate of leakage of potassium ions from sensitive cells (Gale, 1974). Mouse LS cells have been shown to be less sensitive to the action of amphotericin methyl ester (AME) and more sensitive to filipin (FIL) than *Candida albicans* (Gale, 1973, 1974). The suggestion was made that this difference in sensitivity is based upon the different sterols in the membranes of the two organisms, cholesterol being the main sterol in mouse cell membranes, and ergosterol the main sterol of *Candida* plasma membranes (Marriott, 1975). When sterols were added together with AME to suspensions of *C. albicans*, ergosterol was found to be more effective than cholesterol in reducing the action of the antibiotic, suggesting that ergosterol has a higher affinity than cholesterol for AME (Gale, 1974; Gale *et al.* 1975). A similar conclusion was reached by Nozawa *et al.* (1974) after comparing the effects of amphotericin B on the ultrastructure of fungal membranes, containing ergosterol, with those reported by Verleij *et al.* (1973) on cholesterol-containing membranes of erythrocytes or *Acholeplasma laidlawii*. The present communication extends the observations made by the use of K⁺ release measurements on *C. albicans*, and on *Mycoplasma mycoides* subsp. *capri*, grown in the presence of either ergosterol or cholesterol.

The release of K⁺ from *C. albicans* suspensions has been followed by the use of a K⁺-sensitive electrode (Gale, 1974). The addition of AME or FIL is followed by a progressively increasing rate of K⁺ leakage, and a standard rate (s.r.) of release has been defined as 1 nmol K⁺/min/mg dry wt cells over the rate of release in the absence of antibiotic. In the present experiments, the concentration (s.r.c.) of antibiotic required to induce the s.r. of release in a given time (6 to 10 min) at 20 °C was first determined. Mixtures of sterol and polyene were made up in ethanol in the proportions described below and, in each case, an amount of mixture containing sufficient polyene was added to the cell suspensions to give this final concentration (s.r.c.) in the electrode vessel; the effect of the sterol was then assessed by the increase in time required to reach the s.r. of K⁺ release. Controls of ethanol alone were carried out in all cases. All quantities below are expressed in terms of the active component.

Mycoplasma mycoides is sterol-requiring and sterols present in the growth medium are incorporated into the plasma membrane without alteration; the effect of the growth medium on the lipid composition of *M. mycoides* subsp. *capri* strain PG3 has been described by Archer (1975). The release of K⁺ from suspensions of *Mycoplasma* has been studied as for *C. albicans* except that the organisms were suspended in 0.05 M-tris-HCl buffer pH 8.0 containing 0.125 M-sucrose (Archer, 1975). AME and FIL induced a constant rate of K⁺ leakage; the rate varied with antibiotic concentration, but was not time-dependent as with

Fig. 1. Release of K⁺ from *C. albicans*; antagonism of the action of (a) AME and (b) FIL by sterols. The preparation of *Candida* suspensions and assay of K⁺ release were as described by Gale (1974). Sterol and polyene mixtures in the proportions shown were made up in ethanol. The s.r.c. of AME required to induce the s.r. of K⁺ release in 10 min was 0.5×10^{-6} M; the s.r.c. of FIL required to induce the s.r. of K⁺ release in 6 min was 0.9×10^{-5} M. These antibiotic concentrations were used in sterol-polyene mixtures. ●, Ergosterol; ○, cholesterol; ▲, zymosterol.

C. albicans. The concentration of antibiotic producing an increase in the rate of K⁺ release of 2 to 3 nmol/min/mg dry wt organism was determined and the effect of sterols on this rate then assessed either by adding sterol-antibiotic mixtures as described above or by adding the desired amount of sterol in ethanol solution to the electrode vessel immediately before adding the antibiotic solution. Results were the same whichever method of adding sterol was adopted.

Figure 1 shows the effect of increasing amounts of sterol on the time taken to attain a s.r. of K⁺ release from *C. albicans* in the presence of a given concentration of AME (Fig. 1a) or FIL (Fig. 1b). Zymosterol was included in the tests since this sterol is also a component of *Candida* plasma membranes (Marriott, 1975). In each case the time taken to reach s.r. increased in a linear manner with the increasing proportion of sterol. When AME was the antibiotic, zymosterol was the most effective antagonist while ergosterol was approximately twice as effective as cholesterol. The molar ratio of sterol to AME required to double the time taken to produce the standard response was 1.6 for zymosterol, 3.0 for ergosterol and 5.2 for cholesterol. When FIL was the antibiotic, cholesterol was a more effective antagonist than ergosterol; the molar ratio for doubling the response time for ergosterol was 0.24, while cholesterol and zymosterol had similar activities at a molar ratio of 0.09 to 0.1. In antagonism experiments of this nature, although the absolute values of the antagonistic ratios may vary from experiment to experiment, their relative values remain the same (Gale *et al.* 1975).

Freshly prepared suspensions of *Mycoplasma* have negligible leakage of K⁺, and Table 1 compares the effects of adding either AME or FIL, in the presence or absence of sterol, to organisms grown in the presence of cholesterol or ergosterol. The growth media and conditions were adjusted so that the total sterol content (cholesterol or ergosterol) of the membranes was approximately the same in both cases (Archer, 1975). The amount of AME required to release K⁺ at a rate of 2 to 3 nmol/min/mg dry wt organisms was much less for organisms grown in ergosterol than for those grown in cholesterol. The concentration of FIL required to produce this degree of leakage was greater for organisms grown in ergosterol than in cholesterol.

Table 1. *Effect of cholesterol and ergosterol on the release of K⁺ from Mycoplasma mycoides subsp. capri in the presence of amphotericin methyl ester or filipin*

Organisms grown in a modified Edward's medium containing either 0.08 mM-cholesterol or 0.08 mM-ergosterol as sole sterol. Organisms were harvested near the end of the exponential phase of growth, washed 3 times in 0.05 M-tris-HCl buffer pH 8.0 containing 0.125 M-sucrose, and suspended in the same medium at 0.5 mg dry wt/ml in an electrode vessel. K⁺ release was followed with a K⁺-sensitive electrode (Archer, 1975; Gale, 1974)

Antibiotic added ...	AME*								FIL†			
	Cholesterol				Ergosterol				Cholesterol			
	K ⁺ release				K ⁺ release				K ⁺ release			
	nmol/min/mg		%		nmol/min/mg		%		nmol/min/mg		%	
Sterol/polyene molar ratio												
Organisms grown in cholesterol												
0	2.20		100		2.20		100		2.38		100	
0.5	1.82		82.6		1.54		70		1.54		64.8	
1	1.67		75.8		1.14		51.7		0.94		39.3	
5	1.24		56.3		0.86		39		0.51		21.4	
10	0.81		36.8		0.38		17.3		0.31		12.9	
Organisms grown in ergosterol												
0	3.32		100		3.32		100		2.49		100	
0.5	3.30		99.5		2.55		76.8		2.45		98.5	
1	3.15		94.8		2.30		69.3		2.30		92.5	
5	2.89		87.0		2.14		64.4		2.08		83.4	
10	2.38		71.9		1.87		56.4		1.29		51.8	

* AME added at 5 μ M for organisms grown in cholesterol, and 0.5 μ M for organisms grown in ergosterol.

† FIL added at 10 μ M for organisms grown in cholesterol, and 20 μ M for organisms grown in ergosterol.

Table 1 also shows the antagonistic effects of cholesterol and ergosterol against the antibiotics. When tested against *Mycoplasma* grown in cholesterol, the molar ratios of sterol to polyene required to reduce the effect of the antibiotic by 50 % were 1.5 for ergosterol and 6.5 for cholesterol against AME, or 6.3 for ergosterol and 0.7 for cholesterol against FIL. Antagonism was not so marked in tests using organisms grown in ergosterol, but it can again be seen that ergosterol was a more effective antagonist than cholesterol against AME while the reverse was true against FIL.

The conclusions that can be drawn from these and previous results are that AME is more toxic to organisms whose main membrane sterol is ergosterol than to those containing cholesterol, and that ergosterol is a more effective antagonist than cholesterol to AME but a less effective antagonist than cholesterol to FIL. Since the mode of action *in vivo* of these polyenes is believed to arise from the formation of hydrophobic complexes between polyene molecules and sterols (De Kruijff & Demel, 1974) it would appear that AME has a higher affinity for ergosterol than for cholesterol while FIL has a higher affinity for cholesterol than ergosterol. The selective toxicities of these two antibiotics can therefore be attributed to these differences in affinity.

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