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MEMBRANE STABILIZERS INHIBIT POTASSIUM EFFLUX FROM *STAPHYLOCOCCUS AUREUS* STRAIN No. U2275

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The effect of different categories of membrane stabilizers on K^+ loss and growth has been characterized in a culture of *Staphylococcus aureus*. Chlorpromazine, thiopental and tetracaine at low concentrations produced a marked inhibition of K^+ loss and an equivalent increase in the K^+ contents of *S. aureus*. Whereas the inhibitory effect of chlorpromazine on K^+ loss was observed at lower than bacteriostatic concentrations of the drug, thiopental had no effect on growth in the concentration range where K^+ loss was maximally inhibited. It is concluded that the bacteriostatic action of chlorpromazine is probably not related to its membrane stabilizing effect only.

It is well known that chlorpromazine increases the osmotic resistance of erythrocytes and inhibits the passive fluxes of Na^+ and K^+ across the plasma membrane in erythrocytes, nerve and muscle cells [1–3]. Many commonly used drugs share these properties and have been named membrane stabilizers [1,2]. It was recently observed that chlorpromazine at concentrations above 0.2 mM inhibits the growth of *Staphylococcus aureus* and other microorganisms [4]. Hence, it became of interest to determine whether the phenomena of membrane stabilization were of any significance for the growth and the survival of bacteria, and particularly, whether other membrane stabilizers had bacteriostatic actions. It is known that *Escherichia coli* cells growing in media containing such membrane stabilizing agents as barbiturates and local anesthetics undergo a change in the composition of their membrane-lipoproteins [5]. This indicates that bacteria are sensitive to membrane stabilizers.

A strain of *Staphylococcus aureus*, No. U2275 [4,6], was used for the experiments. Chlorpromazine and tetracaine were obtained from D.A.K., Copenhagen, Denmark. Thiopental from Leo Pharmaceutical Corporation, Copenhagen, Denmark.

The release of K^+ from *S. aureus* was determined as follows: 40 μl *S. aureus* broth was diluted into 400 ml ox serum broth containing 40 mM KCl. Aliquots of 200 ml were incubated for 18 h at 35°C. The resulting cultures contained approx. 10^9 c.f.u./ml. Tubes containing 195 ml culture were centrifuged for 20 min at 5°C in a Sorvall centrifuge ($4000 \times g$). The pellets were washed three times by re-suspension in 200 ml 154 mM NaCl followed by re-centrifugation.

Following the 3rd wash, the K^+ concentration of the supernatant was 0.03 ± 0.02 mM. In the experiments concerning tetracaine the bacteria were exposed to the drug (concentration 1 mM) during the wash and centrifugation.

After re-suspension into 5 mM Tris (pH 6.4) the pellets were mixed with the drug solution so as to give a final volume of 85 ml. The tubes were incubated in a shaking water bath at 35°C. After

Abbreviations: c.f.u., colony-forming unit.

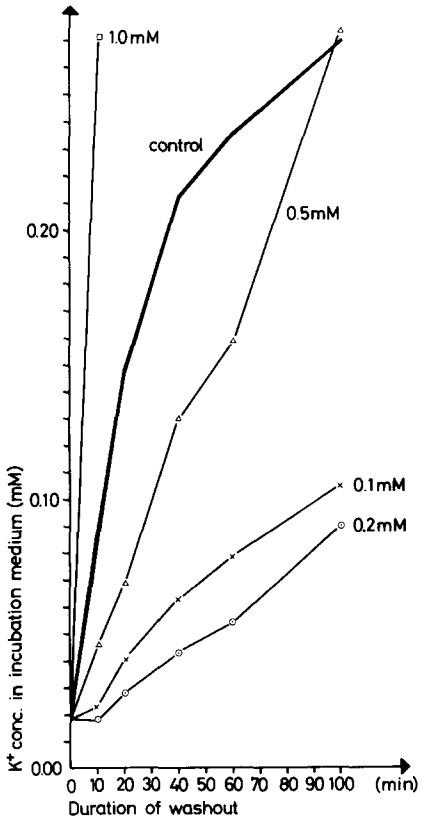

Fig. 1. Effect of chlorpromazine (0.1–1.0 mM) during the time of K^+ release from *S. aureus*.

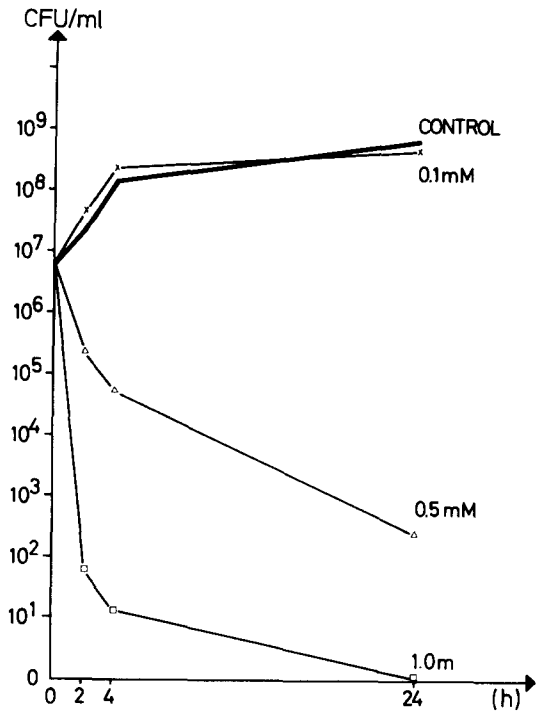

Fig. 2. Effect of chlorpromazine on the growth of *S. aureus* strain No. U 2275.

10, 20, 40, 60 and 100 min, aliquots of 10 ml were withdrawn and filtered through 0.22 μm Millipore filters [7]. The concentration of bacteria in the incubation tubes varied from $0.6 \cdot 10^9$ to $1.1 \cdot 10^9$ c.f.u./ml.

The net K^+ loss from the bacteria was measured in the filtrate by flame photometry, using a Zeiss model M4QIII spectrophotometer with flame attachment.

The method used for the evaluation of growth inhibition induced by the drugs has been described earlier [4].

Fig. 1 illustrates the effect of chlorpromazine on K^+ loss from *S. aureus*; it is seen that in the control experiments there was a rapid loss of K^+ from the bacteria immediately after the resuspension, followed by a somewhat slower release lasting up to 100 min. During this interval the bacteria lost 90% of their initial K^+ contents. From the early rapid phase (0–40 min) of K^+ release it is calculated that the efflux of K^+ takes place at a rate of 0.01 μmol/min (10^9 c.f.u./ml).

At the lowest chlorpromazine concentration (0.1–0.2 mM), there was a pronounced and sustained inhibition of K^+ release. At 0.5 mM the rate of K^+ release showed a delayed increase, and at 1.0 mM chlorpromazine the K^+ loss was stimulated more than 2-fold.

Table I shows the inhibition of K^+ efflux expressed as percent of the controls after 40 min. Up to 90% inhibition of the K^+ loss can be observed, dependent on the drug and the concentration.

Thiopental (0.2 mM) was found to inhibit K^+ loss to about the same extent as tetracaine (5 mM) and chlorpromazine (0.2 mM).

In Fig. 2 it is seen that when *S. aureus* was allowed to grow in the presence of chlorpromazine, no effect was observed at a concentration of 0.1 mM, whereas this concentration clearly inhibited the K^+ loss. With regard to thiopental, no growth inhibition was observed at concentrations up to 1 mM, in spite of the fact that the K^+ efflux was inhibited to an extent of 86%.

TABLE I

THE EFFECT OF SELECTED MEMBRANE STABILIZERS ON K^+ RELEASE FROM *S. AUREUS* 40 MINUTES AFTER INCUBATION

The measured K^+ release is presented as mean ± S.D. of *N* determinations. *P*, significance of the difference between control and experimental results.

Incubation medium	Concn. (mM)	K^+ release (μmol/10^9 c.f.u./ml)		*N*	*P*	Reduction in K^+ release (40 min) (%)
		Experimental, with drug	Control, without drug			
Tetracaine	3.0	0.155 ± 0.028	0.428 ± 0.089	3	<0.01	64
	5.0	0.095 ± 0.005	0.367 ± 0.027	2	<0.01	74
Chlorpromazine	0.1	0.109 ± 0.025	0.202 ± 0.031	6	<0.01	46
	0.2	0.062 ± 0.015	0.197 ± 0.032	5	<0.01	69
	0.5	0.127 ± 0.003	0.187 ± 0.012	3	<0.01	32
	1.0	0.417 ± 0.098	0.196 ± 0.020	4	–	–
Thiopental	0.1	0.113 ± 0.031	0.270 ± 0.057	4	<0.01	58
	0.2	0.049 ± 0.005	0.257 ± 0.005	2	<0.01	80
	0.5	0.023 ± 0.013	0.234 ± 0.032	3	<0.01	90
	1.0	0.034 ± 0.021	0.247 ± 0.035	4	<0.01	86

The present study was initiated by the observation that chlorpromazine inhibits the growth of *S. aureus* and other microorganisms. In order to determine whether this was due to the effects of K^+ transport, the effects of chlorpromazine and other membrane stabilizers have been assessed and compared under conditions where *S. aureus* can be shown to release K^+ into the suspending medium.

S. aureus is coccoid, devoid of any other membrane, but osmotically resistant. Like other microorganisms, it exchanges protons against K^+ across the plasma membrane [8–10], and in the present experiments K^+ loaded bacteria were allowed to release K^+ into an initially K^+ free buffer.

That the bacteria were losing approx. 60–65% of their total contents in 40 min is comparable with the loss of 75% of the total K^+ contents in 40 min in an enriched medium without K^+, reported by Veerkamp [11].

It is seen that the K^+ release from bacteria can be inhibited by the same membrane stabilizing drugs which have already been demonstrated to interfere with K^+ transport across the plasma membrane in eucaryote cells [2]. This brings out some hitherto undetected fundamental similarities between K^+ transport mechanisms in bacteria and eucaryotic cells. The effective doses of chlorpromazine and thiopental are in the same range as reported for muscle cells [2,12]. However, tetracaine appears to be much less potent than in mammalian cells, possibly because this drug cannot readily penetrate into the bacterial membrane. Therefore it was necessary to use tetracaine in the washing procedure, and this is the reason why there is a greater K^+ loss in the tetracaine control.

The present results demonstrate that for all the three drugs tested, a very similar biphasic response is obtained in *S. aureus*. The acceleration of K^+ release is likely to reflect an over-saturation of the lipid bilayer of the plasma membrane.

Whereas the bactericidal effect of chlorpromazine was seen at concentrations from 0.5 mM to 1.0 mM, inhibition of K^+ release was obtained at concentrations down to 0.1 mM. Thiopental was found to inhibit the K^+ release from 0.1 mM to 1.0 mM without any growth or bactericidal effects. Again, this indicates that the phenomenon of membrane stabilization can be produced without interference with the survival of the bacteria.

It has been pointed out that there is synergism between the bactericidal actions of chlorpromazine and penicillin [13,14]. More detailed studies are required to determine whether this is related to the membrane stabilizing action of chlorpromazine or

whether a similar synergism exists between antibiotics and the other membrane stabilizing agents.

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