Streptomycin Lethality and Cyclic AMP

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Received September 7,1972

Summary:

The action of low and moderate concentrations of streptomycin has been studied with Escherichia coli B growing in synthetic media with glucose or glycerol as the carbon source.The inception of lethality depended on the carbon source and the presence of cyclic AMP or ATP in the growth medium.At low concentration of streptomycin (2.5 µg/ml),cells in salts-glycerol media lost their viability following two and a half hour lag period.Glucose repressed the bactericidal effect of streptomycin allowing the cells to grow normally although at a slightly reduced rate.The addition of cyclic AMP to the growth medium reversed glucose repression and rendered the cells sensitive to the action of the drug.At higher concentrations of streptomycin (up to 20 µg/ml)glucose and ATP prolonged and glycerol and cyclic AMP shortened the lag before the cells began to lose their viability.At streptomycin concentration of 5 µg/ml,ATP protected the cells against the bactericidal effect of the drug allowing them to grow normally.This effect of ATP could be abolished by the addition of cyclic AMP.At streptomycin concentrations above 20 µg/ml,the effect of glucose,cyclic AMP and ATP was no longer measurable.

Introduction.

The common characteristics of bacterial operons with catabolite sensitive promoters is that they are repressed by glucose or certain other rapidly metabolized carbon sources (1). This repression called the "glucose effect" or more recently "catabolite repression" can be overcome by cyclic AMP (2). Several observations suggest that ATP may be the catabolite involved in the manifestation of catabolite repression (3).

In this communication we report that the bactericidal effect of low and moderate concentrations of streptomycin is repressed by glucose and ATP and that this repression can be overcome by cyclic AMP.

Materials and Methods.

Escherichia coli strain B was grown in the synthetic medium of Davis and Mingioli (4) supplemented with 0.25% glu-

cose or 0.4% glycerol as the carbon source.All cultures were grown at 37°C with vigorous agitation and were in the exponential phase at a density of $1x10^8$ cells/ml when used for the experiments.Growth was determined in a Klett photometer calibrated with viable counts.Viability was determined by spreading 0.1 ml of the appropriate diluted bacterial cultures on the surface of nutrient agar plates.

Dihydrostreptomycin sulfate,cyclic AMP,AMP and ATP were obtained from Calbiochem.Los Angeles,Calif.All chemicals were of analytical grade.

Results.

E.coli strain B were growing exponentially in salts-glucose and salts-glycerol media.When the titer of $1x10^8$ cells/ml was reached,streptomycin (2.5, 5.0, 10.0 and 20.0 µg/ml) was added to the growing cultures.At zero time and after various time intervals cell viability was determined.The results illustrated in Fig.1 show that when the cultures were growing with glycerol as the carbon source,the addition of streptomycin , 2.5 µg/ml ,caused no significant change in the number of viable cells for about two and a half hours followed by a precipitous decline thereafter of the viable count.Cells growing with glucose as the carbon source were not affected by this concentration of streptomycin.

At higher concentrations of streptomycin (5.0 to 20.0 µg/ml) the duration of the lag before the decline of the viable count depended on the carbon source in the growth medium.Cells growing in glucose as the carbon source survived in the presence of streptomycin almost twice as long as glycerol-growing cells, under otherwise similar conditions.

These results were interpreted in terms of glucose repression of the bactericidal effect of streptomycin.Since the repression by glucose of the synthesis of catabolite enzymes can be overcome by cyclic AMP,it deemed of interest to examine the effect of cyclic AMP on streptomycin lethality.

Fig.2 and 3 illustrate the effect of cyclic AMP on streptomycin lethality.Cells of E.coli B growing in salts-glycerol media,in the presence or absence of cyclic AMP ,were sensitive to streptomycin at a concentration of 2.5 µg/ml and began to lose their viability after a lag of two and a half hours duration.

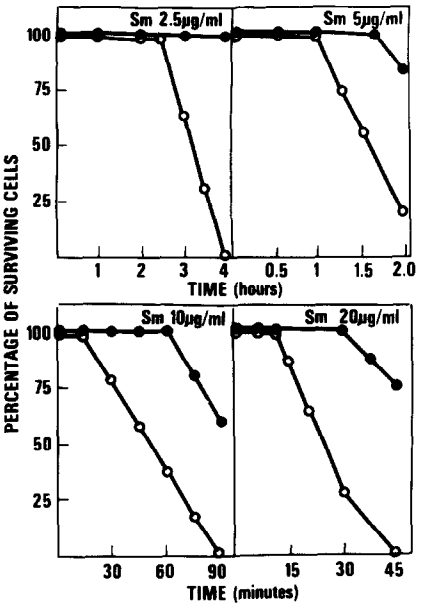

Fig. 1.

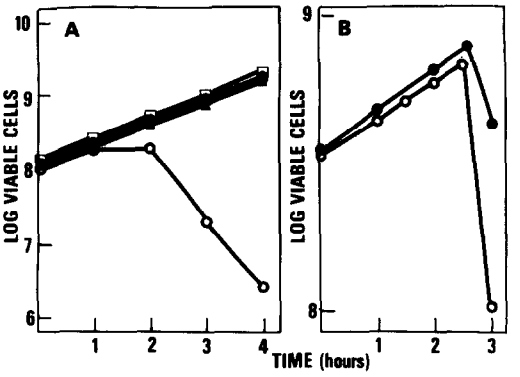

Fig. 2.

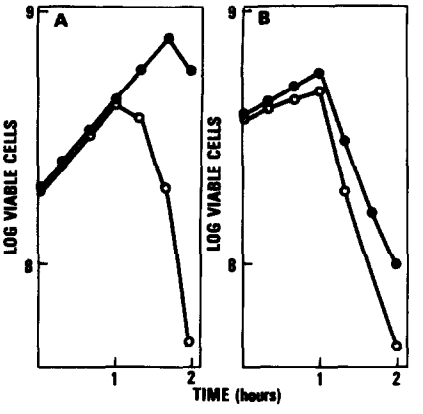

Fig. 3.

Fig.1.The effect of glucose on the viability of E.coli in the presence of streptomycin.The cells were growing in salts-glycerol O--O, or salts-glucose ●--● media.When the titer of $1x10^8$ cells/ml was reached,streptomycin was added to the growth media. At zero time and after various time intervals,samples were taken and cell viability was determined.

Fig.2.The effect of cyclic AMP on the viability of E.coli in the presence of streptomycin.For experimental conditions,see Fig.1. A.-Cells growing in salts-glucose media.Control,without streptomycin,□--□ ;control + cyclic AMP (5 mM),▲--▲ ;streptomycin (2.5 µg/ml),●--●;streptomycin (2.5 µg/ml)+ cyclic AMP(5 mM), O--O; B.-Cells growing in salts-glycerol media.Streptomycin (2.5 µg/ml), ●--●;streptomycin (2.5 µg/ml) + cyclic AMP (5 mM),O--O.

Fig.3.The effect of cyclic AMP on the viability of E.coli B in the presence of streptomycin.For experimental conditions,see Fig.1. A.- Cells growing in salts-glucose media. B.-Cells growing in salts-glycerol media.Streptomycin,5 µg/ml,●--●; ditto, + cyclic AMP (5 mM),O--O.

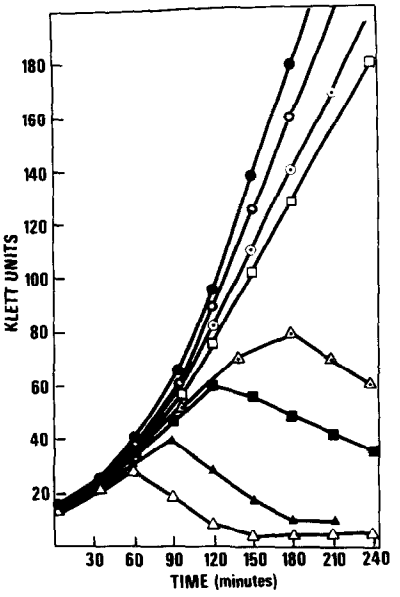

Fig. 4.

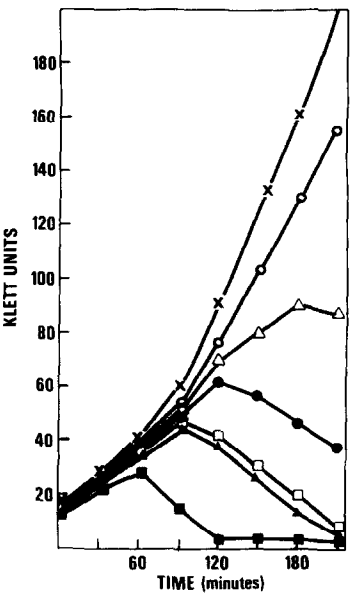

Fig. 5.

Fig.4.The effect of ATP on the viability of E.coli B in the presence of streptomycin.Cells were growing exponentially in salts-glucose medium to a titer of 1×10^8 cells/ml and exposed to streptomycin,5 µg/ml.The concentration of AMP,ATP and cyclic AMP,when present,was 5 mM.Growth was followed in a Klett photometer.With E.coli B,the loss of viability coincides with cell lysis.Control,with or without ATP,●--●;control + cyclic AMP,O--O;control + AMP,⊖--⊖;streptomycin,■--■;streptomycin + cyclic AMP△--△;streptomycin + AMP▲--▲;streptomycin + ATP,□--□ ; streptomycin + ATP and cyclic AMP▲--▲ .

Fig.5.The effect of ATP on the viability of E.coli B in the presence of varying concentrations of streptomycin.For experimental conditions,see Fig.4.Control,with and without ATP, x--x;streptomycin,5 µg/ml,●--●;ditto,+ ATP,O--O;streptomycin, 10 µg/ml,▲--▲;ditto,+ATP,△--△; streptomycin 20 µg/ml,■--■; ditto,+ ATP, □ --□ .

At this concentration,streptomycin was not lethal to cells growing with glucose as the sole source of carbon,but became bactericidal after cyclic AMP had been added to the growth medium.

When glycerol growing cells were incubated with streptomycin at a concentration of 5 µg/ml,the cells began to lose their viability after one hour lag.The duration of the lag was not influenced by the presence of cyclic AMP in the growth medium. When glucose served as the carbon source ,the lag before kil-

ling was prolonged to two hours,and the addition of cyclic AMP reduced it to one hour (Fig.3).At higher concentrations of streptomycin,cell viability was lost so rapidly following exposure to the drug that the effect of cyclic AMP was no longer measurable.

In a recent report Aboud and Burger (3) showed that addition of ATP to spheroplasts of E.coli ,induced for β-galactosidase,reduced the differential rate of enzyme synthesis.Since the effect of ATP was reversed by cyclic AMP,the authors suggested that ATP was the catabolite involved in the manifestation of catabolite repression.

In view of the results of experiments described in the present communication on the effect of glucose and cyclic AMP on streptomycin lethality,it seemed of interest to determine whether ATP would repress the bactericidal effect of streptomycin.It was hoped that in these experiments intact cells could be used,inasmuch as streptomycin is known to alter the permeability barrier and to increase membrane permeability to nucleotides in sensitive cells within minutes (5,6).

The results of the experiments on the effect of ATP on streptomycin lethality are illustrated in Fig.4 and 5.It can be seen from Fig.4 that E.coli B cells grew in the salts-glucose medium supplemented with ATP at the same rate as the control cultures.Cyclic AMP reduced the rate of growth of the cells by about 20% as compared with control cultures.Cells exposed to streptomycin,5 µg/ml,grew for about two hours and began to lyse. Addition of ATP to the growth medium protected the cells against the lethal effect of streptomycin allowing them to grow normally although at a slightly reduced rate.Cyclic AMP completely abolished the protective effect of ATP and rendered the cells sensitive to streptomycin(the cells began to lyse after a lag of about 90 minute duration).AMP prolonged only slightly the lag before lysis,its effect being much less pronounced than that of ATP.

Fig.5 illustrates the effect of ATP on the growth of E.coli incubated with increasing concentrations of streptomycin.ATP protected the cells against the bactericidal effect of streptomycin over a narrow concentration range of the antibiotic.At

streptomycin concentrations above 5 μg/ml, ATP only prolonged the survival time, but could not prevent cell death.

Similar experiments with penicillin and chloramphenicol showed that cyclic AMP had no effect on the action of these antibiotics.

It has recently been reported that the action of cyclic AMP is mediated by a protein, called the catabolite gene activator protein, CAP (7,8). In order to ascertain whether the effect of cyclic AMP on streptomycin lethality is also mediated by CAP we attempted to isolate a mutant of E.coli B which would be defective in CAP production. So far our attempts have been unsuccessful. We decided, therefore, to use the CAP^- mutant of Schwarz and Beckwith (9), generously provided by Dr. Arditti. Both the parent strain, CA-8000, an Hfr Hayes prototroph, and the CAP mutant, CA-7900, were used.

The results obtained with the parent strain, CA-8000, were analogous in all respects to those obtained with E.coli B. At low streptomycin concentration, 2.5 μg/ml, the cells were killed only in the presence of cyclic AMP. At higher streptomycin concentrations, cyclic AMP shortened the survival time of cells exposed to this antibiotic. ATP protected the cells against streptomycin when the concentration of the latter was 5 μg/ml, and prolonged the survival time of cells incubated with higher streptomycin concentrations.

The CAP mutant was sensitive to streptomycin at the concentration of 5 μg/ml. Cyclic AMP did not enhance streptomycin lethality at all concentrations of the drug studied (from 2.5 to 20 μg/ml). ATP, on the other hand, protected the cells or prolonged their survival in the presence of streptomycin, depending on the drug concentration, as in the case of the wild CA-8000 strain.

It should also be mentioned that whereas the parent strain CA-8000 grew in the presence of cyclic AMP at a slightly reduced rate, cyclic AMP had no effect on the growth rate of the CAP mutant.

Discussion.

There are many hypotheses concerning the possible site of lethal action of streptomycin, but existing data have not yet established the validity of a single hypothesis (10). Recently, it has been proposed (11) that streptomycin-stimulated RNA

synthesis may be the primary cause of death of sensitive cells. Although the nature of the RNA the synthesis of which is stimulated by streptomycin has not been elucidated, the authors (11) suggested that it may be messenger RNA which by binding irreversibly to ribosomes was lethal to the bacterial cell.

The results of our experiments which show that cyclic AMP, a compound known to stimulate the synthesis of messenger RNA of many inducible enzymes, enhances the lethal effect of streptomycin suggest that streptomycin may actually induce the synthesis of a specific messenger RNA the synthesis of which is stimulated by cyclic AMP. To explain the lethality of this hypothetical messenger RNA, we propose that it codes for a bactericidal or antibiotic, colicin-like protein.

The search for this hypothetical messenger RNA and the protein it codes for is presently in progress.

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