SHORT COMMUNICATION

Susceptibility of Protein Synthesis in *Escherichia coli* to Tetracycline and Minocycline

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The effects of tetracycline (hydrophilic) and minocycline (hydrophobic) on the synthesis of proteins in *Escherichia coli* were determined. The activity of minocycline against membrane-bound and free ribosomes could not be distinguished from that of tetracycline.

INTRODUCTION

The non-specific passage of antibiotics across the outer membrane of Gram-negative bacteria occurs by two routes: diffusion of hydrophilic molecules through transmembrane pores and penetration of hydrophobic drugs across apolar regions (Nikaido & Nakae, 1979). Although the hydrophobic pathway is virtually inactive in wild-type strains (Nikaido & Nakae, 1979), hydrophobic antibiotics at high external drug concentrations can gain access to the cell interior. Piovant *et al.* (1978) proposed that this results mainly from entry of drugs through hydrophobic envelope regions that connect the cytoplasmic and outer membranes. The preferential activity of hydrophobic inhibitors of protein synthesis against membrane-bound ribosomes (Hirashima *et al.*, 1973; Piovant *et al.*, 1978) is assumed by Piovant *et al.* (1978) to reflect the proximity of junction points to the sites of synthesis of envelope polypeptides.

With regard to the passage of tetracycline across the outer membrane of *Escherichia coli*, we concluded that tetracycline itself diffuses through pores, whereas the more hydrophobic derivative minocycline penetrates through apolar membrane regions (Ball *et al.*, 1977; Chopra & Eccles, 1978). Diffusion of minocycline through apolar outer membrane regions may therefore locate the antibiotic at sites favourable for entry across the cytoplasmic membrane via junction points. We have tested this model for minocycline uptake by comparing its activity against the synthesis of cytoplasmic and envelope proteins with that of tetracycline.

METHODS

Bacteria. *Escherichia coli* K12 strain JC3272 (see Ball *et al.*, 1977) was used throughout the experiments.

Antibiotics. Tetracycline hydrochloride was purchased from Sigma. Minocycline (7-dimethylamino-6-demethyl-6-deoxytetracycline) was a gift from Lederle Laboratories, Gosport, Hants.

Radioactive amino acids. ³H-labelled amino acids (code TRK440) were purchased from The Radiochemical Centre, Amersham.

Inhibition of envelope and cytoplasmic protein synthesis by tetracycline and minocycline. Experiments were based on methods described by Hirashima *et al.* (1973). Bacteria were grown aerobically at 37 °C to a culture density of 1×10^8 cells ml⁻¹ in nutrient broth (Ball *et al.*, 1977). Tetracycline or minocycline was added at 0.2, 0.4 or 0.6 µg ml⁻¹ and incubation at 37 °C was continued for 10 min. ³H-labelled amino acids (1 µCi ml⁻¹; 37 kBq ml⁻¹) were then added and incubation was continued for a further 2 min. After labelling, the cultures were

immediately chilled in an ice bath and non-radioactive Casamino acids (12.5%, w/v) were added. Bacteria were collected by centrifugation and the envelope and cytoplasmic fractions were prepared as described by Ball *et al.* (1977). Radioactivity incorporated into protein of the cytoplasmic and envelope fractions was also determined as described by Ball *et al.* (1977).

RESULTS AND DISCUSSION

Experiments analogous to those performed by Hirashima *et al.* (1973) were conducted to examine the inhibitory effects of tetracycline and minocycline on cell envelope and cytoplasmic protein synthesis. Both drugs inhibited the synthesis of cytoplasmic proteins to a greater extent than envelope proteins, and the pattern of inhibition was similar for both drugs (Fig. 1). These results are therefore not consistent with preferential entry of minocycline through junction regions as, according to Piovant *et al.* (1978), minocycline should be more active than tetracycline against the synthesis of envelope proteins. Furthermore, in contrast to the data of Hirashima *et al.* (1973) and Piovant *et al.* (1978), we found that tetracycline was more inhibitory to the synthesis of cytoplasmic proteins than envelope proteins. The reason for this discrepancy is unclear.

The data in Fig. 1 could be taken to indicate that the outer membrane of *E. coli* does not present a barrier to the entry of minocycline because equivalent external concentrations of tetracycline and minocycline result in comparable inhibitory effects. As noted by Franklin (1973), an estimate of the ability of an antibiotic to penetrate the outer membrane can be made from comparison of the quantity of drug required to inhibit the growth of *E. coli* and a Gram-positive organism. We determined the quantities of drug required to cause 50% inhibition of growth in liquid cultures of *E. coli* K12 JC3272 and *Bacillus subtilis* NCIB 10106 (data not shown). For tetracycline the ratio of *E. coli* to *B. subtilis* inhibitory concentrations was 0.8 whereas for minocycline the ratio was 16.8. These results indicate that the outer membrane of wild-type *E. coli* presents a barrier to the influx of minocycline,

Fig. 1. Ratio of relative rates of protein synthesis in envelope and cytoplasmic fractions from *E. coli* K12 JC3272 inhibited by minocycline (○) and tetracycline (●). Ratios are derived from duplicate determinations for each drug concentration. Experiments were performed as described in Methods.

Mean values for the incorporation of radioactivity into protein (expressed as a percentage of the incorporation in a drug-free control) were as follows:

	Tetracycline concn (μg ml ⁻¹)				Minocycline concn (μg ml ⁻¹)			
	0	0.2	0.4	0.6	0	0.2	0.4	0.6
Envelope protein	100	60	54	33	100	102	78	48
Cytoplasmic protein	100	36	29	16	100	71	40	25

but not tetracycline. Since the growth of wild-type *E. coli* is equally susceptible to inhibition by tetracycline and minocycline this implies that minocycline itself is more active in the prevention of protein synthesis at the ribosome. Similar conclusions were recently drawn by Samra *et al.* (1979).

Since accumulation of tetracycline across the cytoplasmic membrane probably occurs by an energy-dependent carrier-mediated system (Chopra & Howe, 1978), the results presented here (Fig. 1) could reflect transport of both antibiotics by the same carrier. This suggestion is consistent with the data of Samra *et al.* (1979) which show that minocycline causes competitive inhibition of tetracycline transport across the cytoplasmic membrane. Thus, although these antibiotics have divergent routes of entry across the outer membrane, they appear to converge during transport across the cytoplasmic membrane.

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