Increase in Permeability of Escherichia coli Outer Membrane by Local Anesthetics and Penetration of Antibiotics

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The MICs of several antibiotics (both hydrophobic and hydrophilic) which penetrate very poorly into intact Escherichia coli cells were found to be 2- to 10-fold decreased in the presence of low doses of various local anesthetics (procaine, dibucaine, tetracaine, chlorpromazine, and quinine). The concentrations of anesthetics necessary for this effect have no adverse effect on cell growth and are markedly lower than those concentrations used clinically.

The outer membrane of the gram-negative bacterium protects the cell against many antibiotics by the following two different mechanisms (for reviews, see references 1, 3, and 9). (i) Hydrophilic antibiotics with molecular weights greater than 600 are excluded on the basis of size from the water-filled channels of the porins, a specific class of major proteins (7). (ii) The hydrophilic saccharide chains of the lipopolysaccharide (LPS), extending from the outer layer of the outer membrane (7), mechanically prevent the access of many hydrophobic antibiotics, whatever their molecular weight.

Several ways of allowing the disruption of this outer membrane barrier to facilitate the penetration of one or several classes of antibiotics normally unable to reach their final target by either route have been described (1, 3, 9). Besides the introduction of specific mutations which either alter the porin content or shorten the carbohydrate chains of LPS molecules (7), several treatments have been proposed to breach the hydrophobic barrier. The most used (and up to now most useful) treatment is the addition of EDTA, a chelator of divalent cations, in the presence of Tris buffer (5). The release of about 50% of the LPS, probably resulting in the removal of divalent cations which form cross bridges to adjacent LPS molecules, opens the outer membrane to many hydrophobic and large hydrophilic antibiotics (6).

Recently, we have made the fortuitous observation that several agents used as local anesthetics (procaine, tetracaine, and dibucaine), phenothiazine tranquilizers (e.g., chlorpromazine), or antimalarial agents (quinidine) can make the outer membrane of Escherichia coli K-12 permeable to antibiotics which are normally excluded. Besides their specific therapeutic use, all these drugs have several common properties, such as blockage of action potential and modification of membrane fluidity (for a review, see reference 11). Some of these anesthetics have also been reported to have various effects on bacterial cells (4, 10), including an inhibitory effect on cell growth. We have undertaken a series of experiments designed to study the physiological response of E. coli to the various effects induced by these drugs, including inhibition of DNA replication (B. Labedan et al., manuscript in preparation). We observed in control experiments that novobiocin, a well-known inhibitor of subunit B of DNA gyrase (2), was far more efficient in the presence of low concentrations of dibucaine or tetracaine (Labedan et al., in preparation). Since it is well known that novobiocin penetrates rather inefficiently in wild-type gram-negative bacteria (1, 3, 9), we hypothesized that local anesthetics could enhance the entry of this antibiotic. To test this hypothesis, we turned to other antibiotics known to be unable to penetrate the cell envelope.

The increase in permeability of the outer membrane by anesthetics was directly tested by determining in liquid medium the MICs of different antibiotics by using, in parallel, cells growing either in the presence or in the absence of the anesthetics. First, we defined the optimum concentration of each anesthetic (Table 1) as the dose which gave the maximum disruption effect (dose which allowed entry of novobiocin) but which had no effect on bacterial growth in the absence of an antibiotic. Moreover, this concentration was ascertained with two other hydrophobic antibiotics: nalidixic acid and erythromycin. Table 1 also shows that the difference between this optimum concentration and the dose which inhibited growth varied from one anesthetic to another.

The results obtained with all the antibiotics tested in the

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<th>TABLE 1. Optimum concentrations of the different anesthetics</th>
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<td>Procaine</td>
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* The different concentrations of anesthetics were determined under experimental conditions used to measure the MICs of the different antibiotics (Table 2), i.e., use of low inoculum concentrations (around 10⁶ bacteria per ml) in the presence or in the absence, in two parallel series of increasing concentration of anesthetic, of one of the following antibiotics: novobiocin (final concentration, 20 μg/ml), nalidixic acid (1 μg/ml), or erythromycin (10 μg/ml). The dose yielding both 100% of the original viable cell count (in the tube without an antibiotic) and the maximum decrease of MIC of the respective antibiotic (in the tube in the parallel series) was retained as the concentration for optimum membrane disruption.

* Inhibitory concentration of anesthetic yielding 50% of the original viable cell count after overnight culture under the MIC conditions described in footnote a.
presence or absence of tetracaine are shown in Table 2. E. coli K-12 A324 culture (F' pro lacI rpsL) freshly grown in LB medium (7) was diluted to about 10^7 cells per ml. A 0.1-ml portion of this suspension was then added to two identical series of tubes containing 2.5 ml of LB medium supplemented with increasing concentrations of the different antibiotics. Tubes in one of the series (included the control without antibiotic) contained 0.4 mM tetracaine. The tubes were then incubated overnight at 37°C with shaking. The next morning, the optical density of each culture at 620 nm was measured. Similar data (not shown) were obtained with the other local anesthetics. The following three conclusions derived from the results shown in Table 2 are valid for the other anesthetics that we used.

(i) Antibiotics usually ineffective against intact wild-type E. coli cells were found to be more efficient against anesthetic-treated cells. This was especially the case for erythromycin (10-fold decrease of MIC) and for novobiocin and vancomycin (5-fold decrease for each) and was to a lesser extent the case for fusidic acid and nafcillin (3-fold decrease for each). Thus, the presence of local anesthetics seems to facilitate the entry of both hydrophobic (erythromycin, novobiocin, nafcillin, and even the large fusidic acid) and large hydrophilic (vancomycin) antibiotics.

(ii) Hydrophobic antibiotics such as chloramphenicol, rifampin, nalidixic acid, and tetracycline and hydrophilic antibiotics such as cephaloridine and cefotaxime, all of which penetrate the E. coli outer membrane rather effectively showed a slight but reproducible decrease of MIC in the presence of the different local anesthetics. This occurred for antibiotics which cross the outer membrane through porins (cephalosporins, chloramphenicol, and tetracycline) and for those which enter by the hydrophobic route (rifampin).

Therefore, regardless of the nature of the molecular target of the antibiotic, its hydrophobic character, its charge, or its final location (periplasm or cytoplasm) in the cell, we noticed that the presence of the different local anesthetics facilitated entry.

(iii) An unexpected result, obtained with aminoglycoside antibiotics, was that, among the aminoglycoside antibiotics tested, only for spectinomycin (which is actually an aminocyclitol) was the MIC decreased in the presence of local anesthetics. The other aminoglycoside antibiotics were slightly less efficient in the presence of anesthetics. The variations in the MICs were low but reproducible. This slight protective effect was confirmed by measuring the efficiency of plating of bacteria incubated with various doses of either kanamycin or neomycin in the presence of an anesthetic. A possible explanation for this result is that anesthetics interfere with aminoglycoside transport by the mechanism already described for polyanimes and divalent cations (1). Indeed, at the pH (around 7.0) of the growth medium, anesthetics are amphiphilic cations (11). Moreover, spectinomycin, the only exception that we observed, is known to follow rules of transport different from those of the true aminoglycosides (1).

The results presented in Tables 1 and 2 were obtained by using either various E. coli K-12 strains with totally different genotypes or E. coli B or F. Therefore, the facilitating effect induced by the local anesthetics is independent of (i) the presence of the ompC-encoded porin, which is absent from the E. coli B outer membrane (7), and (ii) the nature of the hydrophilic saccharide of LPS molecules, which differ greatly among these different strains (7).

The use of low doses of local anesthetics with antibiotics may be clinically advantageous, especially when it is necessary to broaden and diversify the range of antibiotics that can be used. Interestingly, this facilitating effect occurs at far lower concentrations than are used in anesthetic applications. For example, tetracaine showed maximum efficiency at a dose (0.4 mM = 0.012%) which is far less than is used in therapeutic treatment (both surface [0.1%] and infiltration [2%] anesthesia).

Nonetheless, the increase in permeability of the outer membrane with local anesthetics could be useful in bacterial studies, in which it could conveniently replace EDTA-Tris treatment (5, 6). At the concentrations which we used, the different local anesthetics tested did not inhibit cell growth or other physiological properties of the cell (Labedan et al., in preparation). Therefore, the local anesthetics may also be used to increase the permeability of the outer membrane over long periods of time while maintaining the cell in an active metabolic state.

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LITERATURE CITED