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EXPERIMENTAL ARTICLES

Adaptive Functions of *Escherichia coli* Polyamines in Response to Sublethal Concentrations of Antibiotics

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Abstract—*Escherichia coli* exposure to sublethal antibiotic concentrations induced an increase in cell polyamine contents. Maximum accumulation of putrescine and spermidine in response to antibiotics-induced oxidative stress preceded the increment of cadaverine, the content of which was dependent on the *rpoS* expression level and reached the maximum in response to fluoroquinolones. The polyamine positive modulating effects on *rpoS* expression increased in the following order: cadaverine–putrescine–spermidine. The reason for cadaverine accumulation was the increase in activities of lysine decarboxylases CadA and Ldc. High cadaverine accumulation in the cells exposed to fluoroquinolones and cephalosporins resulted in the reduction of porin permeability; so it was considered as a response aimed at cell protection against antibiotic penetration into the cell. Netilmycin, unlike other antibiotics, did not substantially affect the lysine decarboxylase activity and cellular polyamine pools.

Key words: polyamines, lysine decarboxylase, porin transport, σ^S, expression, antibiotic resistance.

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Polyamines such as putrescine, spermidine, spermine, and cadaverine are major cationic compounds in all living organisms from viruses to humans [1]. Involvement of polyamines in a number of cellular processes is well explored in both eukaryotes and bacteria [2]. Lately, considerable attention is drawn to their functioning as gene expression modulators [3] and regulators of porin channel activity [4], which may be of importance in the development of antibiotic resistance in microorganisms.

Earlier, protective functions of polyamines have been demonstrated in *Escherichia coli* cultures under stress conditions, specifically, oxidative [5, 6] and acidic [7] stresses. Among the described responses of *E. coli* under the given conditions is an increased activity of the enzymes responsible for polyamine synthesis resulting in a higher intracellular polyamine pool. We have shown that oxidative and acidic stress conditions were accompanied by an increased resistance towards certain groups of antibiotics [8]. It remained unclear whether bacteria are able to react to antibiotics by increasing the polyamine pool in the absence of stress stimuli; the response could demonstrate polyamines' involvement in bacterial adaptive reaction to antibiotic effect.

The type of bacterial response towards a given antibiotic is presently associated with its effective concentration. While not significantly influencing the bacterial growth rate, subinhibitory concentrations of antibiotics still induce considerable alterations in gene expression; therefore an opinion has developed that the natural function of antibiotics is intercellular communication via a quorum sensing type of mechanism [9]. Data on pseudomonads using quinolones as signal molecules in intercellular messaging (fluoroquinolones are known to be wide-range antibiotics) [10] supports this concept.

Various low-molecular weight compounds, products of bacterial metabolism, may act as effectors of gene expression either by transmitting quorum sensing signals or by modulating gene expression patterns [10]. Apparently, polyamines may also be referred to as part of this group; polyamine modulation for the expression of numerous target genes is well acknowledged and supports the distinguishing of a specific polyamine modulon [11].

We have demonstrated earlier that in a polyamine-dependent *E. coli* mutant putrescine increased the expression of the *rpoS* gene encoding the σ^S subunit of RNA polymerase; this phenomenon was accompanied by an increase in the minimal inhibitory concentration (MIC) of fluoroquinolone antibiotics [8]. Increase in levofloxacin MIC values was registered upon culturing under acidic conditions, resulting in a considerable increase in the intracellular cadaverine concentration. These data indicate involvement of polyamines and σ^S in the development of antibiotic resistance, although the mechanisms still remain unclear. The aim of the present work was to answer the following questions.

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Bacterial strains used in the work

E. coli strains	Genotype	Source, reference
EH40	GC4468 (λEH40:*soxS*::*lacZ*)	Demple B. [12]
RO91 (pBR322)	MC4100(λRZ5:*rpoS742*:*lacZ*[hybr])	Hengge-Aronis R. [13]
RO200	MC4100(λRZ5:*rpoS742*::*lacZ*)	
HT306	*thr-1*, *araC14*, *ΔspeD98*, Δ(*gpt-proA*)*62*, *lacY1*, *gln V44*(AS), *galK2*(Oc), λ⁻, Δ(*SpeB-SpeA*)*97*, Δ(*SpeC-glcB*)*63*, *rpsL25*(strR), *xylA5*, *mtl-1*, *thi-1*, *ampCp-1*, *cadA2*	Tabor H. [14]
SHT03	HT306, but *lacZ*⁻DE3 (λRZ5:*rpoS742*:*lacZ*[hybr])	Shumkov M.S. [8]

What is the nature of the interrelation between the *rpoS* expression level and cadaverine accumulation? Are antibiotics able to influence the cellular concentration of polyamines at neutral pH values? Does the transporting activity of porin channels change in response to sublethal concentrations of antibiotics and does it depend upon the type of antibiotic?

MATERIALS AND METHODS

Objects of the study. Strains of *E. coli* used in the present work are listed in Table 1, with an indication of their genotypes and source.

Cultivation of microorganisms. Prior to the experiments, *E. coli* strains maintained on LB agar slants were inoculated into a tube containing 5 ml of LB broth. After 11-hour cultivation at 37°C, the cells were transferred into a flask with 100 ml of LB broth and cultured for 13 h more in a thermostatic shaker (120 rpm) also at 37°C. The culture was then used for subsequent inoculation into 50 ml of LB broth. The studied antibiotics were introduced in the concentrations specified in the figure captions; the culture's optical density (OD_{600}) was 0.3.

In order to study polyamine functions in the regulation of *rpoS* gene expression, a polyamine-dependent *E. coli* strain SHT03 was grown on an M-9 medium containing 1 μg/ml thiamine, 100 μg/ml proline, and 1 μg/ml pantothenate [14].

The cell biomass was evaluated by optical density (OD_{600}) on a PD-303UV spectrophotometer (Apel, Japan).

The following reagents were used in the work: ampicyllin, streptomycin, tetracycline, levofloxacin, pefloxacin, cefotaxim, netilmycin, cefasolin, putrescine, cadaverine, spermidine by Sigma (Germany).

Activity of β-galactosidase was determined in the cells pretreated with a mixture of SDS (Sigma, United States) and chloroform by Miller's method [15].

Polyamine content. Polyamine concentration was determined by two methods, thin layer chromatography [16] and HPLC using an LC-10Avp chromatograph (Shimadzu, Japan). Pretreatment of the samples for analysis included extraction with subsequent derivatization of polyamines with dansyl chloride [16]. Polyamines were separated on a Luna C18(2) column (250 × 4.6 mm, 5 μm; Phenomenex, United States) at 25°C. Water and acetonitrile were applied to the column at the rate of 1 ml/min with a linear gradient of acetonitrile concentration from 40 to 100% within 20 min with subsequent equilibration with 40% acetonitrile for 10 min. The dansylated polyamine derivatives were detected using an RF-10AXL flow fluorimetric detector (Shimadzu, Japan) at 400 nm excitation wavelength and 516 nm emission.

Determination of lysine decarboxylase activity. Bacterial cell cultures were centrifuged for 8 min at 5000 rpm, 0°C, and washed twice with 100 mM citrate–phosphate buffer (pH 7.0). Resuspended cells were sonicated (22 kHz, 10 mA, 30 s). Cell homogenates were centrifuged (16000 *g*, 20 min), lysine decarboxylase activity was determined in the supernatant. Total volume of 0.5 ml in the incubation mixture for determination of the enzymatic activity contained 100 mM citrate–phosphate buffer of the given pH (see Results), 0.04 mM pyridoxal phosphate, 1 mM dithiothreitol, 10 mM L-lysine, and the supernatant containing 100 μg protein. The reaction was induced by an addition of the substrate, the mixture was incubated at 37°C for 60 min, and the reaction was terminated by an addition of perchloric acid to the final concentration of 0.4 M. Denatured protein was precipitated by centrifugation (16000 *g*, 10 min). The enzyme specific activity was calculated as the amount of the product (cadaverine) generated by 1 mg of the protein per minute.

Protein content was determined according to the Lowry procedure [17].

Transport activity of porin channels of the *E. coli* outer membrane was determined according to a modified method of Zimmermann and Rosselet [18] based on photometric measurement of cephalosporin concen-

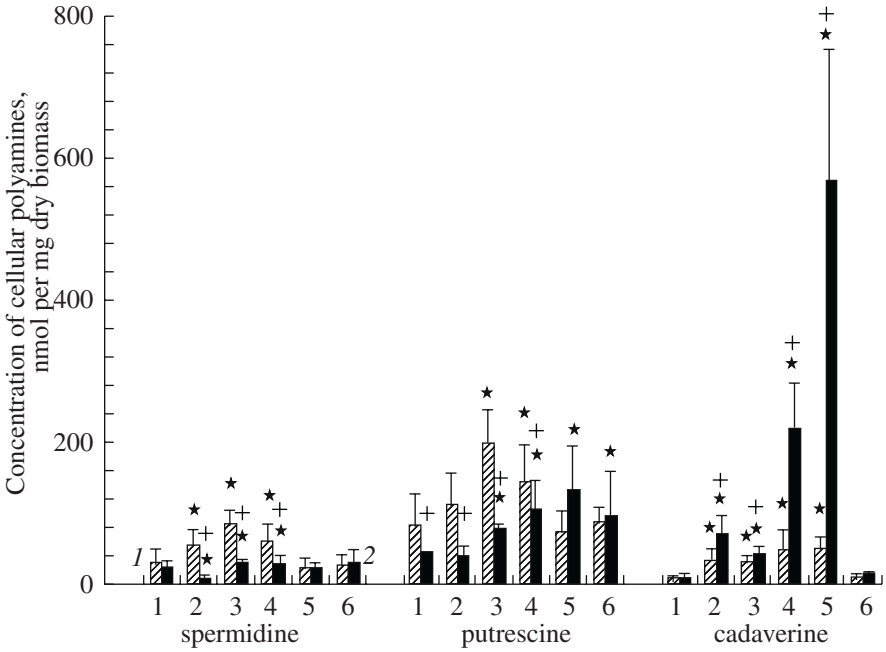

Fig. 1. Changes in the intracellular polyamine content in *E. coli* strain RO91 in response to sublethal concentrations of antibiotics in the medium 2 h (hanched columns) and 6 h (shaded columns) after antibiotic introduction. Bacteria were cultured on LB medium without antibiotics (1) and containing 0.7 μg/ml cefotaxim, 2 μg/ml cefazolin, 0.012 μg/ml levofloxacin, 0.038 μg/ml pefloxacin, and 0.08 μg/ml netilmycin (2–6, respectively). ★ designates a statistically significant difference from the control group, +, designates the difference between 6-h and 2-h groups, $p \le 0.05$.

tration in the medium in the course of antibiotic transfer through porin channels of the outer membrane of gram-negative microorganisms and cleavage by periplasmic β-lactamase. This was performed using cefazolin with an absorption maximum at 270 nm. *E. coli* strains used for measurement were transformed with the pBR322 plasmid carrying the β-lactamase gene.

Results were processed statistically using the standard Statistica for Windows 6.0 software package (StatSoft, Inc., 2001). The data in the figures present the mean results from a series of at least three similar experiments as an average ± standard square deviation. The significance for the differences of the compared groups in the case of multiple comparisons was determined using either Newman–Keuls or Dunnet criterion. To validate the time dynamics, the paired Student's *t*-test was used. The differences were considered significant at $p \le 0.05$.

RESULTS

Antibiotics effect on cellular polyamine concentrations. The concentration of antibiotics resulting in 50% inhibition of biomass accumulation after 4 h of incubation according to OD_{600} data were used as sublethal concentrations of antibiotics (SLCA).

The lowest response towards polyamines was observed for the aminoglycoside antibiotic netilmycin, whose effect was observed only towards putrescine and only after 6 h of observation (Fig. 1). The rest of the antibiotics provoked by far more significant modifications in the cellular pool of polyamines specific for the antibiotic class. Fluoroquinolone introduction induced considerable accumulation of cadaverine, which exceeded the levels of spermidine and putrescine. In contrast, predominance of putrescine and, a lesser accumulation of cadaverine was typical of cephalosporin antibiotics. Cephalosporins and levofloxacin also excited a spermidine response. Variations in intracellular polyamine profiles with time are evidence for the presence of two opposite trends. Putrescine and spermidine cellular content peaked after 2 h of exposure to most antibiotics (with the exception of netilmycin and pefloxacin) and decreased by the 6th hour. Cadaverine accumulated gradually reaching its maximum by the end of observation.

Thus, all the antibiotics under study induced an increase in polyamine intracellular concentration; the magnitude of response and its specificity towards various polyamines depended upon the class of antibiotics. The data indicate the probable involvement of polyamines into the processes of adaptation to SLCA. A similar response of polyamines has been earlier observed under various stress conditions, including oxidative stress with polyamines exhibiting a protective activity [5, 6].

Effect of antibiotics on the expression of the oxidative stress gene *soxS*. Induction of antioxidant genes is a characteristic feature of oxidative stress. This consideration provided basis for studying the expression of

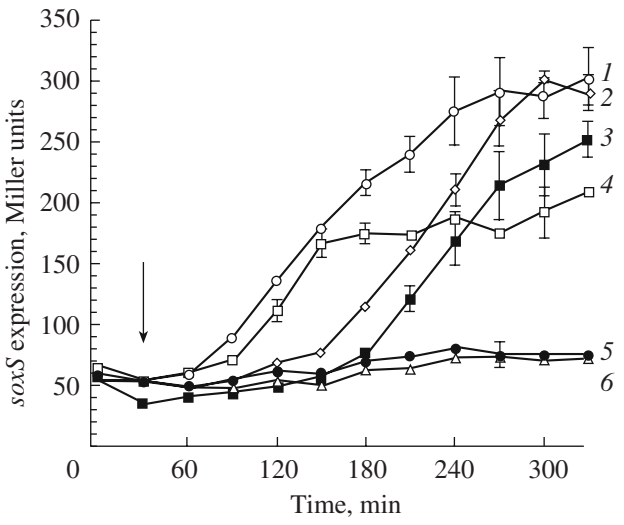

Fig. 2. Changes in the *soxS* gene transcription in *E. coli* strain EH40 in response to sublethal concentrations of antibiotics in the medium. Bacteria were cultured on LB medium without antibiotics (*5*) and in the presence of 0.7 μg/ml cefotaxim, 0.012 μg/ml levofloxacin, 0.038 μg/ml pefloxacin, 2 μg/ml cefazolin, and 0.08 μg/ml netilmycin (*1–4, 6*, respectively). Arrow indicates the time of antibiotic addition.

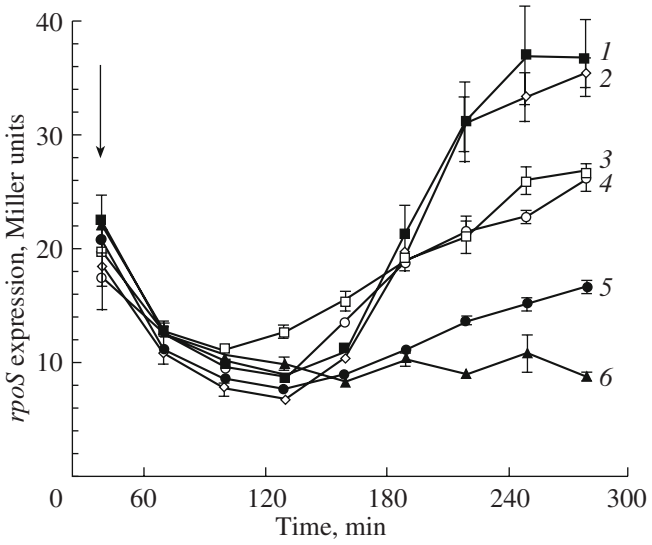

Fig. 3. Changes in the *rpoS* gene transcription in *E. coli* strain RO200 in response to sublethal concentrations of antibiotics in the medium. Bacteria were cultured on LB medium without antibiotics (*5*) and in the presence of 0.012 μ/ml levofloxacin, 0.038 μg/ml pefloxacin, 0.7 μg/ml cefotaxim, 2 μg/ml cefazolin, and 0.08 μg/ml netilmycin (*1–4, 6*, respectively). Arrow indicates the time of antibiotic addition.

the gene *soxS* encoding the transcription regulator which controls antioxidant protection genes of the *soxRS* regulon.

According to the experimental data, those antibiotics transported through porin channels (cephalosporins and fluoroquinolones studied in this case) caused a significant increase in the *soxS* expression as a form of response towards the oxidative stress mostly pronounced for cefotaxim (Fig. 2). In contrast, netilmycin, transported mainly across the lipid bilayer of the outer membrane, didn't affect the expression of the antioxidant defense gene. Thus, the aminoglycoside causes no oxidative stress in *E. coli*, which is also in accordance with the absence of a pronounced response on the part of cellular polyamines (Fig. 1).

Therefore, in *E. coli* sublethal doses of cephalosporin and fluoroquinolone antibiotics generate oxidative stress which manifests itself, among other things, in increased synthesis rates of the polyamines carrying protective functions in this very type of stress.

Effect of antibiotics on the *rpoS* expression level and the influence of polyamines. Earlier, a probable relationship between the *rpoS* expression level and the minimum inhibition concentration was pointed out [8]. Therefore, variations in the *rpoS* expression were studied as a possible *E. coli* adaptive response towards SLCA.

As follows from the experimental data, fluoroquinolones (levofloxacin and pefloxacin) brought about a pronounced induction of the *rpoS* at the transcriptional level (Fig. 3), which was considerably higher than the cell response towards cephalosporins. These trends occur only in the case when antibiotics are transported though the porin channels. An aminoglycoside netilmycin didn't induce the *rpoS* and even somewhat suppressed its expression.

A polyamine-dependent *E. coli* mutant SHT03 was used to study the effect of polyamines on *rpoS* expression. The positive effect of equimolar concentrations of biogenic polyamines on gene expression increased in the series cadaverine–putrescine–spermidine (Fig. 4). The data are evidence for the dominating role of putrescine and spermidine, rather than cadaverine, in positive modulation of the *rpoS* expression; the phenomenon might also be revealed under antibiotic treatment conditions.

Effect of antibiotics on the activity of lysine decarboxylase. The most pronounced concentration increase in response to treatment with fluoroquinolones was observed for cadaverine which is the product of lysine decarboxylase activity.

Lysine decarboxylase activity was measured in *E. coli* RO91 extract in a wide pH range; two peaks at pH 5.5 and 7.5 were revealed, corresponding to the optimal values for two acknowledged forms of the enzyme, CadA and Ldc [19], (Fig. 5). Under SLCA treatment, activity of both forms increased. Various types of antibiotics affected the ratio between CadA and Ldc activity differently (Fig. 6). Cephalosporins induced a significantly higher activity level of CadA than of Ldc, while a fluoroquinolone-generated response was just the opposite. Netilmycin displayed a

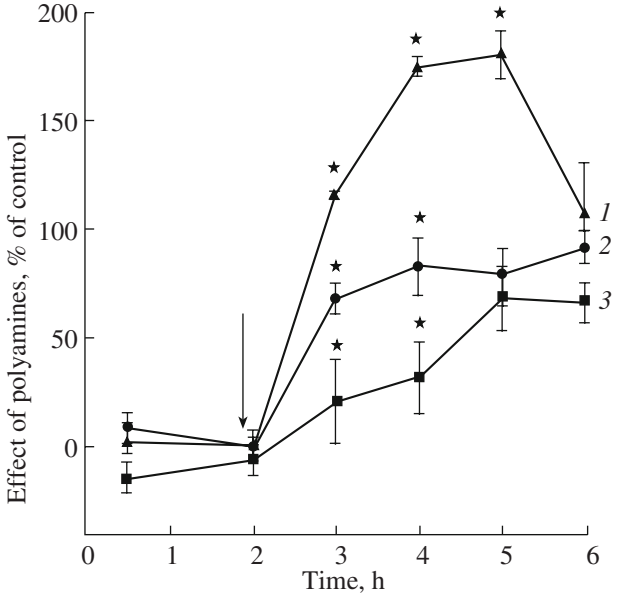

Fig. 4. Effect of equimolar concentrations of polyamines (10 μM) on *rpoS* expression in a *E. coli* polyamine-deficient strain SHT03: spermidine (*1*), putrescine (*2*), and cadaverine (*3*). ★ designates statistically significant difference in *rpoS* expression against the control group, $p \le 0.05$. Arrow indicates the time of polyamine addition.

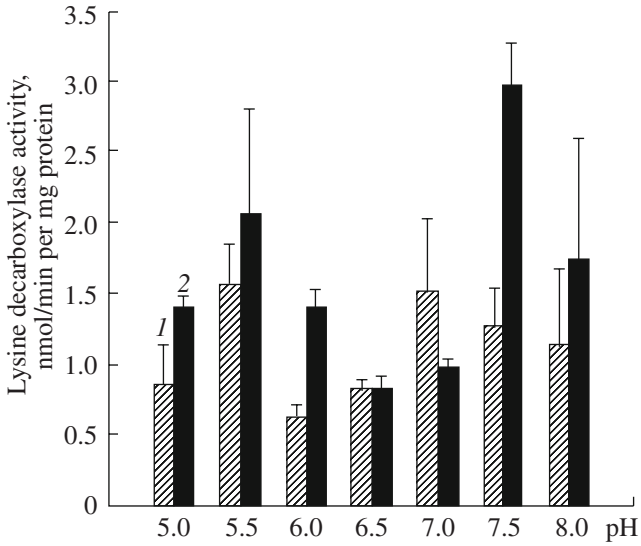

Fig. 5. Effect of pH on lysine decarboxylase activity of *E. coli* strain RO91 in the absence (*1*) and in the presence (*2*) of 0.7 μg/ml cefotaxim. Lysine decarboxylase activity was measured after 6 h of cultivation.

weak inhibitory effect towards both forms of the enzyme.

Variations in the transport activity of porin channels under antibiotic treatment. Earlier, exogenous polyamines added into a bacterial culture were shown to promote a decrease in the activity of the outer membrane porin channels [8]. For this reason, we studied *E. coli* porin permeability under sublethal concentrations of those antibiotics which induced an increase in endogenous cadaverine.

A significant suppression of porin permeability was observed during treatment with fluoroquinolones (Fig. 7), which induced an increase in cellular cadaverine concentrations (Fig. 1). These data prove the importance of endogenous polyamines in porin transport down-regulation as a mechanism for the cell adaptation to sublethal concentration of antibiotics.

Surprisingly, an aminoglycoside antibiotic, netilmycin, while displaying little effect on polyamine concentration (Fig. 1) was quite effective in porin permeability suppression (Fig. 7). The rational explanation of the data should might be netilmycin ability to interfere with the transport activity of porin channels concurrently passing the lipid bilayer of the outer membrane.

Unfortunately, the features of the method for determining the activity of the porin channels implying the use of plasmid β-lactamase didn't allow for evaluation of porin permeability variations under cephalosporin treatment.

Therefore, the raise in cadaverine cellular concentration under sublethal doses of fluoroquinolones may be regarded as one of *E. coli* protective responses aimed at the limitation of antibiotic penetration through porin channels.

DISCUSSION

Early studies of polyamines describe their nonspecific regulatory effects on the processes of transcription and translation [1]. However, the modulatory effects of these compounds were recently shown to be specifically aimed at the group of genes of a so called polyamine modulon [11]. Nowadays, polyamines are known to regulate specifically the cellular adaptive functions involved in bacterial stress response [5, 8].

The present study revealed increased levels of polyamines in cells and stimulation of the enzymes of polyamine synthesis in response to sublethal concentrations of antibiotics (Figs. 1 and 6). Earlier, we demonstrated a similar response of the polyamine synthesis apparatus towards oxidative stress [5]. Certain antibiotics are known to provoke oxidative stress reactions [20], which is in accordance with the theory of oxidative stress as realization of a nonspecific suicidal response of bacteria in response to sublethal external influence. Under the described conditions, inhibition of constructive metabolism is accompanied by an accumulation of excess energy and intensification of the respiratory chain, resulting in generation of reactive oxygen species as a byproduct [21].

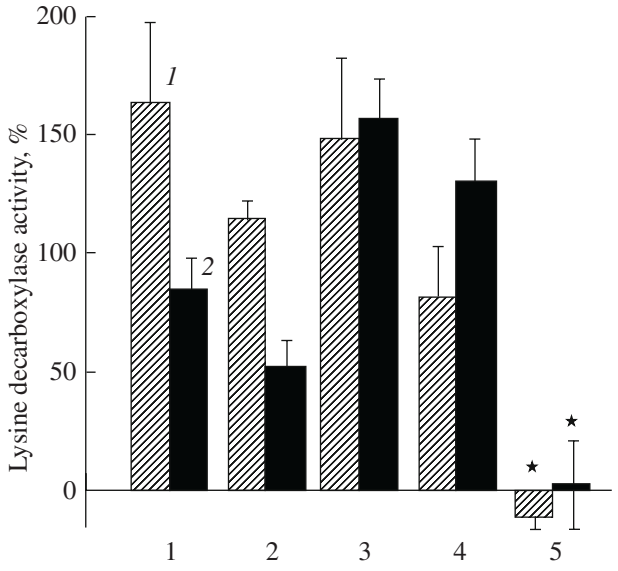

Fig. 6. Change in activity ratio between two forms of lysine decarboxylase, CadA and Ldc, in *E. coli* RO91 cells in the presence of antibiotics of various classes. Bacteria were cultured on LB medium without antibiotics and containing 0.7 μg/ml cefotaxim, 2 μg/ml cefazolin, 0.012 μg/ml levofloxacin, 0.038 μg/ml pefloxacin, and 0.08 μg/ml netilmycin (1–5, respectively). Activity of lysine decarboxylase forms was measured after 180 min of cultivation. The reaction medium of pH 5.5 was used for CadA activity determination (*1*) and of pH 7.5 for Ldc (*2*). Average values of activity expressed as percent to control are presented. ★ designates the absence of statistically significant differences against the control group, $p \le 0.05$.

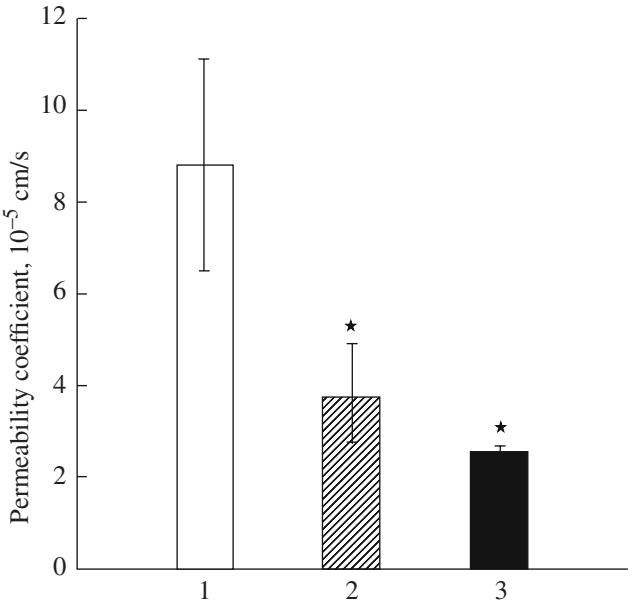

Fig. 7. Effect of sublethal concentrations of antibiotics on porin permeability of *E. coli* RO91 pBR322 cell wall. Bacteria were cultured on LB medium (1) without antibiotics and containing 0.038 μg/ml pefloxacin (2) and 0.08 μg/ml netilmycin (3). Porin transport activity was determined after 360 min of cultivation. ★ designates statistically significant differences against the control group, $p \le 0.05$.

Most of the antibiotics studied in the present work, with the exception of netilmycin, induced the expression of *soxS* encoding one of *E. coli* master regulators of antioxidant protection (Fig. 2). Consequently, oxidative stress is a possible reason for polyamine accumulation in response to antibiotic treatment.

No oxidative stress was observed in the case of netilmycin treatment, possibly because sublethal concentrations of aminoglycosides, unlike those of other antibiotics, do not induce an accumulation of excess energy. The idea is supported by the data on the ability of the polycation aminoglycosides to penetrate the lipid bilayer of the membrane at the expense of proton motive force ($\Delta\psi$) [22], which may lead to $\Delta\psi$ dissipation and disruption of oxidative phosphorylation. In turn, the absence of a pronounced increase in cellular polyamines in response to oxidative stress reduces the efficiency of the OppA-coupled transport of aminoglycosides through the plasma membrane, the content of OppA protein being positively regulated by polyamines [23]. The outlined chain of events may probably be regarded as a bacterial response towards sublethal concentrations of aminoglycosides, which is supported by the data on anaerobic cultures with low $\Delta\psi$ values [24], which are more resistant to this class of antibiotics, as well as *E. coli* mutants with impaired polyamine synthesis [25].

The known role of polyamines in protection against oxidative stress is in connection with their functioning as scavengers for reactive oxygen species and as positive regulators of antioxidant genes transcription [6]. Moreover, polyamines were also reported acting as positive regulators of expression of the *rpoS* gene coding for a multiple stress response regulator, RNA-polymerase σ^S-subunit [3]. Earlier, possible involvement of RpoS was in *E. coli* adaptation to certain antibiotics was reported [8]. All of the antibiotics applied in the present research were shown to alter the *rpoS* expression (Fig. 3), however, fluoroquinolones activated the expression at a higher level than cephalosporins.

A study of polyamine modulation of *rpoS* expression in cultures of polyamine-dependent mutants of *E. coli* revealed their increased positive modulatory effect in the series cadaverine–putrescine–spermidine (Fig. 4). These data, together with the significant precedence of putrescine and spermidine peak concentrations in comparison to cadaverine (Fig. 1), prove their leading role in positive modulation of the *rpoS* gene expression similar to the effect described for the *soxS* [6].

Cadaverine is a lysine decarboxylase product. For the moment, two forms of the enzyme have been described in *E. coli*, an inducible (CadA) and a "constitutive" form (Ldc), with different optimum pH values [19]. CadA is known to be induced under acidic conditions; yet, recent studies have demonstrated induction of the *cadA* gene expression in response to oxidative stress [26], thus it may be under regulation of SoxS. The physiological role of Ldc is not clear; its constitutive nature is also under question. The *ldc* gene was shown to be under RpoS control [27].

The ratio between the *rpoS* (Fig. 3) and *soxS* (Fig. 2) gene expression for various antibiotics is in complete agreement with the activity ratio between CadA and Ldc (Fig. 6). A higher *soxS* induction level in the presence of cephalosporins determines the domination of CadA activity. Meanwhile, strong induction of *rpoS* in the presence of fluoroquinolones resulted in an increase in Ldc activity in the presence of high CadA activity. The findings are evidence for the SoxS-dependent regulation of lysine decarboxylase to be predominant for cephalosporins, and the RpoS-dependent, for fluoroquinolones. The presented results are in accordance with the literature data on CadA and Ldc belonging to the *soxRS* and *rpoS* regulons, respectively [26, 27].

Apparently, a significantly higher expression level of *rpoS* in response to fluoroquinolones than to cephalosporins is associated with different gene induction mechanisms. The key to fluoroquinolones effect should be in the structural similarity of these antibiotics to the quorum sensing signal's molecules belonging to the group of 4-quinolones (PQS) and described for the first time in pseudomonads [28]. Recently, quinolone interaction with a LuxR-like protein SdiA in *E. coli* was shown to induce an early transfer of the bacterial culture into the stationary growth phase [29], which the authors attributed to an increase in the *rpoS* expression. It was also accompanied by an increased resistance to antibiotics caused by activation of a multiple drug efflux pump AcrAB.

We demonstrated that, along with the specified antibiotics resistance mechanism via the activation of efflux pumps, a different type of mechanism that is RpoS- and SoxS-dependent, also functions in *E. coli* cells via lysine decarboxylase induction. As a consequence, cadaverine intracellular content increases considerably (Fig. 1) causing significant suppression of the porin transport (Fig. 7) and increased antibiotic resistance in *E. coli* [8]. Furthermore, SoxS-dependent synthesis of cadaverine is a nonspecific bacterial response to the addition of the antibiotics transported through porin channels, while cadaverine synthesis conditioned by the induction of the RpoS-regulated enzyme Ldc is primarily characteristic of fluoroquinolones.

In contrast to other antibiotics, netilmycin suppressed the *soxS* and *rpoS* genes (Figs. 2 and 3). The absence of induction of any of the two forms of lysine decarboxylase against this background confirms once again the presence of a relation between SoxS and RpoS content and CadA and Ldc activities, respectively.

In spite of the absence of the cellular cadaverine increase in response to netilmycin, addition of this antibiotic to *E. coli* culture caused a significant suppression in porin transport, as in the case of fluoroquinolones. The most appropriate reason for this effect may be direct inhibition of porin channels permeability by the aminoglycoside. This being true, netilmycin should be pictured not only crossing the lipid bilayer of the outer membrane directly, but also to some extent interacting with porin channels and thus blocking their transport functions. Circumstantial evidence to support the hypothesis may be found among the data on the electrostatic interactions between aminoglycoside molecules and functional groups of OmpF [30] in the process of transport through the porins, although relatively large antibiotic molecules are unable to penetrate through the gap in the porin channel.

The following sequence of events seems to describe reasonably the generation of response to sublethal concentrations of cephalosporins and fluoroquinolones in *E. coli*. The primary reaction towards antibiotics is the uncoupling of energetic and constructive metabolic processes, which results in oxidative stress. As a response to oxidative stress, first of all, putrescine and spermidine levels rise, which positively regulate the *soxS* and *rpoS* expression; in turn, the products of these genes control the expression of the lysine decarboxylase genes. Increase in activity for both forms of lysine decarboxylase causes a high cellular content of cadaverine, a suppressor of porin permeability, which inhibits the antibiotics penetration into the cell.

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