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EXPERIMENTAL ARTICLES

Effects of Monoamine Neuromediators on the Growth-Related Variables of *Escherichia coli* K-12

A. M. Anuchin, D. I. Chuvelev, T. A. Kirovskaya, and A. V. Oleskin[1]

Sector for Biosocial Studies, Department of Microbial Physiology, Faculty of Biology, Moscow State University, Moscow, 119991 Russia

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Abstract—The monoamine neuromediators serotonin (5-HT), histamine, dopamine (DA), and norepinephrine (NE), added to an *Escherichia coli* K-12 strain MC 4100 culture upon inoculation, stimulate cell proliferation (determined from CFU formation) and biomass accumulation (monitored nephelometrically) during the late lag phase and the early exponential growth phase. These effects are less significant in the late exponential and stationary phase cultures. According to the concentration dependence of the stimulatory effects, the neuromediators can be classified into two groups: (i) the catecholamines DA and NE, whose effects increase almost linearly with increasing concentrations within the range of 0.1–100 μM, and (ii) histamine and 5-HT, which are characterized by bell-shaped concentration dependence curves with maxima at 0.1 (histamine) and 1 μM (5-HT). On an agar-containing medium, the growing *E. coli* population includes solitary cells and compact cell groups (microcolonies). In this system, both tested catecholamines exert a relatively weak stimulatory influence that manifests itself as an increase in the number of both solitary cells and cell groups, and occurs at concentrations of 10 μM and higher. In analogy to the culture grown on the liquid medium, 5-HT and histamine are distinguished by nonlinear concentration dependence curves: their effects peak at 0.1 μM (histamine) or 1 μM (5-HT); an increase in the neuromediator concentrations results in a decrease in effects that are enhanced by further increasing the concentrations to the submillimolar range. DA increases the percentage of solitary cells, whereas the other tested amines promote cell group formation. The results are interpreted in terms of specific (probably receptor-dependent) mechanisms of action in the neuromediators involved.

Key words: neuromediators, serotonin, catecholamines, dopamine, norepinephrine, histamine, microbial communication, stress, *Escherichia coli.*

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Most natural microbial populations do not exist in isolation, because they form part of biocenoses and ecosystems. They interact with other microbial populations, as well as animal, plant, or fungal macroorganisms. The aspect of these interactions that is presently being studied intensively is the exchange of chemical regulatory agents.

Biogenic amines (serotonin, dopamine, norepinephrine, adrenaline, histamine, etc.) are evolutionarily conserved compounds that perform communicative and regulatory functions in various types of animals [1], plants [2], fungi, and protozoa (see [3]).

In a large number of animals and in humans, biogenic monoamines serve as neurotransmitters and/or (histo)hormones [1]: they are present in various amounts and combinations ("cocktails") in the functional zones of the brain and beyond the central nervous system, e.g., in the gastrointestinal tract (GIT). The composition of these cocktails is responsive to alterations in the state of the macroorganism. For example, it changes in response to stress. In all likelihood, neuromediators interact with local symbiotic or parasitic microbiota that can release their own mediators that influence the host organism.

The data available in the literature are consistent with this suggestion but they are still incomplete. The growth of enteropathogens (*Shigella, Salmonella, Aeromonas hydrophila*, and pathogenic *E. coli* strains such as O157:H7) is stimulated by norepinephrine, which also increases the production of Shiga-like toxins and adhesins [4–8]. Dopamine produces a weaker growth-stimulating effect, while serotonin produces no significant effect [5, 7]. These data are apparently in line with the suggestion that active pathogenic microflora is adapted to a lifestyle that is characteristic of the GIT under stress stemming from infection. Therefore, it prefers norepinephrine, which is produced by the macroorganism in response to infection: this substance induces rapid growth and virulence factor synthesis.

[1] Corresponding author; e-mail: e-mail Aoleskin@rambler.ru

We demonstrated earlier that, in contrast to pathogenic microflora, the growth of nonpathogenic *Escherichia coli* K-12 (strain MC4100) is accelerated by the addition of serotonin [9], a mediator normally contained in the brush border of intestinal mucosa. No data have yet been obtained on the effects of other neuromediators on nonpathogenic *E. coli.* However, there are reports on the stimulatory effect of serotonin on the growth of other microorganisms: the yeast *Candida guillermondii* [10] and the bacteria *Enterococcus faecalis* [10] and *Rhodospirillum rubrum* [9].

The goal of this work was to carry out a comparative analysis of the influence of several neuromediators on the growth-related variables of *E. coli* K-12 on a liquid and solid medium.

MATERIALS AND METHODS

The subject of this study was the nonpathogenic strain *Escherichia coli* K-12 MC4100 from the collection of the Winogradsky Institute of Microbiology, Russian Academy of Sciences.

Studies with *E. coli* grown in a liquid medium. The bacteria were cultivated at 33°C on an LB medium. Its composition (g/l) was as follows: tryptone, 10; yeast extract, 5; and NaCl, 6 (pH 7.0). The growth of the culture was assayed as (i) colony-forming unit (CFU) number by inoculating diluted cell suspensions on petri dishes, and (ii) optical density at 540 nm (OD_{540}), using a single-beam Jasco-4 spectrophotometer. Inoculum (1 ml of the 10^{10} cells/ml suspension) was added to 100 ml of the medium; i.e., the final concentration of *E. coli* cells was 10^8 cells/ml at inoculation.

Freshly prepared aqueous solutions of dopamine hydrochloride, serotonin hydrochloride, histamine hydrochloride, and norepinephrine bitartrate (Sigma, United States) were added to final concentrations of 0.01, 0.1, 1, 10, and 100 μM. The monoamines were sterilized by ultrafiltration, and the pore diameter was 0.22 μm. An equivalent volume of distilled water was added to the control system. Samples for determining OD_{540} and CFU were taken 1.5 h (late lag phase), 3 h (early exponential phase), 6 h (late exponential phase), and 24 h (stationary phase) after inoculation.

Studies with *E. coli* grown on a solid medium. Several thin microscope slides were placed in each petri dish before filling it with the agarized LB medium. The agar layer over the slide was 0.2 ± 0.1 mm thick. The number of both compact groups of five or more *E. coli* cells and solitary cells was determined microscopically. Freshly prepared aqueous solutions of neuromediators were added to final concentrations of 0.01, 0.1, 1, 10, and 100 μM. A 24-h culture grown in the liquid LB medium was diluted with distilled water to a concentration of 10^3 cells per 1 ml of medium, and 0.1 ml were added to petri dishes with the LB agar. Hence, each petri dish contained approx. 10^2 *E. coli* cells upon inoculation. The inoculum was carefully spread over the medium surface with a spreading rod.

At regular intervals, the slides were removed from the petri dishes under sterile conditions. They were examined with a light microscope (Carl Zeiss, Jena, Germany) in a bright field. Preliminary studies demonstrated that the cell density in the control system (without neuromediators) was 31 ± 3 solitary cells and 10 ± 2 compact cell groups after 17 h of cultivation. At this point in time (17 h), control and experimental (neuromediator-containing) samples were compared.

The mean values were calculated based on the data obtained in 15–20 fields. Three series of each experiment were conducted, and the results presented below are averaged values. Their statistical treatment was based on the Student test ($P < 0.05$).

RESULTS

Effects of monoamine neuromediators on CFU formation and OD_{540} increase in *E. coli* K-12 cultures grown on a liquid medium. Strakhovsky et al. [10] attempted to enhance the effect of serotonin by adding serotonin, apart from the inoculation moment, at several points in time during the growth of the yeast *Candida guillermondii.* However, according to our earlier data on the effect of serotonin on the growth of the bacterium *Rhodospirillum rubrum* [9], a "stepwise" addition of serotonin does not increase its effect. Therefore, in this study, we added neuromediators only upon inoculation.

The concentration dependence curves of the DA and NE effects on CFU number and OD_{540} were almost linear after both 1.5 (Figs. 1a and 2a) and 3 h (Figs. 1b and 2b) of cultivation. From the results, it is evident that NE caused a 21% increase in the CFU number at a concentration of 100 μM, after 1.5 h of cultivation producing a weaker growth-stimulating effect than DA, that caused a 55% increase in CFU number at the same concentration. The data on the 6 h (late exponential phase) and 24 h (stationary phase) *E. coli* cultures demonstrated that the DA and NE effects on both CFU and OD_{540} were attenuated in aging cultures. For instance, DA only increased the CFU and the OD_{540} values of a 6-h culture by 10.5%. These data did not significantly differ from the control system in terms of statistics for the confidence level $P > 0.99$ and, therefore, are not presented in the form of bar charts.

The effects of histamine (Figs. 3a, 3b) and serotonin (5-HT; Figs. 4a, 4b) were characterized by a nonlinear concentration dependence with a maximum at 0.1 (histamine) and 1 μM (5-HT). Interestingly, histamine produced the strongest effect among the tested amines. At the optimum concentration (0.1 μM), it caused a twofold stimulation of the CFU formation and the OD_{540} increase in 1.5-h *E. coli* cultures (Fig. 3a). The effects of histamine and 5-HT, like those of the catecholamines NE and DA, were attenuated in 6-h and 24-h *E. coli* cul-

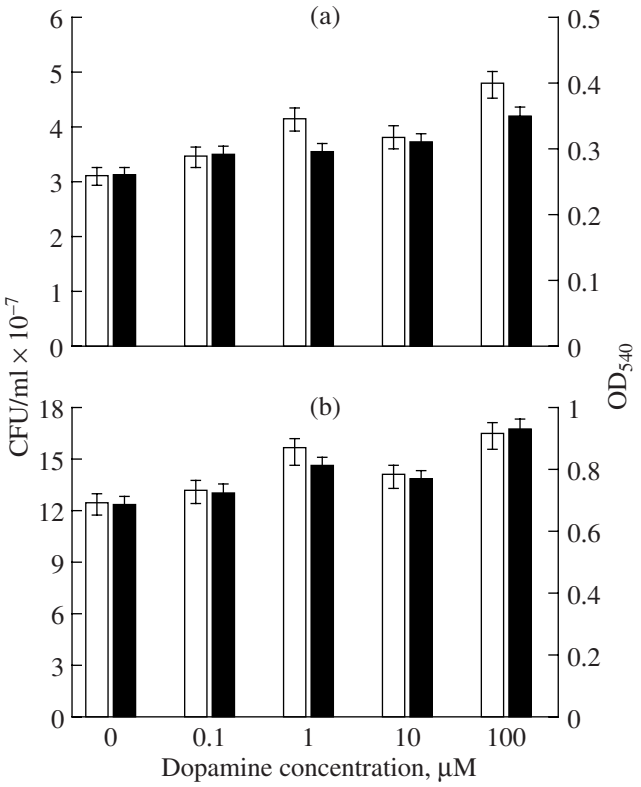

Fig. 1. Growth of *E. coli* K-12 submerged culture after the addition of various concentrations of dopamine. Light columns, biomass (OD_{540}); dark columns, CFU. (a) 1.5 h of cultivation; (b) 3 h of cultivation.

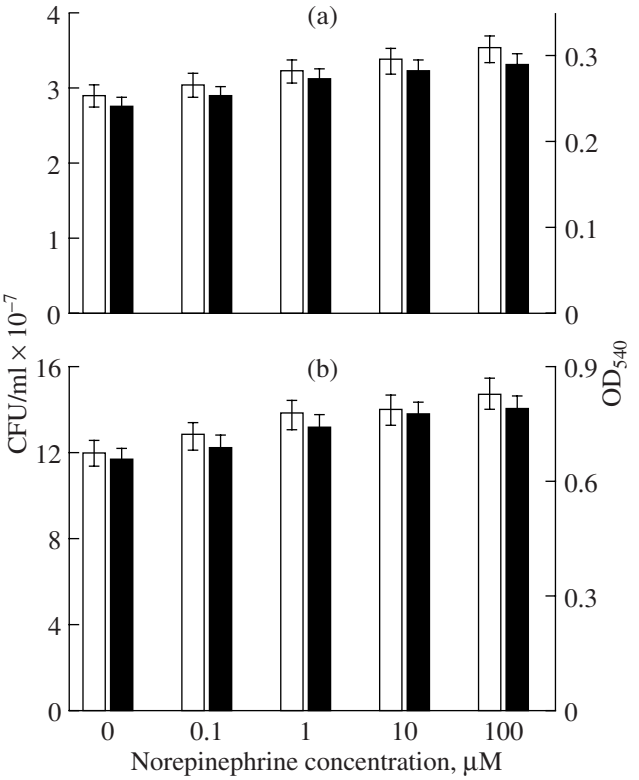

Fig. 2. Growth of an *E. coli* K-12 submerged culture after the addition of various concentrations of norepinephrine. Light columns, biomass (D_{540}); dark columns, CFU. (a) 1.5 h of cultivation; (b) 3 h of cultivation.

tures. No statistically significant differences between the growth-related variables of the control and mediator-treated samples were revealed (data not shown).

Hence, the tested neuromediator amines added to *E. coli* K-12 cultures upon inoculation stimulated the proliferative activity of their cells (CFU formation) and biomass accumulation (OD_{540} increase) in young (1.5 and 3 h) cultures. The amines differed in terms of the concentration dependences of their effects and were classified into two groups: (i) the catecholamines DA and NE with a linear concentration dependence of the growth-stimulating effect and (ii) histamine and 5-HT that lack catechol structures and are characterized by a nonlinear (bell-shaped) concentration dependence curve.

The drastically decreased neuromediator effect on the CFU formation and OD_{540} increase in aging (6 and 24 h) *E. coli* cultures may be due to the mechanism of action of the monoamines that (i) accelerate the lag phase of bacterial cultures, presumably by increasing the number of cells that are capable to divide (the CFU number); (ii) accelerate cell division during the lag phase and the early exponential phase, i.e., operating as growth-stimulating agents, as was earlier suggested for serotonin [9]; and (iii) accelerate the desorption of the cells that attach to the walls of the cultivation vessel during the lag phase [11]. All the mechanisms may function in parallel. Mechanism (i) was tested in this work by cultivating bacteria on a solid medium.

Effects of monoamine neuromediators on CFU formation and OD_{540} increase in *E. coli* K-12 cultures grown on a solid medium. An *E. coli* population grown on a solid medium contains solitary cells and dense cell groups (microcolonies). It was earlier established that serotonin stimulates the formation of matrix-embedded cell groups [9]. In this section, we compare the effects of different neuromediator monoamines on the formation of solitary cells and dense cell groups, i.e., on the growth pattern of microbial populations on a solid medium.

Similar to the liquid medium, addition of the catecholamines DA and NE to the agarized medium stimulated growth of the *E. coli* population, increasing the numbers of solitary cells and their dense groups (Figs. 5a and 6a). The effects of both catecholamines were characterized by a linear concentration dependence and manifested themselves to a maximum extent at concentrations of 10–100 μM. The stimulatory effect

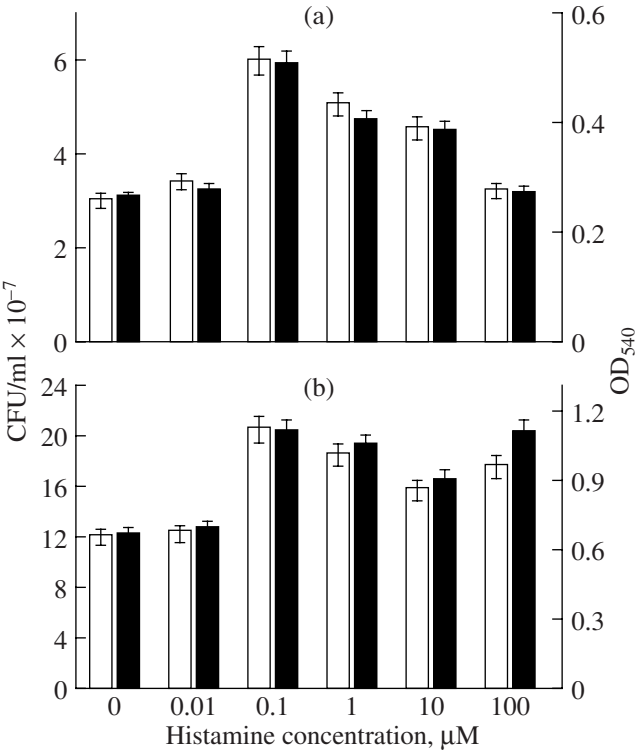

Fig. 3. Growth of an *E. coli* K-12 submerged culture after the addition of various concentrations of histamine. Light columns, biomass (D_{540}); dark columns, CFU. (a) 1.5 h of cultivation; (b) 3 h of cultivation.

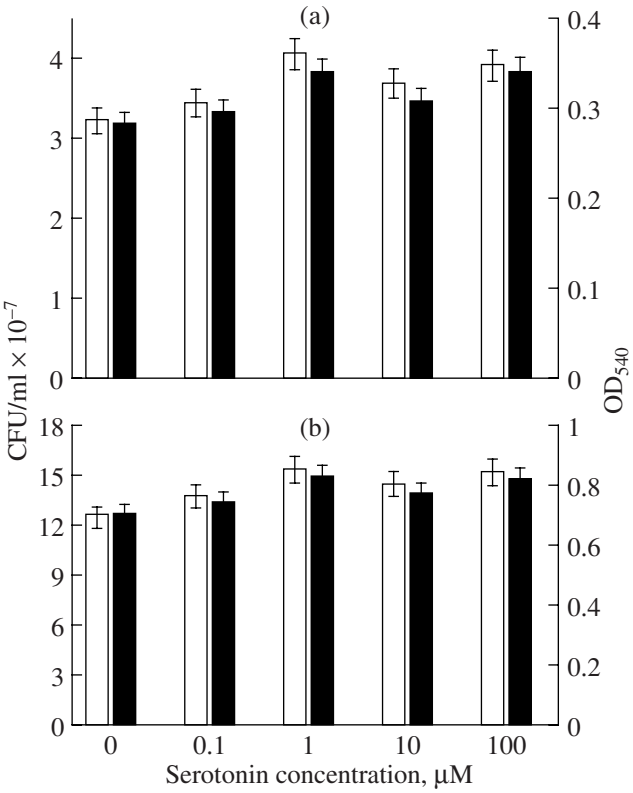

Fig. 4. Growth of an *E. coli* K-12 submerged culture after the addition of various concentrations of serotonin. Light columns, biomass (D_{540}); dark columns, CFU. (a) 1.5 h of cultivation; (b) 3 h of cultivation.

of NE or DA was threefold at a concentration of 100 μM.

The catecholamines significantly differed in terms of their effects on the ratio between the numbers of solitary cells and their compact groups (Figs. 5b and 6b). In comparison to the control system, this ratio increased in the case of DA (0.1–1 μM) and decreased in the case of NE.

In contrast to the catecholamines, 5-HT (Fig. 7a) and histamine (Fig. 8a) demonstrated nonlinear concentration dependence curves of their stimulatory effects on the growth-related variables of the tested bacteria on the solid medium. The plots for the number of solitary cells and for the number of microcolonies were characterized by peaks at 0.1 μM (histamine) or 1 μM (5-HT); further increase in amine concentrations resulted in a decrease in the amplitude of the effect, followed by its increase at submillimolar concentrations. Histamine was the most efficient of the tested neuromediator amines in respect to stimulating the growth of *E. coli* K-12 populations both on liquid and solid media.

Both amines influenced the ratio of the number of solitary cells to the number of microcolonies on the solid medium, although to a different extent. Like NA, histamine (Fig. 7b) decreased this ratio within the whole tested concentration range; i.e., it predominantly stimulated microcolony growth. Serotonin produced a comparatively weak stimulatory effect on microcolony formation (Fig. 8b).

DISCUSSION

According to the data in the literature, the growth of pathogenic enterobacteria is maximally stimulated by norepinephrine, whose release is characteristic of a macroorganism's response to infection. Dopamine, a minor component of the response, is active at comparatively high concentrations. For instance, norepinephrine and dopamine at concentrations of 3 and 100 μM, respectively, stimulated the growth of the pathogenic strain *E. coli* JPN10 (O44:H18) on the liquid SAPI medium with blood serum (the inoculum concentration was 144 cells per 1 ml) [7]. Serotonin had almost no effect on the growth of enterobacteria; it only slightly stimulated the growth of the intestinal pathogen *Aeromonas hydrophila* at a concentration of 1 mM [5].

According to our own data, the nonpathogenic symbiotrophic strain *E. coli* K-12 MC 4100 prefers a different neuromediator "landscape." Serotonin normally

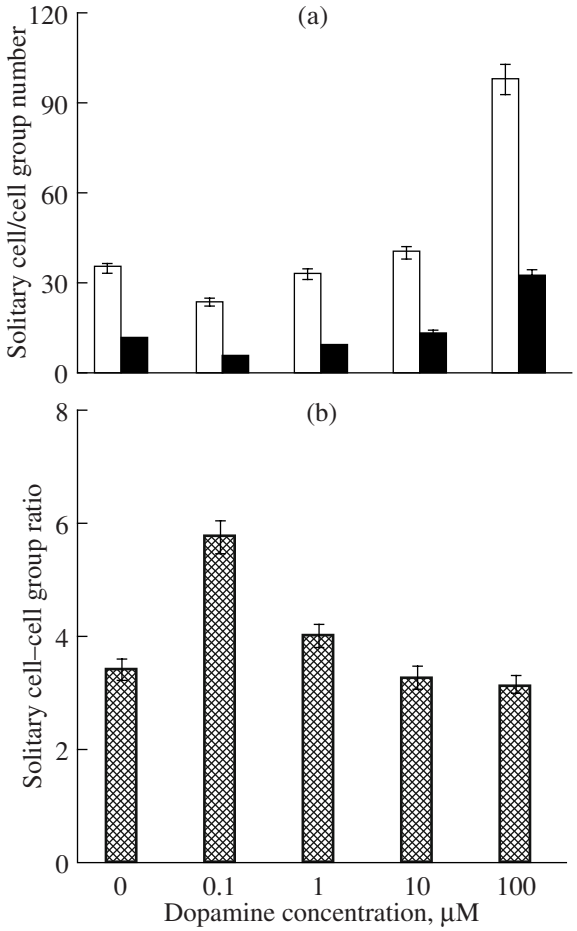

Fig. 5. Effect of dopamine on the growth pattern of an *E. coli* population on a solid medium. (a) solitary cell number (light columns) and cell group number (dark columns); (b) ratio between the solitary cell number and the cell group number.

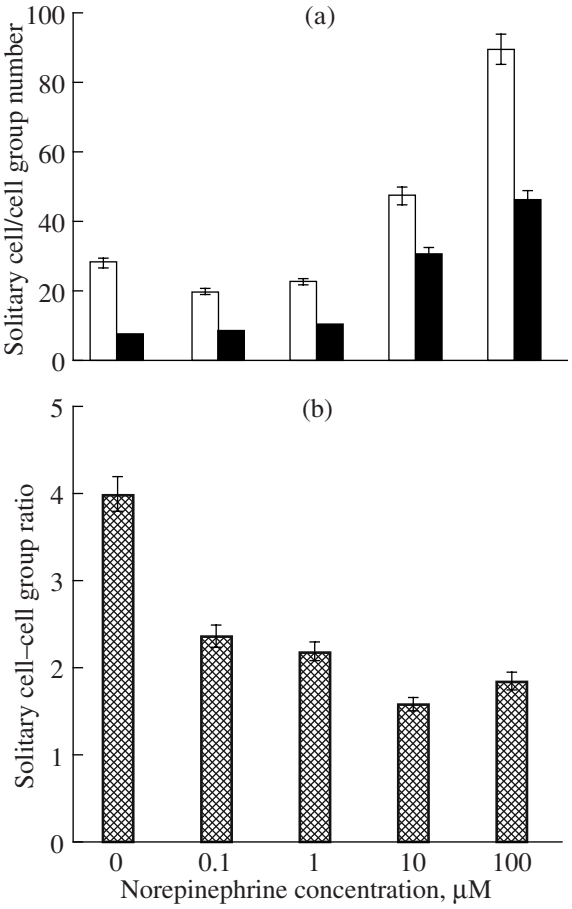

Fig. 6. Effect of norepinephrine on the growth pattern of an *E. coli* population on a solid medium. (a) solitary cell number (light columns) and cell group number (dark columns); (b) ratio between the solitary cell number and the cell group number.

contained in the chromaffin granules of GIT mucosa cells was no less efficient than norepinephrine in all tested systems (on the liquid and the solid medium, Figs. 4 and 8). Dopamine, a minor component of the response to infection, stimulated proliferative activity and biomass accumulation to a greater extent than the major component, norepinephrine (Figs. 1 and 2). Histamine, a characteristic factor of local inflammation (which also results in the production of additional amounts of serotonin), was the most efficient of the tested neuromediators and also was characterized by the lowest active concentration (Figs. 3 and 7).

Presumably, the nonpathogenic strain *E. coli* K-12 MC 4100, in contrast to pathogenic strains, is adapted not to serious infection, but to light local inflammation. It is characterized by the synthesis and release of histamine and serotonin and, to a lesser extent, of catecholamines that are extruded into the intestinal lumen from nerve terminals damaged by inflammation. The light local inflammation of intestinal mucosa may be due to microtraumas caused, e.g., by coarse food.

What is the mechanism of action of the monoamine neuromediators on bacterial populations? Lyte et al. [4, 8] attribute their influence on microorganisms to the facilitation of iron ion transfer into the cell by chelating iron by catechol rings. Accordingly, the effect is expected to be produced by amines that possess the catechol structure, i.e., dopamine and norepinephrine, not serotonin and histamine. However, according to our data, the strongest stimulatory effect was caused by the catechol ring-lacking histamine; a statistically significant effect was also produced by serotonin.

The two catecholamines (dopamine and norepinephrine) differ in terms of their influence on the growth-related variables of bacteria grown on an agarized medium: dopamine increases the percentage of solitary cells at a concentration of 0.1 μM (Fig. 5), while norepinephrine, like serotonin and histamine, increases the percentage of cell groups (Figs. 6–8).

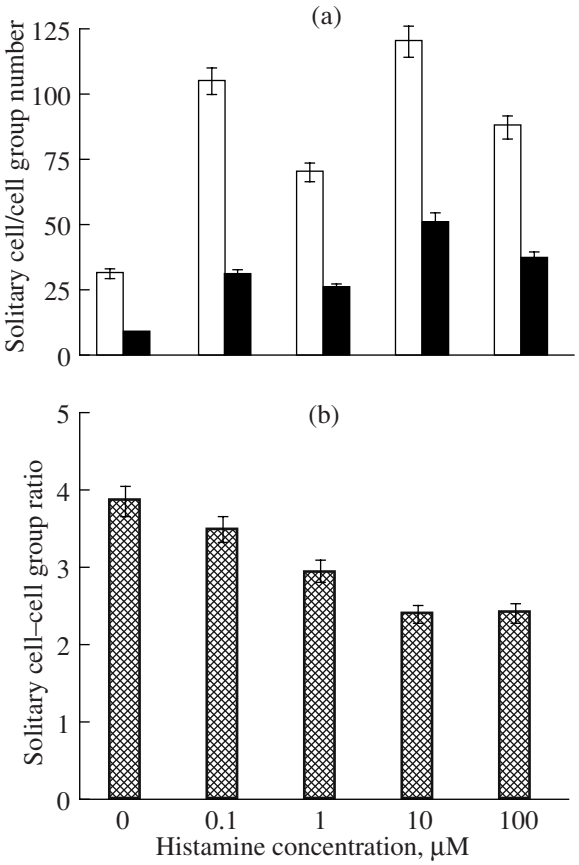

Fig. 7. Effect of histamine on the growth pattern of an *E. coli* population on a solid medium. (a) solitary cell number (light columns) and cell group number (dark columns); (b) ratio between the solitary cell number and the cell group number.

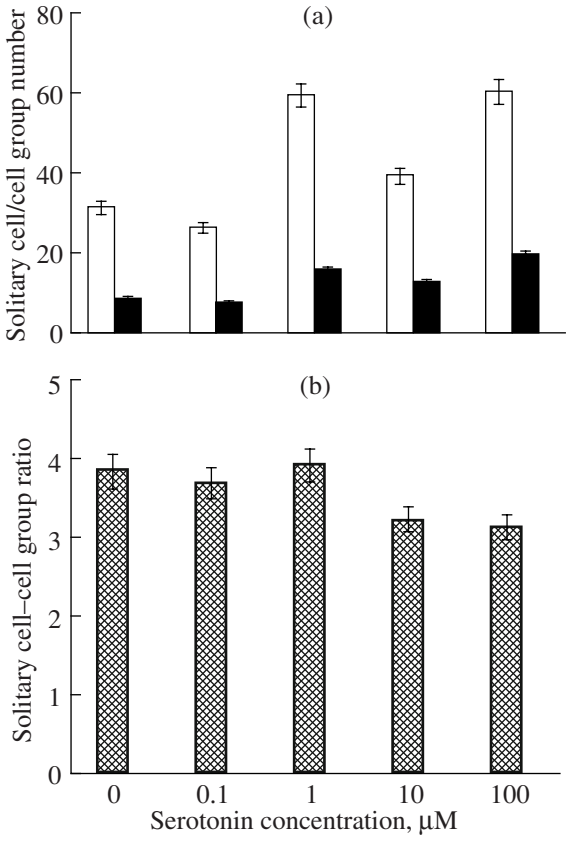

Fig. 8. Effect of serotonin on the growth pattern of an *E. coli* population on a solid medium. (a) solitary cell number (light columns) and cell group number (dark columns); (b) ratio between the solitary cell number and the cell group number.

These differences may be due to different effects of the amines on the adhesive properties of bacterial cells.

Hence, the effects produced by the neuromediators do not necessarily require a catechol structure. Taking into account the difference between the effects of two chemically similar compounds, dopamine and norepinephrine (which only contains an extra OH group in the side chain), we suggest that these effects at least partly result from specific interactions involving receptors on the surface of bacterial cells that individually recognize neuromediators. In a similar fashion, animal and human cells use different groups of membrane receptors to bind dopamine and norepinephrine. They possess D receptors (D_1, D_2, D_3, etc.) for dopamine and α- and β-adrenoreceptors for norepinephrine [1], even though the adrenoreceptors also bind dopamine with a lesser degree of affinity. It was demonstrated in Lyte's laboratory [12] that the stimulatory effects of norepinephrine and dopamine on the growth of pathogenic enterobacteria (*E. coli* O157:H7, *Salmonella enterica*, and *Yersinia enterocolitica*) are prevented by α-adrenoreceptor blocking agents and an animal cell-characteristic dopamine antagonist, respectively.

Our earlier data also pointed to the possible role of membrane-dependent neuromediator binding. It was established that dopamine and serotonin, but not norepinephrine (the least efficient growth stimulator in this work), stimulated the respiratory activity of *E. coli* cells [13]. Serotonin concentrations of about 100 μM decreased the membrane potential of the purple bacterium *Rhodospirillum rubrum* [9].

Forming part of a complex microbial symbiotic community in the animal/human GIT, the *E. coli* population is probably responsive to changes in the neuromediator ratio in the "cocktail" produced by the host and, presumably, by the bacteria per se [14]. Increasing or decreasing the microcolony percentage in populations seems to be one of the mechanisms enabling structured microbial communities forming colonies, biofilms, or flocks to vary their growth patterns, which should promote their evolutionary adaptation to changing environmental conditions.

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