Effect of a Peptide Extract of Fetal Brain Tissue on the Intracellular pH of Mouse Peritoneal Phagocytes

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The intracellular pH (pH_i) of mouse peritoneal neutrophils, initially 0.2 U, drops after a 15-min incubation of these cells with a peptide extract of fetal brain tissue. Treatment with the preparation leads to appreciable changes in the distribution of neutrophils by the examined parameter. For macrophages, the acidifying effect of the agent and its effect on the pattern of cell distribution in terms of pH values are far less expressed. The effects of the agent in dilutions $1:10^2$ and $1:10^4$ on the mean pH_i are virtually the same.

Key Words: *peptides; neutrophils; macrophages; intracellular pH*

The recent upsurge in the use of bioactive peptide regulators of biogenic nature in medicine has sparked interest in the mechanisms of their action. Among the important trends of research is analysis of the effects of agents on the principal systems regulating cellular functions, one of which is the system of pH regulation. Due to the widespread use of fluorescent probes, experiments in recent years have persuasively demonstrated that many processes taking place in the cell with a modified function are somehow related to changes in the intracellular pH. Such processes include adhesion and fusion of cells, alteration of their shape, formation of the cytoskeleton, transport of ions and low-molecular compounds, activity of cell metabolism, processes of proliferation, and many others [9,13]. Shifts in the intracellular pH values registered during such events are commonly 0.1 to 0.2 U. Of special interest are the cells helping provide for the immune homeostasis of the organism, including phagocytic cells (neutrophils and macrophages), the specific aspects of whose functional activity are realized with the active participation of H^+ ions [3,7,8,14,15].

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Among the mechanisms of pH regulation in the cells a key role is played by various systems of H^+ transport across the plasma membrane (proton transfer conjugated with other ions and system of transport H^+-ATPases) [13]. The system of electroneutral Na^+/H^+ exchange revealed for many cell types [12] has been the focus of attention in recent years, and the increase of intracellular pH observed during exposure of the cell to a number of physiologically active agents has been shown to result from intensification of Na^+/H^+ metabolism.

Hence, published data indicate that hydrogen ions, along with Ca^{2+} ions and cAMP, play an important role in the regulation of cellular functional activity. In this study, therefore, we aimed to elucidate the effect of a peptide extract of fetal tissue on the intracellular pH of mouse peritoneal neutrophils and macrophages.

MATERIALS AND METHODS

Previously described methods [6,7] with some modifications were used. Experiments were carried out on peritoneal neutrophils and macrophages of outbred male albino mice. In order to obtain the cells of the first type, the animals were intraperitoneally injected 2 ml of peptone solution (50 mg/ml). After 3 h the

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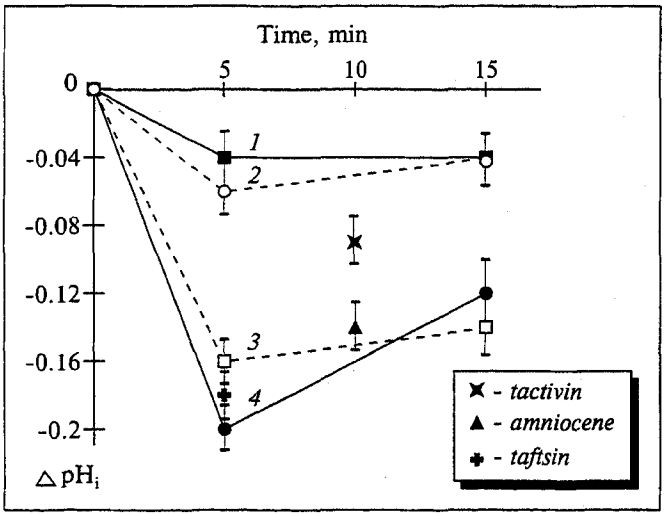

Fig. 1. Effect of peptide extract of fetal brain tissue on intracellular pH of mouse peritoneal macrophages. Abscissa: time after replacement of incubation medium with medium containing peptide extract in dilutions $1:10^2$ (*2, 4*) and $1:10^4$ (*1, 3*). Ordinate: changes of intracellular pH of neutrophils (*3, 4*) and macrophages (*1, 2*) in comparison with the respective controls (ΔpH_i). For comparison data are presented on the effects of the regulatory tetrapeptide taftsin (0.1 μg/ml) and the bioactive agents amniocene (diluted $1:10^8$) and tactivin (1.0 μg/ml).

animals were sacrificed and peritoneal fluid rich in neutrophils was removed. Peritoneal macrophages were prepared by washing out the cells from the abdominal cavity of just-sacrificed intact (unstimulated with peptone) mice using Hanks' solution (containing 20 mM HEPES, pH 7.2), 2 ml per mouse. The concentration of cells in the resultant suspension was brought to 10^6 cells/ml with Hanks' solution.

The suspension (20 μl) was placed on slides and incubated 45 min in a humid chamber, after which the slides were washed in Hanks' solution to remove the cells that failed to adhere. The preparations of peritoneal phagocytes adhering to slides were kept in Hanks' solution during the experiment.

A peptide extract of fetal brain tissue obtained by T. P. Klyushnik at the Research Center of Mental Health, Russian Academy of Medical Sciences, was used. Experiments to study the effects of the fetal tissue peptide extract on the cells were carried out as follows. An aliquot of solution was removed from a sealed flask with the extract (2 mg/ml) under sterile conditions and two consecutive dilutions were prepared in Hanks' solution: $1:10^2$ and $1:10^4$ (20 and 0.2 μg/ml, respectively).

The fluorescent pH indicator fluorescein was added as a result of a 15-min incubation of phagocytes on the slides with fluorescein diacetate (in a final concentration of 5 μg/ml). After nonbound stain had been washed off, the slides were placed in a chamber of special design which was filled with Hanks' solution and the fluorescence of 10 cells was recorded for control. Then the solution in the chamber was replaced with another one containing the peptide extract in an appropriate dilution and the intensity of fluorescence of individual cells was measured during 20 min.

Intracellular pH was measured using a variant of the microfluorometric method [6] under a LYuMAM-I3 fluorescence microscope fitted with a photometric attachment with a kit of optical-interference filters.

The data are presented as the arithmetic mean values of the examined parameters and their mean square errors.

RESULTS

Figure 1 presents the data analyzing the effect of a peptide extract of fetal brain tissue possessing high biological activity [10] on the intracellular pH of peritoneal phagocytes. Effects of the peptide extract in dilutions $1:10^4$ (0.2 μg/ml) and $1:10^2$ (20 μg/ml) were investigated. These dilutions were selected because, as had been previously shown, well-expressed cell responses to the regulatory tetrapeptide taftsin [7,11] and the drug tactivin, representing a complex of thymic regulatory peptides [1], are observed with such concentrations. Figure 1 shows that the efficacy of the drug differed for the two cell types examined. Exposure of neutrophils led to a reliable ($p<0.05$) drop of pH_i after just a 5-min incubation, this drop still being evident by the 15th min of incubation. It is noteworthy that the agent showed a similar efficacy toward the cells in both dilutions tested. On the other hand, the acidifying effect of the agent on peritoneal macrophages was far less expressed. For these cells, the effects of the agent in the two concentrations studied were again virtually the same. The differences in the effects of peptide extracts on neutrophils and macrophages seem to be due to a different sensitivity of these types of cells. One of the causes may be "sensitization" of neutrophils, but not of macrophages, to the exposure as a result of preliminary stimulation of mice with intraperitoneal peptone during the isolation of cells [4]. Data on the similar acidifying effects of taftsin, amniocene, and tactivin on the intracellular contents (Fig. 1) were also obtained in experiments with stimulated peritoneal macrophages of mice.

It should be noted that the effects of many bioactive factors on cells are associated with an initial acidification of the intracellular contents followed by recovery of the initial pH_i to the baseline level and then even beyond. The duration of the initial phase of acidification under different conditions and under the effects of different factors varies within a wide range. Similar changes of pH_i are observed un-

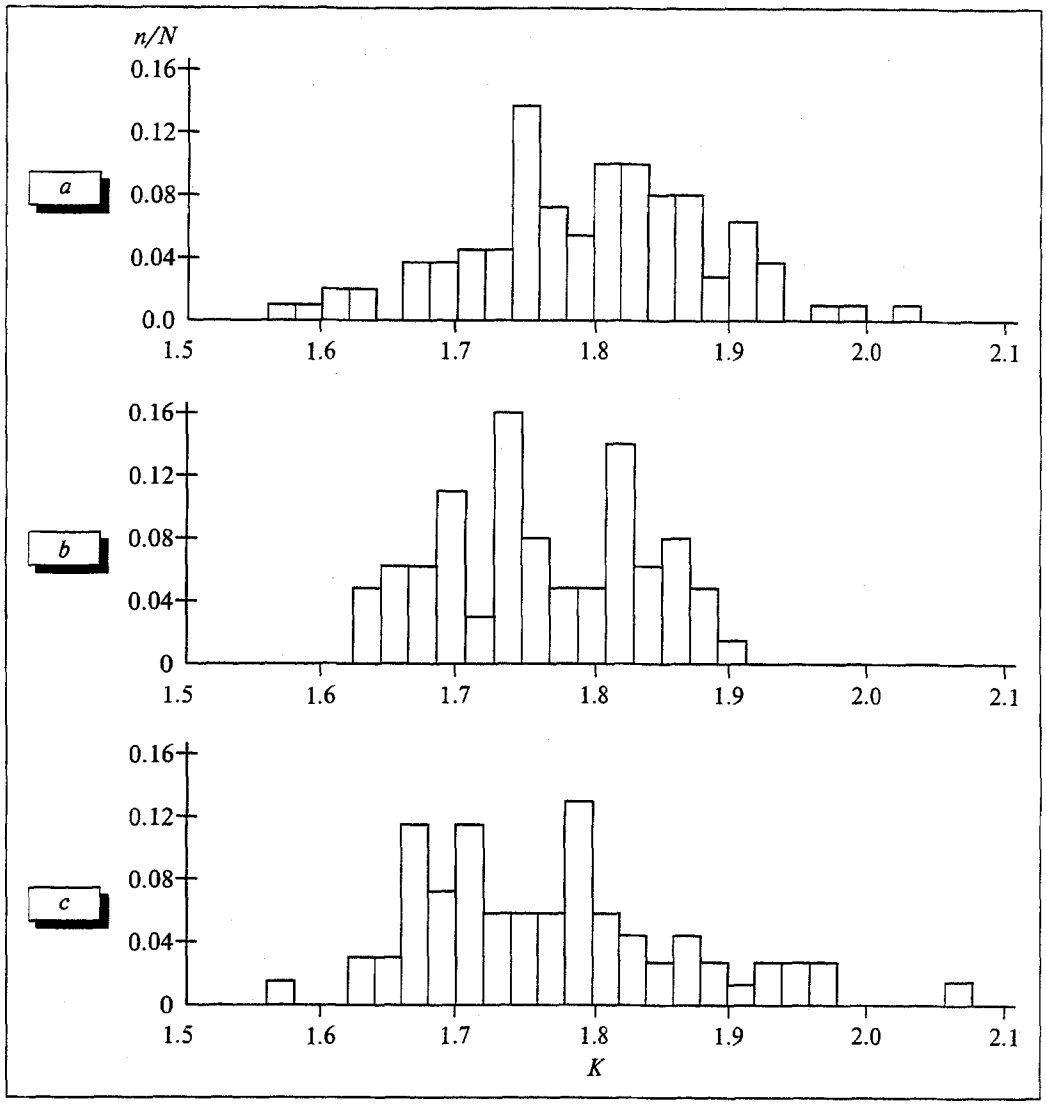

Fig. 2. Histogram showing distribution of macrophages by the *K* parameter proportionate to their intracellular pH during exposure to peptide extract of fetal brain tissue. Here and in Fig. 3: abscissa: *K* value (ratio of intensity of fluorescence of fluorescein-stained cells at wavelengths 520 and 570 nm); ordinate: ratio of the number of cells with preset *K* value (*n*) to total number of cells examined (*N*). *a*) control; *b* and *c*) effects of peptide extract in dilutions $1:10^4$ and $1:10^2$, respectively.

der the influence of the chemotactic tripeptide N-formyl-Met-Leu-Phe [14], the regulatory phagocytosis-stimulating tetrapeptide taftsin (our unpublished data), low-intensive x-ray [5] and laser [8] exposure, and many other bioactive agents.

It has been shown for formyl-peptide and taftsin [2,14] that their interactions with cellular receptors are associated with a short-term increase of the intracellular concentration of Ca^{2+} due to its release from the intracellular depot and intensive release from the incubation medium. Initially the fall of the intracellular pH is caused by the activation of different biochemical processes leading to the formation of H^+ and by the release of protons into the cytoplasm on account of the Ca^{2+} exchange for H^+ of mitochondrial and other organelles. A further gradual rise of pH_i is caused by the activation of mechanisms including the Na^+/H^+ exchange system. Both phases of changes are evidently functionally important and reflect the restructuring of cell functions directly after the exposure and the subsequent boost of activity due to the increase of pH_i. According to published data [9], an increase of the total functional and metabolic activity of cells is associated with a rise of their pH_i, while a decline of activity is associated with acidification of the intracellular contents.

Mindful of the marked structural and functional heterogeneity of neutrophils and macrophages [4], we analyzed the effect of the peptide extract on the pattern of their distribution in terms of intracellular pH values, summarizing the data obtained for the interval of 5 to 15 min incubation of cells with the agent. The cell distribution histograms are presented in Figures 2 and 3. Analysis showed that exposure of macrophages to the peptide extract in a $1:10^4$ dilution is associated with a negligible increase of the standard coefficient of asymmetry (from -0.59 to -0.11) and a decrease of the standard excess coefficient (from -0.01 to -1.73), this indicating a flattening of the peak

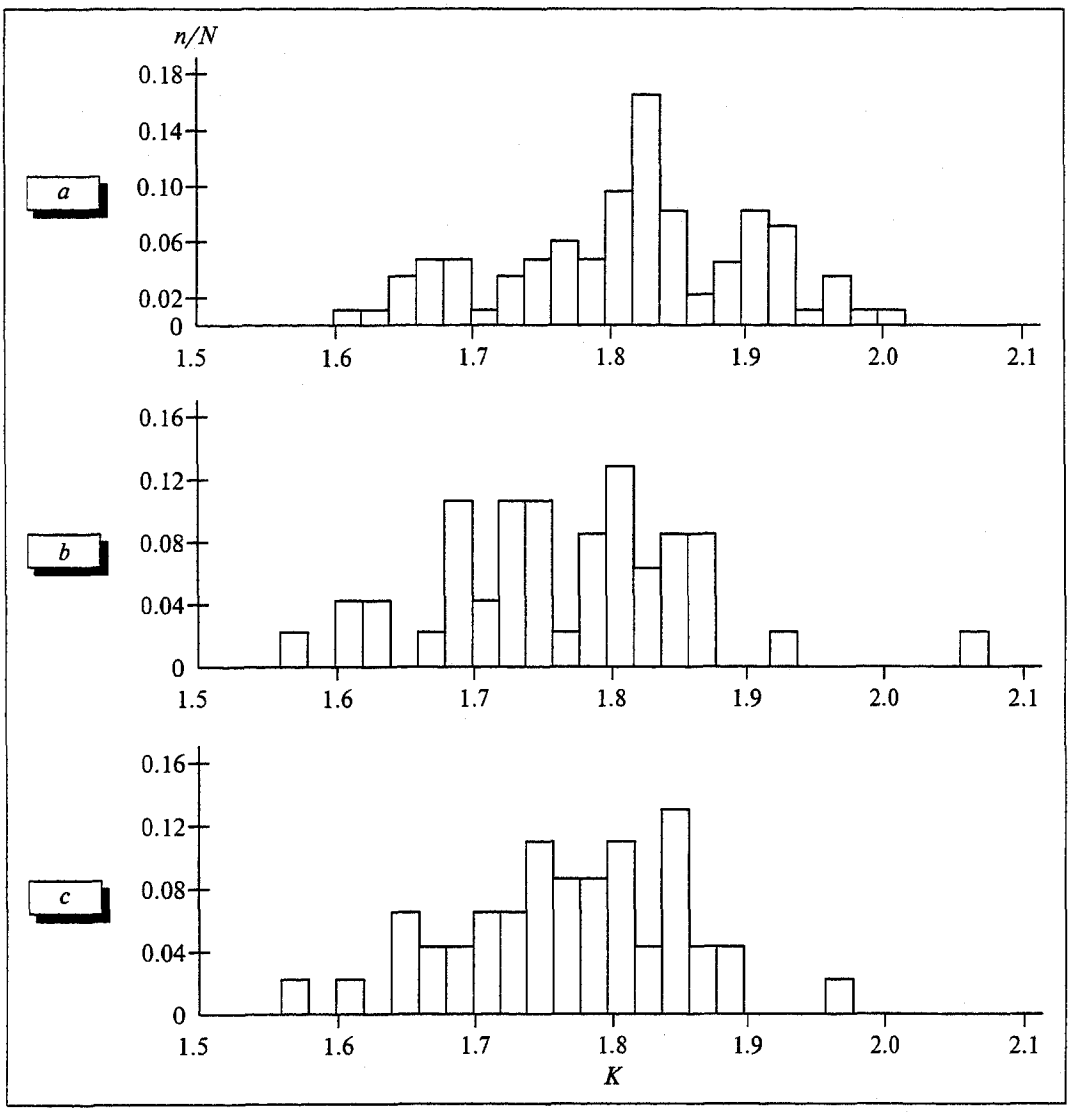

Fig. 3. Histogram showing distribution of neutrophils by the *K* parameter proportionate to their intracellular pH during exposure to peptide extract of fetal brain tissue.

of distribution. Moreover, due to the disappearance of cell subpopulations with the most alkaline and acid pH_i values, the distribution becomes narrower than in the control. An increase of the concentration of peptide extract ($1:10^2$ dilution) led to a decrease of the mode of distribution (from 1.83 to 1.71). This was paralleled by a recovery to the control level of the minimum, maximum, and amplitude of distribution and was followed by a marked increase of the asymmetry and excess coefficients (to 2.19 and 0.34, respectively). These changes point to a decrease of the most frequent value in the distribution, an increase of positive asymmetry, and a sharpening of the peak of the distribution curve.

In contrast to macrophages, exposure of stimulated neutrophils to the peptide extract was associated with not only more pronounced changes of the mean pH_i values, but also more significant changes in the pattern of cell distribution in comparison with the control (Fig. 3). Upon exposure to the agent in the $1:10^4$ dilution we observed a decrease of the median (from 1.83 to 1.77), mode (from 1.83 to 1.69), and geometric mean (from 1.82 to 1.77) of distribution. The values of the upper (from 1.9 to 1.83) and lower (from 1.77 to 1.71) quartile decreased. The standard asymmetry and excess coefficients increased and became positive (from 0.91 to 1.25 and from -0.96 to 2.17, respectively). This indicates that, along with a drop of the mean value of distribution, the value of the most probable distribution decreases as well, whereas the positive asymmetry of distribution and the sharpness of the peak of the distribution curve increase. Exposure of cells to the agent in the $1:10^2$ dilution did not cause any appreciable changes in the mean values or the median and geometric mean in comparison with the $1:10^4$ dilution, although the mode of distribution increased almost to the control level. The values of the upper and lower quartiles did not differ for the two dilutions either. At the same time, exposure to the agent in a higher concentration leads to an ap-

preciable decrease of the asymmetry and excess coefficients (to -0.62 and 0.03, respectively). The data indicate an increase of the most probable distribution value and a decrease of asymmetry and flattening of the peak of the distribution curve.

Hence, exposure of stimulated mouse peritoneal neutrophils to a peptide extract of fetal tissues leads to a reduction of their mean intracellular pH between the 5th and 15th min of exposure and alters the pattern of cell distribution in terms of this parameter. These effects are far less expressed in unstimulated peritoneal macrophages. No appreciable differences were observed in the effects of the peptide preparation in dilutions $1:10^4$ and $1:10^2$ on the mean pH_i of phagocytes. The results appear to reflect the first phase (reduction of pH_i) of modification of cell condition in response to the peptide extract of fetal brain tissue.

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