scent HDL. The content of total serum Ch dropped by 38% ($p<0.01$) and by 41% ($p<0.01$) after the administration of compound II and compound I, respectively. However, the level of α-Ch did not drop, and in the case of compound II it even rose 22%, i.e., the drop of total Ch occurred due to LDL and VLDL Ch. Both test compounds increased the content of Ch in the liver, whereas accumulation of TG was observed only in the case of compound I (Table 2).

Thus, the effect of compounds I and II on serum LP and on the content of Ch in blood serum and in the liver surpasses that of estradiol, which does not normalize the LP spectrum in ovariectomized animals and even alters it adversely in experimental animals. Compound II possesses a less pronounced hypolipidemic activity in comparison with compound I; however, its effect on the serum LP spectrum is more favorable.

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Effect of Tuftsin on the Functional Activity and Intracellular pH of Murine Peritoneal Macrophages

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The effect of tuftsin and its tripeptide analog in various concentrations (from 0.001 to 10.0 μg/ml) on phagocytosis and on the intracellular pH is studied in murine peritoneal macrophages. Tuftsin causes a uniform dose-dependent increase of these two parameters in the cells. This effect is maximally pronounced at concentrations of the peptide close to its physiological level (about 0.3 μg/ml) and gradually decreases as its content in the incubation medium is lowered or raised. On the other hand, the tripeptide analog of tuftsin does not exhibit such an effect on the cells and under the same conditions suppresses phagocytosis and acidifies their intracellular medium.

Key Words: *tuftsin; phagocytosis; intracellular pH; macrophages*

Recently, increasing attention has been paid to the mechanism of action of the biologically active peptide regulators. Tuftsin, an endogenous tetrapeptide with a pronounced phagocytosis-stimulating activity, is among these. Despite a rather long history of studies, the molecular mechanism of its action upon the cell is far from clear [8]. A powerful tool for solving this problem is an analysis of the effect of the peptide on the major regulatory sys-

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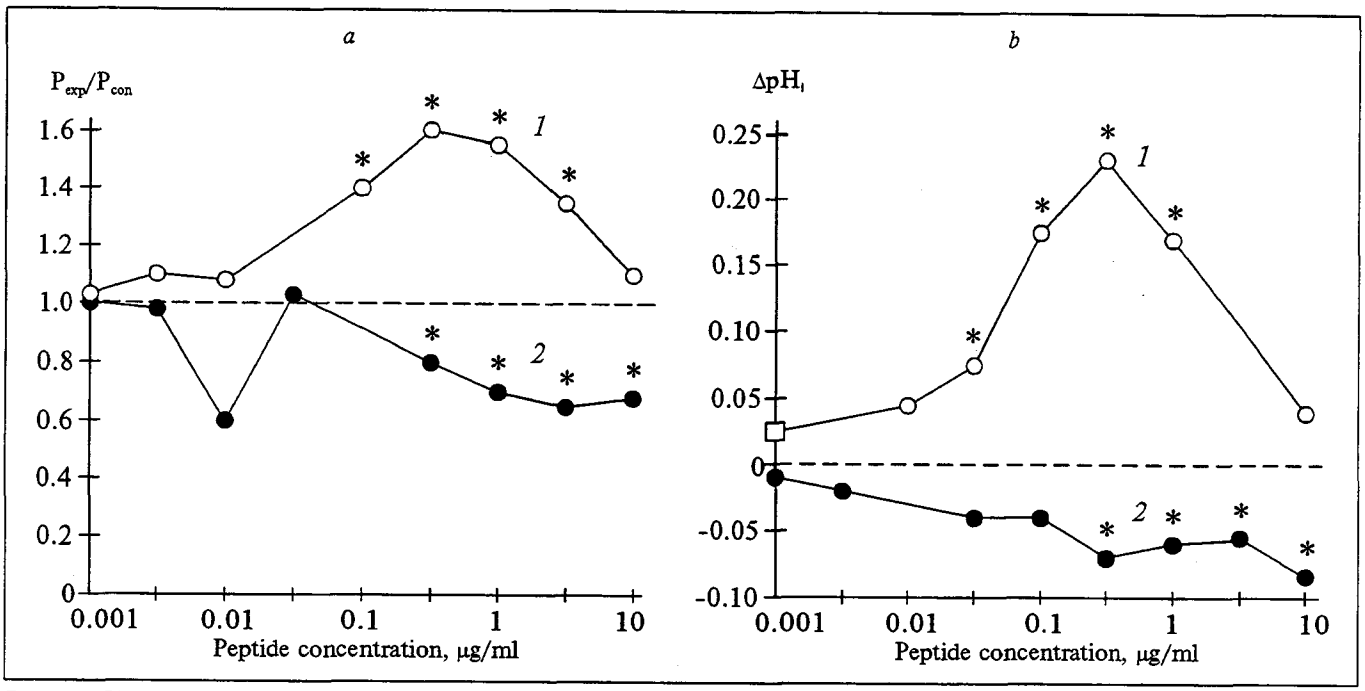

Fig. 1. Effect of various concentrations of tetrapeptide tuftsin (*1*) and its tripeptide analog (*2*) on level of phagocytosis (*a*) and pH_i (*b*) of murine peritoneal macrophages. P_{exp}/P_{con}: ratio between fractions of cells binding latex particles in the presence of peptide and in its absence. dpH_i: changes of intracellular pH of macrophages vs. the control. An asterisk shows the reliability ($p<0.05$) of differences.

tems of cell functions. Among them is the system regulating the intracellular pH, which is involved in different aspects of cell functions, is closely connected with other regulatory systems, and, in the opinion of some workers, may claim to be the crucial system of cell regulation [2,5,7].

In view of the foregoing, the aim of the present study was to investigate the effect of tuftsin and its tripeptide analog on the specific functional activity and intracellular pH of murine peritoneal macrophages.

MATERIALS AND METHODS

Male nonpedigree albino mice weighing 20-25 g were used in the study. Investigations were performed on stimulated peritoneal macrophages. They were obtained by injecting 2 ml of peptone solution (50 mg/ml) in the animals 72 h prior to isolation of cells. Immediately after the animals had been sacrificed, macrophages were obtained by washing the cells out of the peritoneal cavity with Hanks solution containing 10 mM HEPES (pH 7.2) (2 ml per animal). The concentration of cells in the suspension obtained therewith was adjusted to 10^6 cells/ml. The suspension in a volume of 50 μl was placed on a cover slip and incubated for 20 min in a humidity chamber; the cover slips were then washed in Hanks solution to remove nonadherent cells. From then on, all the procedures with cells were performed at 22°C. After 15 min of adaptation, the preparations of glass-adherent peritoneal macrophages were placed in incubation chambers; after a 30-min incubation with peptide they were washed with Hanks solution and used in the experiments.

Tetrapeptide tuftsin (TKPR) and its tripeptide analog (KPR), obtained at the Department of Chemistry of Natural Compounds (State University, Leningrad), were used in the study.

The phagocytic activity of macrophages was measured by the latex-binding test (diameter of latex particles 1.13 μ). For this purpose, a suspension of particles in Hanks solution (some 3×10^7 particles per ml) in a volume of 100 μl was added to a monolayer of cover slip-adherent cells and, after a 30-min incubation in the humidity chamber, the preparations were washed free of unbound particles. The cell preparations were then air-dried, fixed in methanol (5 min), and stained after Romanovskii-Giemsa. The phagocytic activity of macrophages in the preparation was determined by quantitating the percentage of macrophages, binding a minimum of three latex particles.

The intracellular pH of macrophages was measured microfluorometrically with fluorescein diacetate [1]. Fluorescein, a luminescent pH indicator, was introduced in the cells by incubating macrophages with fluorescein diacetate (in a final concentration of 5 μg/ml) for 15 min. After the

unbound indicator had been washed out, the cover slips were placed in a specially designed chamber filled with Hanks solution, and the intensity of fluorescence of individual cells was measured at wavelengths 520 and 570 nm with a LYuMAM-IZ luminescent microphotometer. The intracellular pH was determined with the use of calibration curves. The technique was previously described in detail elsewhere [3]. In our experiments the intracellular pH of macrophages in the control was 7.18±0.01.

The results were statistically processed. The data obtained were presented as the sum of the arithmetic mean and mean square deviation of the parameters.

RESULTS

Figure 1 presents the results of studies of the effect of tetrapeptide tuftsin and its tripeptide analog on the level of phagocytosis and on the intracellular pH (pH_i) of murine peritoneal macrophages. Interaction of tuftsin with the cells resulted in a dose-dependent increase of their phagocytic activity (P_{exp}/P_{con}). The maximally pronounced phagocytosis-stimulating effect of the peptide (almost 1.6-fold) was observed at tuftsin concentrations of about 0.3 μg/ml, this closely approaching the physiological level of the peptide in the blood of healthy persons (0.26 μg/ml) [8]. When the concentration of peptide in the incubation medium was raised or lowered, its phagocytosis-stimulating effect gradually waned, almost fading away at concentrations of 0.01 and 10 μg/ml. The data presented in Fig. 1 also demonstrate that changes in the pH_i of macrophages are dose-dependent, being similar to the pattern of changes in the level of phagocytosis. It is evident that tuftsin causes an increase in the pH_i, which is most marked (by some 0.23 unit of pH) in the range of the concentrations eliciting the maximum phagocytosis-stimulating effect of the peptide.

A study of the effect of the tripeptide analog of tuftsin on phagocytosis in macrophages produced an entirely different picture. Beginning with a concentration of about 0.1 μg/ml, the tuftsin analog gradually lowered the level of phagocytosis, which constituted some 70% of the control for peptide concentrations from 1.0 to 10.0 mg/ml. The analog exerted a similar effect upon pH_i, gradually lowering it (a decrease by 0.1 unit of pH being attained) when the concentration of the test agent was raised.

Analysis of our findings pinpoints a similarity of the concentration dependence of peptide-induced changes for the functional activity of marophages to that for pH_i. The tuftsin-induced increase in the level of cell phagocytosis is attended by an increase in pH_i, whereas the decrease in the level of phagocytosis caused by the tripeptide analog of tuftsin results in a drop of pH_i. Our results further corroborate the common pattern noted in diverse types of the cells, whereby an increase or decrease of pH_i (within the physiological range) goes along with a corresponding change of the functional-metabolic activity of the cells [2,5,7].

The presence of a peak on the curves showing the level of phagocytosis and pHi as functions of the tuftsin concentration in the incubation medium is of great interest. Although there is a large body of evidence on the phagocytosis-stimulating effect of tuftsin [4,8,9], only in two reports (focused on such parameters of macrophages as phagocytosis of IgG-covered sheep erythrocytes [6] and the formation of superoxide anion [10]) have we found indications that, once the maximum effect has been attained, raising the concentration of peptide mitigates its stimulating effect. At the same time it should be mentioned that, according to these data, the maximum stimulation of phagocytosis was observed for a tuftsin concentration almost one order of magnitude lower than in our case (0.025 μg/ml), whereas the maximum stimulating effect of the peptide with respect to O_2 formation in macrophages was recorded at a concentration of agent of about 0.188 μg/ml, which was close to that observed in our experiments.

The direct correlation between the peptide-induced effects, with respect to their intensity and direction, on phagocytosis and on the level of pH_i in macrophages implies that the intracellular pH contributes to the biological effect of tuftsin and its tripeptide analog.

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