Effect of Carnosine on Intracellular pH in Cultured Human Embryo Pulmonary Fibroblasts

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Carnosine, a dipeptide known to contribute to the regulation of repair processes [13], has been shown to improve the healing of skin wounds, burns, trophic ulcers, and surgical wounds [6]. This dipeptide proved more effective than other bioactive compounds (dalargin, tryptophan, insulin, nerve growth factor) in promoting the healing of a stab-and-slash wound in the lung of a guinea pig [2]. Local injection of the drug into the wound area enhanced healing and led to minimal cicatricial changes. Numerous fibroblasts with intracellular organelle hyperplasia were observed in a surviving pulmonary tissue culture and a 5-7-fold increasse of the proliferation of these cells in comparison with the control [3]. This necessitated a study of the intracellular processes which boost fibroblast proliferative activity under the influence of carnosine. A relationship exists between cell proliferation and the intracellular pH [12]. Studies of the effects of growth factors, regulatory peptides, and certain drugs on the multiplication of cultured cells showed that the earliest of all cell surface reactions was the activation of Na^+, H^+ metabolism, resulting in cytoplasm alkalization. This may promote the passage of cells into the S phase and their subsequent division [13].

The present study focused on changes in the intracellular pH in cultured human embryo pulmonary fibroblasts as one of the components of the mechanism of action of carnosine.

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MATERIALS AND METHODS

The object of investigation was a monolayer diploid culture of human embryo pulmonary fibroblasts (HEPF) obtained from strain library at the Institute of Virology of the Russian Academy of Sciences. Cells were grown on slides in penicillin vials in Eagle's medium with 10% embryonal serum and antibiotics at 37°C. Intravitam cell pH changes were measured in individual cells using fluorescein diacetate (FDA) in a concentration of 1×10^{-5} M on a cyto-pH-fluorometer produced by the histology department of the Biological Faculrt of the M. V. Lomonosov Moscow University [5]. When FDA penetrates into cells it is hydrolyzed in them to fluorescein, whose fluorescence spectrum depends on the pH. The ratio between the intensity of fluorescein fluorescence at wavelengths 518 and 570 nm was transformed into pH values using calibration curves obtained as a result of fluorometry of fluorescein solutions in buffers with a pH from 5.8 to 7.6. FDA was added to the incubation medium for 1.5 min, and then the preparation was washed in a fresh portion of conditioning medium. The slide with the culture was removed from the vial and mounted with the cell down on a special chamber made from a slide with two strips of the same glass glued to it 10 mm

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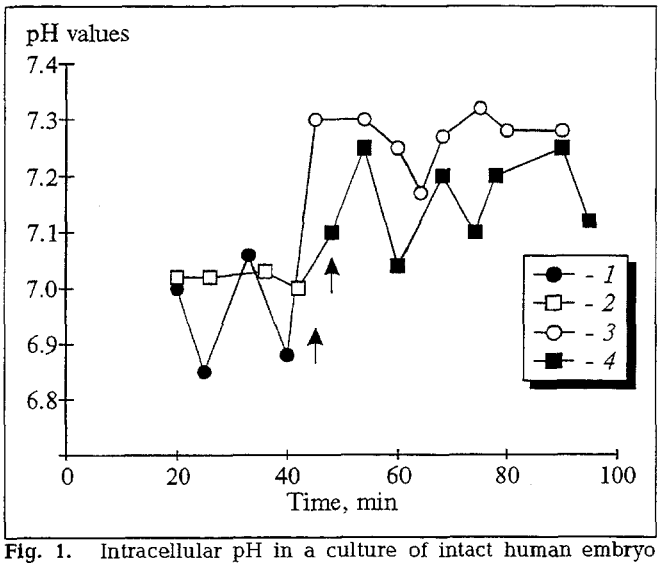

Fig. 1. Intracellular pH in a culture of intact human embryo pulmonary fibroblasts. *1*) slide 1; *2*) slide 2; *3*) after the addition of 10 μg/ml and *4*) after the addition of 20 μg/ml purified carnosine. The arrow shows the moment of carnosine addition. Dots on the diagram represent mean value for 5 cells measured in succession.

apart [4]. This permitted the introduction of medium or necessary reagent to the cells under the slide, delivering them with a strip of filter paper applied to the opposite end of the chamber without removing the preparation from under the microscope. The intracellular pH was measured for 20-30 min in intact preparations (control) and for one hour after the original medium was replaced with the carnosine-containing medium (1, 5, 10, 20, 40, 50, 100, and 150 μg/ml) on days 1, 2, 3, 5, and 7 of culture growth.

Carnosine purified to 98% and unpurified with 13% admixtures (mainly histidine, alanine, tyrosine, and metals: iron, copper, lead) were used in the experiments.

RESULTS

On day 1 the control fibroblast population was characterized by weakly acid intracellular pH values (6.9±0.04), which may be attributed to the as yet incomplete process of cell spreading on the glass and to adaptation of the cell culture. By day 2 of fibroblast culture growth the intracellular pH values had increased to 7.04±0.03 on average, as a greater number of cells were in the S phase (the differences were significant, p=0.01). Other authors have reported an increase of the intracellular pH in the S phase [10]. When the fibroblasts progressed to the stationary growth phase, the population's intracellular pH gradually dropped from day 3 to day 5 from 6.89±0.03 to 6.75, respectively.

Addition of purified carnosine in a concentration of 1 μg/ml to the fibroblast culture induced no changes in the intracellular pH. A concentration of 5 μg/ml raised the pH by 0.08 U on average over the first 8 minutes. When the dipeptide was used in a concentration of 10 μg/ml, the maximal alkalization effect was attained: by 0.22 U on average during the first 6 min of observation (Fig. 1). An increase of the dipeptide concentration from 50 to 100 μg/ml resulted in a less intensive cellular alkalization in comparison with the previous concentration: by 0.1 U in the first 8 min of observation.

Similarly, no changes in the intracellular pH were observed when 1 μg/ml of unpurified carnosine was added to the culture. Concentrations of 5-20 μg/ml induced different reaction: acidification, alkalization, or no effect. In seven cases the cellular pH dropped by 0.11 U on average within the first 4 min of observation (Fig. 2), and in four cases the addition of 5-20 μg/ml dipeptide raised the pH by 0.15 U on average during the first 5 min. No effect was observed in four cases. Concentrations from 40 to 150 μg/ml lowered the intracellular pH by 0.12 U on average during the first 6 min of observation in the majority of cases. When the carnosine content in the incubation medium was increased to 100-150 μg/ml, the acidification effect was not enhanced and the intracellular ΔpH was 0.1 U during the first 5 min of observation. Interestingly enough, the addition of carnosine in concentrations of 5, 50, and 100 μg/ml induced in some experiments a biphasic cellular reaction of the acidification-alkalization type. The

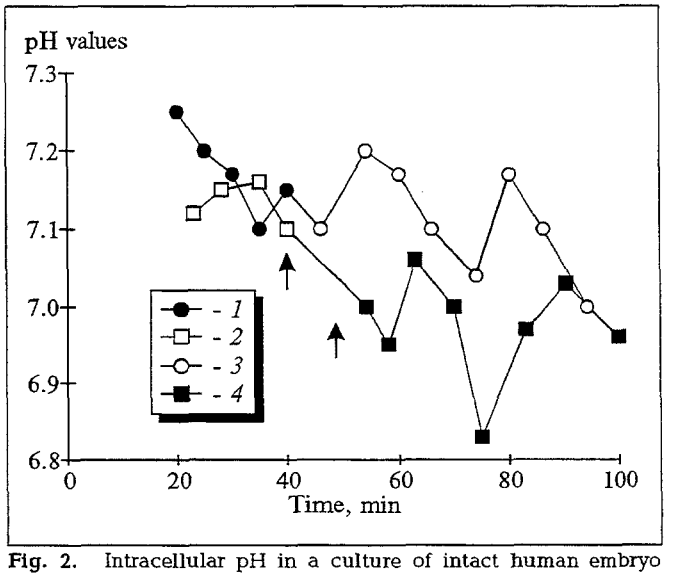

Fig. 2. Intracellular pH in a culture of intact human embryo pulmonary fibroblasts. *1*) and *2*) as in Fig. 1; *3*) after the addition of 10 μg/ml unpurified carnosine; *4*) after the addition of 50 μg/ml unpurified carnosine.

intracellular pH rose in these cases an average 11 min after the addition of the drug.

These data suggest a carnosine-effected increase of fibroblast intracellular pH starting from the very first minutes of observation, this reflecting early changes in cultured cells developing in response to the addition of peptide growth factors [11]. The degree of intracellular pH changes in the fibroblasts depended on the carnosine concentrations, lower concentrations being more effective [7]. Some authors report, however, that increasing the growth factor concentrations results in a still more intensive pH rise [1]. However, in these cases the doses used were several orders lower than in our experiment. The fact that unpurified carnosine induced various types of responses is explained by the presense of metal umpurities in the preparation, which resulted in a nonspecific cellular reaction: acidification. The rise of the intracellular pH which occurred immediately or after a brief acidification period in the experiments with low doses of carnosine was most likely due to the direct effect of the dipeptide. The concentrations of impurities in the preparation in this case were rather low and the cells' adaptability was sufficiently high. When higher doses were added, the amount of impurities could no longer be of no consequence to cells and caused a prolonged acidification.

Hence, the carnosine-induced rise of the fibroblast intracellular pH may be one of the causes of activated proliferation. Cellular pH-metry may be used as a rapid test to assess the degree of purity of a preparation.

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