Biological Activity of Amino Acids in Organotypic Tissue Cultures

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We studied the effect of 20 standard L-amino acids on proliferation of the nervous, cardiovascular, urogenital, digestive, and immune system tissues from young and old animals in organotypic cultures. The effect of amino acids on tissue culture proliferation depended on their origin and animal age.

Key Words: *organotypic culture; amino acids; bioactivity*

The maintenance the biological integrity of the body at the cellular level is regulated by signals preserving complex balance between the two major physiological processes, proliferation and programmed cell death. The cell-cell signaling at the para- and autocrine levels is regulated by signaling molecules, cytokines and cytomedins, peptide bioregulators maintaining the cell renewal homeostasis. These peptides, in turn, are hydrolyzed to amino acids also acting as regulators of cell proliferation and apoptosis [7,9,12,14].

Organotypic culturing of tissue fragments is the most adequate method of rapid evaluation of the effect of bioactive substances [3,4-6]. Changes in cell proliferation can be a primary integral indicator for bioactivity assessment of the test substance and the reason for testing its other effects.

Here we performed screening study of the effect of 20 L-amino acids on cell proliferation in organotypic cultures of the nervous, cardiovascular, urogenital, digestive, and immune system tissues from young and old animals.

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MATERIALS AND METHODS

Organotypic culturing was performed as described elsewhere [1,6]. A total of 4800 organotypic cultures of ectodermal (cerebellum, cerebral cortex, and subcortical structures), mesodermal (heart, cartilage, prostate, testes, skin, and spleen), and endodermal (pancreas, and liver) tissues from young (3 months) and old (24 months) Wistar rats. Tissue samples isolated under sterile conditions were cut into small fragments (~1 mm^3) and transferred to collagen-coated Petri dishes (Biolot). The nutrient medium contained 35% Eagle's medium, 35% Hanks solution, 25% fetal calf serum, 0.6% glucose, 0.5 U/ml insulin, and 100 U/ml gentamicin.

Cultures of all tissues were divided into two groups: control and experimental (addition of one of 20 amino acids). We used L- and D-amino acids (Sigma): glycine (Gly), alanine (Ala), asparagine (Asn), histidine (His), lysine (Lys), serine (Ser), glutamine (Gln), arginine (Arg), proline (Pro), aspartic (Asp) and glutamic (Glu) acids, tyrosine (Tyr), cysteine (Cys), valine (Val), threonine (Thr), methionine (Met), leucine (Leu), isoleucine (Ile), phenylalanine (Phe), and tryptophan (Trp). For evaluation of effective concentrations, the amino acids were added to the culture medium in a concentration range of 0.01-10 ng/ml.

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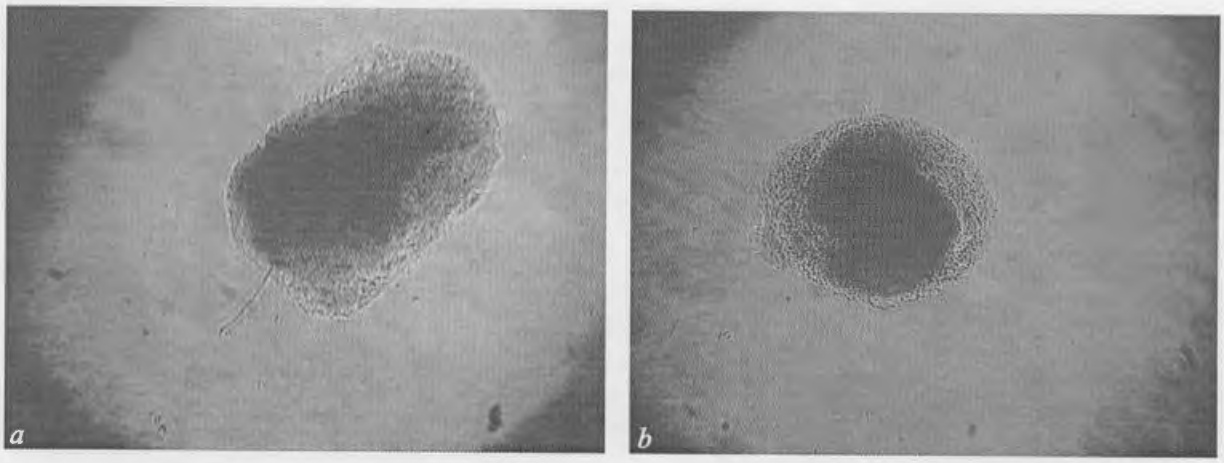

Fig. 1. Vital microscopy of explants of brain cortex from old rats on day 3 in culture, ×70. *a*) control, *b*) addition of 0.05 ng/ml leucine, vital light microscopy. Central dark fragment is the explant and cell monolayer around it is the growth zone.

The concentration of 0.05 ng/ml was effective for all amino acids. Petri dishes with cultures and 3 ml culture medium were incubated for 3 days at 37°C and 5% CO_2 and then were examined under a phase-contrast microscope.

For evaluation of proliferation in cultures, area index (AI) was calculated as the ratio of the total explant area (including the zone of the area of its central zone). The explants were visualized using a microtelevision attachment (series 10, MTN-13, Alpha-Telecom). AI was calculated using PhotoM 1.2 software. For each substance, 20-25 experimental and 20-23 control explants were analyzed. The type of distribution was determined using Shapiro–Wilk test; for verification of statistical homogeneity of samples, nonparametric univariate analysis of variance (Kruskal–Wallis test) was used. AI in the control was taken as 100%.

RESULTS

On day 1 of culturing, flattening of the explants on the collagen substrate and dislocation of proliferating and migrating cells constituting the growth zone from the explant edge were observed. In the peripheral zone of the explants from different tissues, the peripheral growth zone and the explant capsule represented by 1-2 layers of fibroblasts that do not form a continuous layer. The growth zone consisted of migrating and

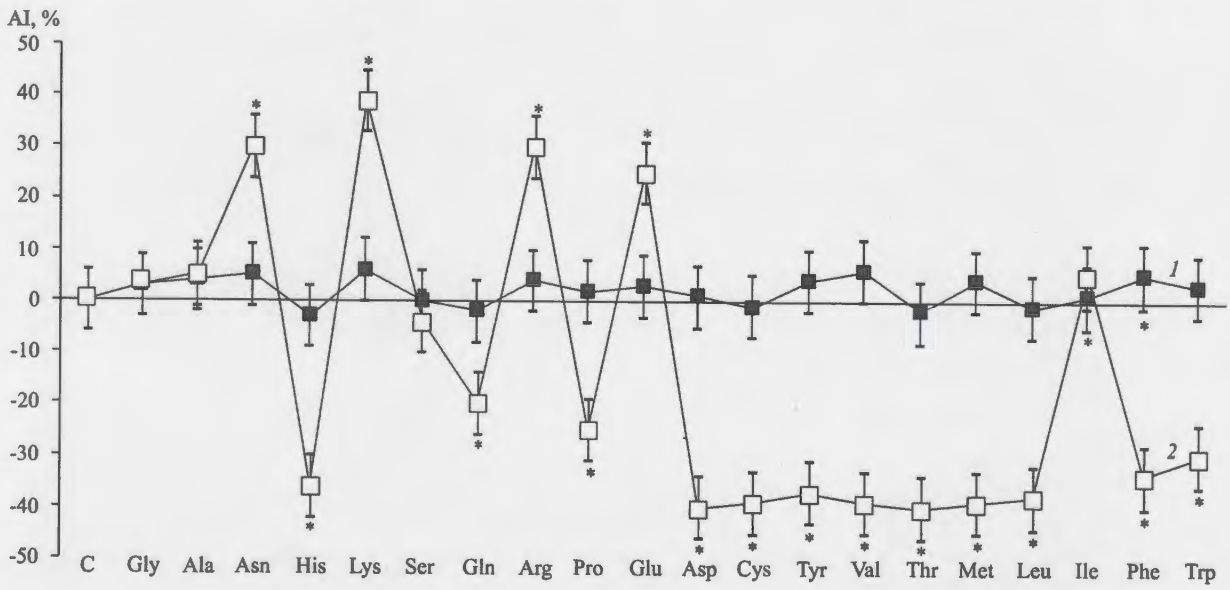

Fig. 2. Effect of L- and D-amino acids on AI in organotypic culture of the spleen from young Wistar rats. Zero line: control (C); *1*) D-amino acids; *2*) L-amino acids. *$p<0.05$ in comparison with the control.

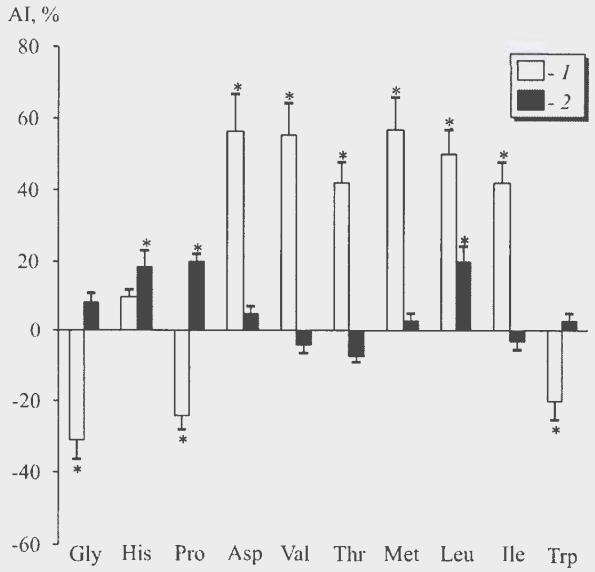

Fig. 3. Effect of amino acids on cell proliferation in organotypic cultures of the brain cortex from young and old rats. Here and in Figs. 4 and 5: *1*) tissue explants from young rats; *2*) tissue explants from old rats. *$p<0.05$ in comparison with AI of control explants.

proliferating tissue-specific cells, as was shown earlier in histological and immunocytochemical studies [1]. In 3 days, AI of experimental explants increased in comparison with AI of control explants in case of stimulation of cell proliferation in the growth zone (Fig. 1) and decreased in case of its suppression.

Specificity of the effect of L-amino acids in organotypic cultures of rat tissues was confirmed by studying the effect of 20 D-amino acids in a concentration of 0.05 ng/ml on the proliferative processes (Fig. 2). None of the tested D-amino acids affected the explants in the tissue culture: AI remained at the control level in all cases.

Thus, only standard L-amino acids can modulate cell proliferation in organotypic cultures of various tissues, which agrees with previous reports [8].

The changes in AI produced by L-amino acids were different in cultures of different tissues. In the nervous tissue of the cortex, subcortical structures, and cerebellum (ectodermal tissues), cell proliferation was stimulated by hydrophobic amino acids Asp, Val, Thr, Met, Leu, Ile (Fig. 3), while acidic amino acids with changed side chain (Glu, Asp, Arg, Lys) stimulated proliferation of endo- and mesodermal tissue cultures (Figs. 4 and 5).

It was found that the cell response to amino acids depended not only on the tissue genesis, but also on its regeneration potential. In tissues with high regeneration potential, *e.g.* spleen and liver (tissues of meso- and endodermal genesis, respectively), in contrast to the nervous tissue (ectodermal genesis), hydrophobic amino acids suppressed proliferation (Figs. 2 and 4). Continuous renewal of the cell composition of these organs requires constant cell elimination. The picture observed in the postmitotic myocardium (tissue of mesodermal genesis characterized by the absence of cell division in postnatal ontogenesis), differed from that in tissues with high regeneration potential. The growth of myocardial explants was stimulation in the presence of Asp, His, Lys, Ser, Arg, Glu, Ile in the culture medium (Fig. 5). Addition of other amino acids to the culture medium had no effect on cell proliferation in myocardial tissue culture and hence, none of them produced the inhibitory effect on postmitotic myocardial tissue.

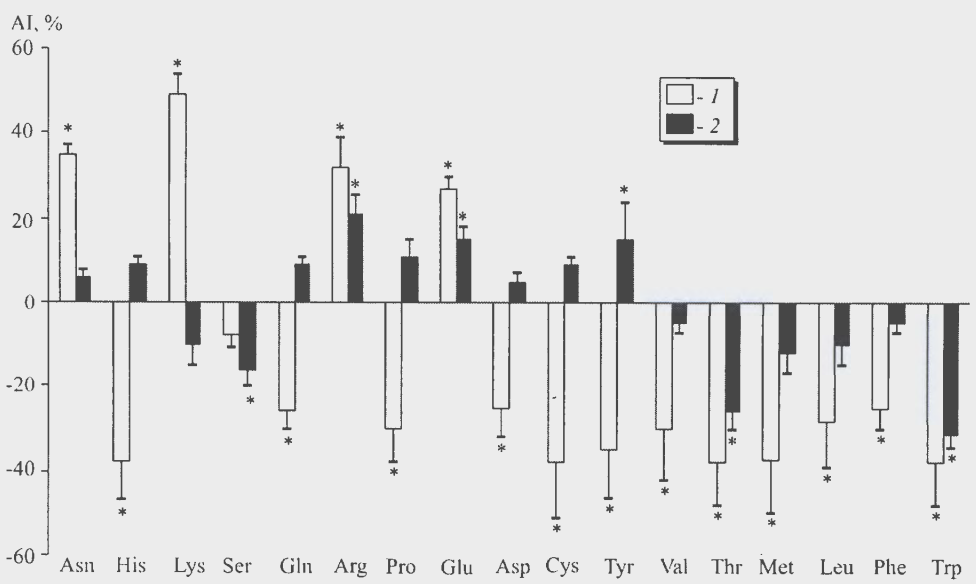

Fig. 4. Effect of amino acids on cell proliferation in organotypic cultures of the spleen from young and old rats.

Significant differences were observed in the influence of amino acids on tissues from young and old animals. In tissues obtained from old rats, the number of stimulating amino acids decreased, while in tissues from young animals cell proliferation was stimulated by all amino acids, except Ala and Phe. In tissues obtained from old rats, four amino acids produced no stimulating effect: Ala, Asp, Val, and Met.

When the frequency of amino acids was taken into account, the number of amino acids increasing AI in the tissue explants old animals sharply decreased (Fig. 6). The most abundant amino acid Arg stimulated cell proliferation in 9 tissues from young animals and in 6 tissues from old rats. The differences in the action of Glu were even more pronounced: it increased AI in 6 tissues from young animals and in only one tissue from old animals. In tissue cultures from old animals, the stimulating effect of Pro and Asp was 4-fold less incident, Lys and Tyr 3-fold less incident, and Leu and Ile 2-fold less incident than in young tissues.

In organotypic cultures of tissues from old animals, each amino acid producing the stimulating effect on AI was 1.5-6.0-fold less abundant than in tissues from young animals. The fact that the number of amino acids actively modulating proliferation decreased in tissues of old rats suggests that the factors regulating cell proliferation should be replenished during aging. These factors include short peptides inducing proliferation and differentiation and suppressing apoptosis in tissue cultures during aging [7,12,13]. The effect of short peptides, as well as some amino acids, is based on their penetration into the cell cytoplasm, nucleus, and nucleolus and interaction with DNA and RNA [2,10]. It was demonstrated that the interaction of short peptides with DNA double helix is determined by electrostatic interaction of polar side chains of the peptides with sugar-phosphate DNA backbone and the formation of hydrogen bonds with peptide carboxyl and amino groups with nucleic bases of DNA [2,11].

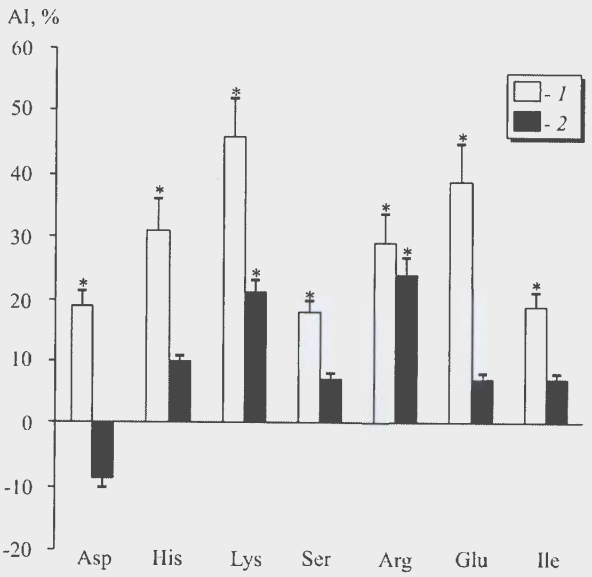

Fig. 5. Effect of amino acids on cell proliferation in organotypic cultures of the myocardium from young and old rats.

Thus, the most abundant amino acids Lys, Arg, Glu, Leu, Ile stimulating proliferation in organotypic cultures of tissues from old animals provide the basis for the synthesis of new short peptides intended for stimulation of the regeneration processes in tissues during aging.

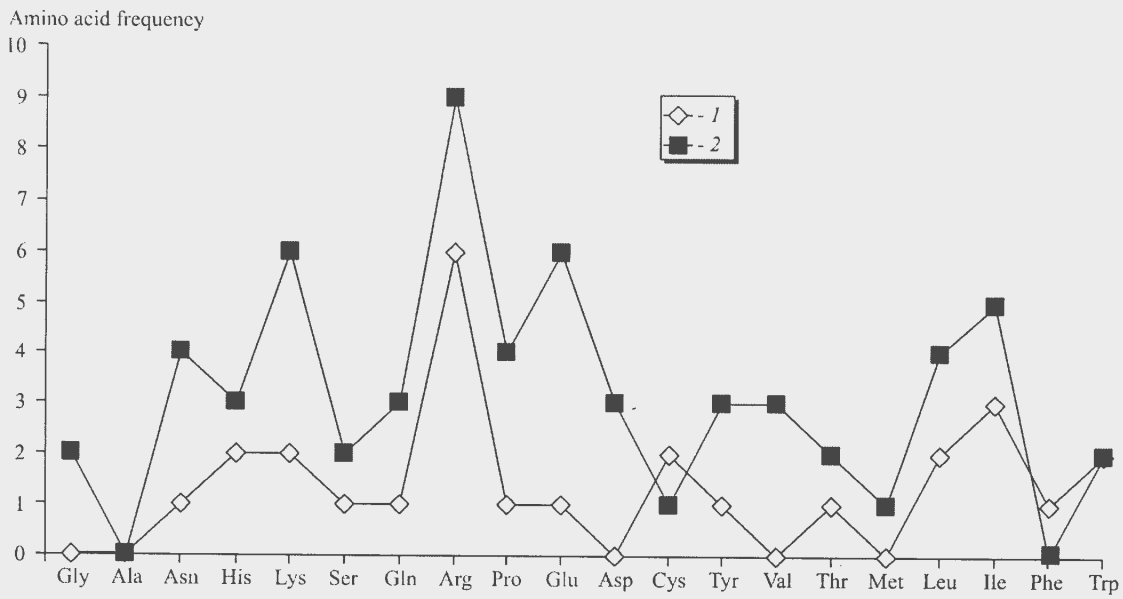

Fig. 6. Frequency of the stimulating effects of amino acids on cell proliferation in organotypic cultures from young (*1*) and old (*2*) rats.

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