BIOGERONTOLOGY

Effect of Amino Acids and Antibodies against Nerve Growth Factor Receptors on the Development of Organotypic Culture of Lymphoid Tissue

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We studied the effects of 20 L-amino acids on organotypic culture of splenic lymphoid tissue from 3-month-old rats were studied in the presence of apoptosis-inducing monoclonal antibodies against low-affinity receptors for nerve growth factor NGFRp75. The influence of amino acids stimulating cell proliferation in explants (lysine, asparagine, and glutamic acid) did not depend on NGFRp75. Hydrophobic amino acids inhibiting the growth zone in isolated application and abolished the inhibition of explant development in the presence of antibodies against NGFRp75. These amino acids can mediate the pro-apoptotic effect on lymphoid tissue via low-affinity receptors for nerve growth factor.

Key Words: *lymphoid tissue culture; amino acids; nerve growth factor receptors*

The regulation of reparative processes in tissues via stimulation or inhibition (apoptosis) of cell proliferation involves various proteins, including growth factors and oligopeptides [2,7,8,11]. Published data suggest that amino acids (AA) also play a role in these processes [1,5,13]. Experiments on PC3 and DU145 cells of androgen-independent prostate cancer showed that methionine deprivation activated apoptosis in PC3 cells, while deprivation of tyrosine and phenylalanine primarily affected DU145 cells [13]. The stimulatory or inhibitory (increased apoptosis) effect of AA with charged side chain (lysine, arginine, asparagine, and glutamic acid) in organotypic culture of rat spleen depends on tissue maturity [3,4]. Organotypic culturing of tissues is one of the most reliable methods for screening of bioactive substances, because the criterion of bioactivity is changes in cell count in the explant growth zone due to cell proliferation or apoptosis [3,5,15].

Low-affinity receptors for nerve growth factor (NGF), NGFRp75, are located on lymphocytes and mononuclear cells from the spleen and lymph nodes. They are structurally similar to receptors for tumor necrosis factor [6,10]. It was hypothesized that NGFRp75 modulate the function of high-affinity tyrosine kinase receptors for NGF. Recent experiments with monoclonal antibodies and antisense oligonucleotides showed that NGFRp75 can also induce apoptosis in nerve and lymphoid tissues by regulating the expression of genes for Bcl-2, Bcl-xl, and Fas [9,12,14,15].

In the present work, the effects of 20 L-AA in combination with monoclonal antibodies against low-affinity NGFRp75 were studied on the culture of mature lymphoid tissue to reveal the existence of common signal pathways.

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MATERIALS AND METHODS

Experiments were performed on the organotypic culture of 400 explants from the spleen of 3-month-old Wistar rats. Spleen fragments (1 mm^3) were prepared under sterile conditions and placed in collagen-coated Petri dishes. The nutrient medium contained 35% Eagle medium, 35% Hanks solution, 25% fetal bovine serum, 0.6% glucose, 0.5 U/ml insulin, and 100 U/ml gentamicin. L-AA (Sigma) were added to the culture medium. Titration showed that the effective concentration of all AA in the culture of lymphoid tissue was 0.05 ng/ml. The area index (AI) was statistically insignificant or did not differ from the control in the presence of L-AA in lower or higher concentration (up to 5 ng/ml). Further increase in the concentration of L-AA to 10-20 ng/ml was accompanied by inhibition of growth. These changes were related to the damaging effect of the test substances in high concentrations (even upon stimulation with AA in a concentration of 0.05 ng/ml). Mouse monoclonal antibodies against NGFRp75 (DacoCytochromation) were administered in an effective concentration of 200 ng/ml. Antibodies in this concentration abolished the stimulatory effect of 20 ng/ml NGF. The nutrient medium (3 ml) and the test substances in the specified concentration were added to Petri dishes with experimental explants and the nutrient medium alone (3 ml) was added to Petri dishes with control explants. Hence, the explants of both groups developed in a similar volume of the medium. Petri dishes were maintained in a thermostat at 37°C and 5% CO_2 and examined under a phase contrast microscope after 3 days.

AI was calculated as the ratio of the entire explant area (including the zone of migrating cells) to the area of the central zone of the explant. Explants were visualized using a microscope equipped with a microtelemetric eyepiece (series 10, MTN-13, Al'fa-Telekom). AI was calculated by means of PhotoM 1.2 software. Each substance was examined on 20-25 experimental explants and 20-23 control explants. The significance of differences in AI for the control and experimental explants was estimated by Student's *t* test.

RESULTS

Spreading of the explants on the collagen substrate and transfer of proliferating and migrating lymphocytes and macrophages from the marginal area of the explant in the growth zone occurred on day 1 of culturing (Fig. 1). AI of experimental explants exceeded the control on day 3 of the study (only upon stimulation of the development of the growth zone). During suppression of the development of the growth zone, AI was below the control level.

Addition of antibodies against NGFRp75 in effective concentration to the culture medium decreased the growth zone of spleen explants by 30±3% (n=21, p<0.05) compared to the control (n=23).

Administration of AA to the culture medium increased or decreased AI (Table 1). AI increased

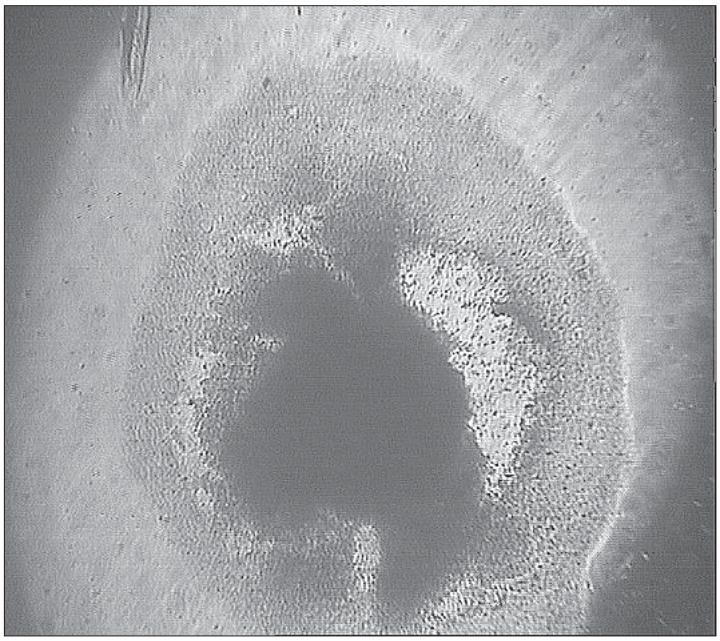

Fig. 1. Vital microphotography of spleen explant and growth zone on day 3 of culturing in the presence of 0.05 ng/ml lysine. Phase contrast microscopy, ×70.

TABLE 1. AI of Rat Spleen Explants after Administration of AA Alone or in Combination with Antibodies against NGFRp75 (*M*±*m*)

AA	AI (%) after treatment with	
	AA	AA+antibodies against NGFRp75
Glycine	2±1	12±3
Alanine	2±1	5±1
Asparagine	34±4*	28±5*
Histidine	-35±7*	23±3*
Lysine	40±5*	30±7*
Glutamine	-20±3*	25±3*
Serine	-8±1	11±4
Arginine	35±7*	-20±3*
Proline	-22±2*	44±5*
Glutamic acid	28±6*	34±6*
Aspartic acid	-28±3*	-12±3
Cysteine	-30±5*	4±1
Tyrosine	-29±2*	-14±3
Valine	-35±5*	6±2
Threonine	-35±7*	12±5
Methionine	-35±3*	12±6
Leucine	-34±5*	10±4
Isoleucine	-5±1	11±5
Phenylalanine	-35±7*	6±2
Tryptophan	-28±5	5±3

Note. *$p<0.05$ compared to the control (0%).

after treatment with lysine, arginine, asparagine, and glutamic acid in effective concentrations (Table 1). Glycine, alanine, isoleucine, and serine had no effect on the development of explants. Under these conditions, AI did not differ from the control. The remaining AA (histidine, proline, and hydrophobic AA) significantly suppressed the development of the explant growth zone. The area of growth zone significantly decreased after administration of valine, threonine, or methionine to the culture medium. These changes manifested in a decrease in AI compared to the control (Table 1).

Other results were obtained after combined administration of AA and antibodies against NGFRp75 to the culture medium. Asparagine, lysine, and glutamic acid also produced a stimulatory effect in the presence of antibodies against NGFRp75. AI increased by 28-34% compared to the control. Hence, administration of these AA alone or in combination with antibodies against NGFRp75 produced a similar effect. The exception was arginine. Individual treatment with arginine significantly increased AI of the explants. However, AI decreased after combined administration of arginine and antibodies against NGFRp75 (Table 1).

Glycine, alanine, isoleucine, and serine had no effect on explant development in the presence of antibodies against NGFRp75. Under these conditions, AI did not differ from the control.

Antibodies against NGFRp75 modified the effect of those AA that inhibited the growth zone under conditions of individual administration to the culture of lymphoid tissue. For example, the inhibitory effect was abolished after administration of hydrophobic AA (aspartic acid, cysteine, tyrosine, valine, threonine, methionine, leucine, phenylalanine, and tryptophan) in combination with antibodies against NGFRp75 to the culture medium. Under these conditions the growth zone of the explants was comparable to the control. AI did not differ from the control. Individual treatment with histidine and proline was accompanied by inhibition of explant development. After combined administration, these AA significantly stimulated the growth zone. AI increased by 23-44%.

Our results suggest that the effect of AA stimulating cell proliferation (lysine, asparagine, and glutamic acid; except arginine) is not mediated by low-affinity receptors for NGF. These AA with a charged side chain and low hydrophobicity may be considered as the simplest regulators of physiological function in cells and tissues. They are most abundant in repeating sequences of protein structures [1,2,12]. We believe that regulatory activity of these AA does not involve growth factors. By contrast, antibodies against NGFRp75 abolish the inhibition of explant development under the influence of those AA that decrease the growth zone after individual administration. AA not having a positively charged side group and situated at the end of the spectrum of increasing hydrophobicity (5.3-7.5 kJ/mol) probably mediate the proapoptotic effect via low-affinity receptors for NGF. Hence, the blockade of receptors with antibodies abolished the inhibitory effect of these AA in tissues.

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