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Activity of Nerve Growth Factor in Rat Regenerating Liver

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, № 3, pp. 345-347, March, 1996
Original article submitted April 18, 1994

Two bioassays of specific activity in the presence and absence of specific antiserum, incubation with pheochromocytoma PC-12 cells, proteolytic digestion, and ultrafiltration demonstrate that nerve growth factor isolated from the liver exhibits the basic characteristics of classic mouse nerve growth factor. High activity of this factor is observed in the operated lobe during the first day (3-20 hours) and on days 3-10 of regeneration, i.e., before and after the phase of hepatocyte proliferation.

Key Words: *nerve growth factor; regeneration of the liver; pheochromocytoma; organ cultures*

A few published data [6,8-10] suggest that exogenously injected nerve growth factor (NGF) accelerates the initial stages of regeneration in organs and tissues. This effect is most likely due to an increase in the innervation of the damaged area and the development of so-called hyperneuria [1]. However, endogenous (synthesized in the tissue) NGF, which may be obtained during posttraumatic regeneration of the liver [4], has not yet been studied.

The aim of the present study was to perform a physiological and biochemical analysis of isolated hepatic NGF and to elucidate its possible effect on the repair processes in the liver.

MATERIALS AND METHODS

Random-bred albino rats were divided into several groups. In group I during the first few hours (3, 8, 15, and 20) and on days 1, 3, 5, 7, 10, 14, 21, and 30 of posttraumatic regeneration of the liver (50% resection of the left lobe) an NGF-like substance was isolated and a comparative analysis of NGF activity in the liver lobes and in the serum was carried out with the treatment of selected samples (control and days 3 and 7) with anti-NGF antiserum. In group II NGF activity was measured in a modified organ culture of the liver. In group III NGF isolated from the liver was tested in a culture of pheochromocytoma PC-12 cells. In group IV the obtained factor was treated with pronase to determine its nature and subjected to ultrafiltration on PM-10 membrane filters. Each group of rats had respective controls.

Hepatic NGF was isolated by chromatography on sorbents produced at the Institute of Biochemistry of the Academy of Sciences of the Republic of Uzbekistan (Patent No. 1172114 issued by the Research Institute of the State Patent Expert Commission, USSR, 1982). The activity of the end product was biologically tested by culturing the spinal ganglia of 8-day-old chick embryos in the presence of the dissolved test sub-

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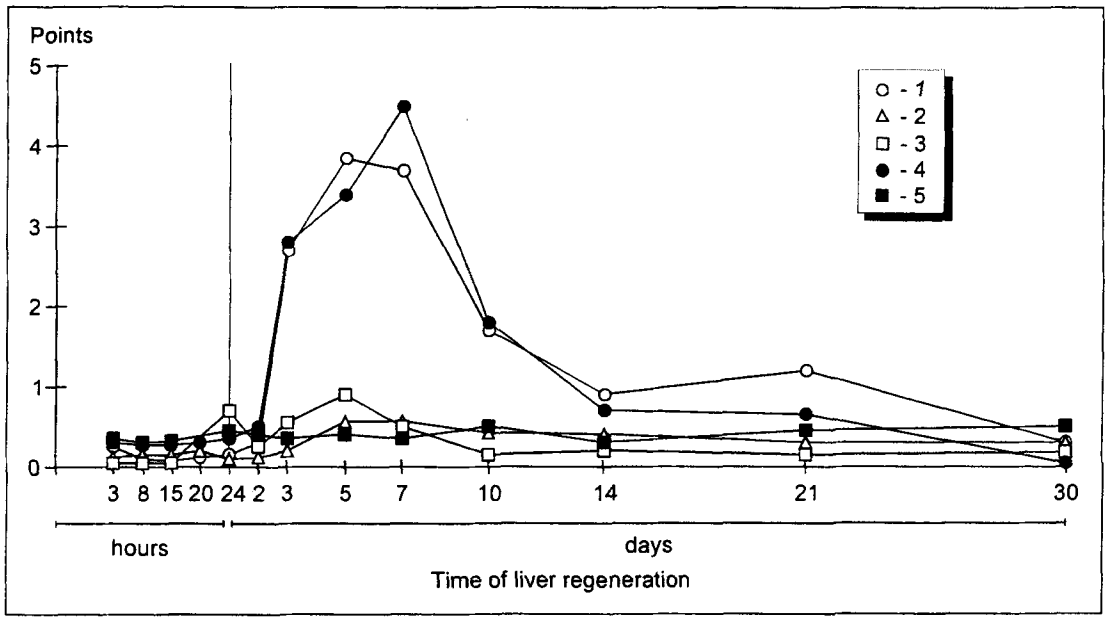

Fig. 1. Comparative analysis of nerve growth factor activity in dried fractions from the liver lobes and from the serum. *1*) left lobe; *2*) right lobe; *3*) central lobe; *4*) serum; *5*) intact liver.

stance using a technique described elsewhere [2]. The rate of growth was visually determined 18-24 hours later using a 5-point scale [5] by evaluating the length and branching of neurites, and their number. Anti-NGF antiserum was obtained from rabbits immunized with high-molecular mouse NGF in a dose of 40-60 mg/kg in Freund's complete adjuvant. The appearance of antibodies in the blood was detected by Ouchterlony double radial immunodiffusion. In group II chick spinal ganglia were cultured without the test substance but in the presence of a 1-2 mm^3 sample of regenerating liver so that one or several ganglia were placed on a collagen substrate at a distance of 1-2 mm from the bioptate. In series III pheochromocytoma PC-12 cells in RPMI-1640 medium were incubated for 3 days in the presence of a varying concentration of the test NGF (7 days postresection), after which the index of cells with processes was calculated.

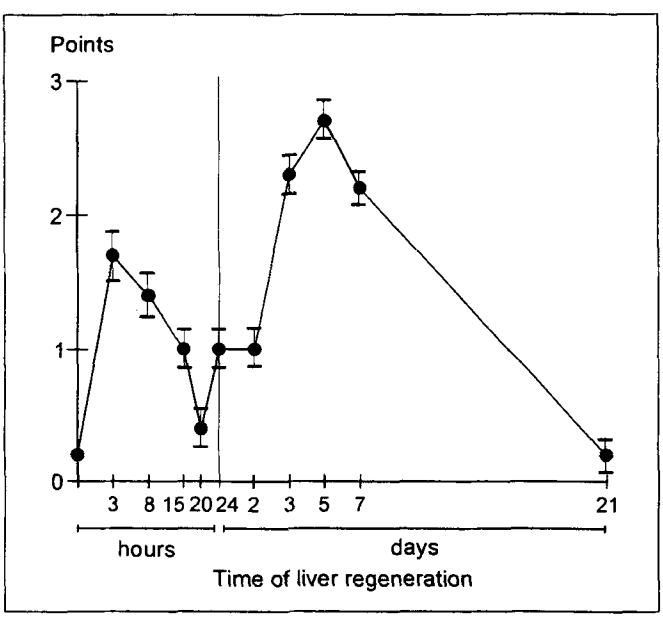

Fig. 2. Activity of nerve growth factor in bioassay of tissue liver explants.

RESULTS

Biological testing in group I revealed no NGF activity in the operated left or intact right and central lobes during the first hours and days postoperation (Fig. 1). But starting from day 3 the level of NGF-like factor in the regenerating lobe sharply increased and attained the maximum on days 5-7. The concentration of 8 μg/ml is optimal for achieving neurite-stimulating and neuronotropic effects of the fraction from regenerating liver. The biological activity of the dry substance was 70 biological units per milligram of preparation. Starting from day 10 NGF activity in the regenerating lobe fell off, returning to baseline on days 20-30. Practically no NGF activity was observed in the intact right and central lobes during the repair period but the very low values observed during the first 10 days increased by one order of magnitude. On the other hand, NGF activity in the blood was proportional to that in the liver (Spearman correlation coefficient 0.925) and there was a parallel rise and fall of the level of NGF-like factor during the main times of regeneration.

Experiments with cell culture demonstrated that anti-NGF antiserum blocked the neurite-stimulating activity of the NGF-like fraction isolated from the regenerating liver on days 3 and 7. This effect was observed not only for dry extracts of the liver but also when the antiserum was added to the culture medium

containing the NGF-like fraction isolated from the blood and submaxillary salivary gland at the same times.

Experiments in group II showed that culturing of explants from the liver with the spinal ganglia from 8-day-old chick embryos as a variant of organ culture is a more sensitive method than classical bioassay. First, the neurite "halo" around the ganglion had a tendency to grow toward the liver bioptate. Second, some NGF activity was observed during the first few hours of regeneration and induced a mixed glial and neurite growth around the ganglia. Thus, samples of the left lobe obtained during the first postoperative hours induced a weak NGF activity and strong glial growth around the ganglion. Starting from day 3, however, this mixed response gives way to neurite growth directed primarily toward the liver explant (Fig. 2). Anti-NGF antiserum added to this culture blocked the neurite-stimulating activity of NGF released by liver explants obtained on days 3 and 7 day postsurgery.

The response of cultured pheochromocytoma PC-12 cells to the addition of NGF-active fractions from regenerating liver to the medium was variously expressed. The maximal growth (14.5 and 14.4%) was induced by the fraction obtained from a 7-day liver regenerate (protein concentration 0.63 and 10 μg/well), the control index of neurite-bearing cells being 5.4% [7]. This index for the dry fraction from the submaxillary salivary glands of male mice, isolated by us on the sorbent, was also 14.4% at a protein concentration of 0.4 μg/well, while the index of neurite-bearing cells for the fraction from intact liver was within the control values.

Finally, proteolytic digestion of the liver fractions with pronase and ultrafiltration through a PM-10 membrane filter showed that NGF from the liver, similarly to the classic NGF from mouse submaxillary gland, is a protein but not a peptide compound.

Thus, two bioassays (organ culture and classic bioassay) in the presence and absence of anti-NGF antiserum, incubation with PC-12 pheochromocytoma cells, proteolytic digestion, and ultrafiltration demonstrated that nerve growth factor isolated from the liver possesses the main characteristics of classic mouse NGF.

TABLE 1. Cell Response of Pheochromocytoma PC-12 Culture Induced by Dry Fraction from the Liver and Salivary Glands

Concentration, μg protein/well	Index of neurite-bearing cells, %
Dry fraction from intact liver	
5	4.2±0.3
2	5.1±0.5
0.9	4.8±0.5
0.3	4.5±0.4
Dry fraction from mouse submaxillary salivary glands	
0.4	14.4±0.3
0.2	8.7±0.2
0.1	12.7±0.6
Dry fraction from regenerating liver, day 7	
20	cell death
10	14.4±0.5
5	12.6±0.8
2.5	11.6±0.2
1.25	13.1±0.4
0.63	14.5±0.4
0.31	7.8±0.5
0.16	5.6±0.3

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