GROWTH-STIMULATING ACTION OF BALIZ-2 ON SYMPATHETIC GANGLIA IN CULTURE

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UDC 615.275.015.4:616.834-003.9-092.4

KEY WORDS: neurotrophic factors; sympathetic ganglia; culture.

The preparation Baliz-2, a product of microbiological synthesis, has a distinct stimulating action on inhibited repair processes [1, 2]. It has been approved for use in medical practice as an antibacterial, wound-healing, and antiburn remedy. Analysis of the action of Baliz-2 on repair processes in nerve tissue requires the initial study of its affect on development and growth of peripheral ganglia in culture. It can be concluded from investigations on organotypical cultures of sympathetic and spinal ganglia [3-5] that this model may be successfully used as a test system for screening biologically active compounds.

Comparison of the action of Baliz-2 with that of such well known neurotrophic factors as the nerve-growth factors [10] isolated from brain extract [13], from injured nerve tissue [12], and from conditioned media [7, 9], and also with that of many other peptide neurotrophic factors [4, 5, 11] may provide fresh opportunities in the search for ways of restoring the structures and functions of injured nerve tissue.

EXPERIMENTAL METHOD

Experiments were carried out on 124 explants of sympathetic ganglia of newborn Wistar rats. Tissue was cultured by the method described previously [3, 4] on coverslips covered with collagen in 35-mm Petri dishes in 600 µl of growth medium. The Beliz-2 was used in concentrations of 0.01, 0.001, and 0.0001% (original commercial concentration 0.9%). The preparation was obtained in the Department of Biologically Active Substances, Research Institute of Physical and Organic Chemistry, and it consists of a mixture of organic keto acids.

Sympathetic ganglia were cultured for 4-7 days. Intravital observations on the time course of growth of the cultures were made on the 1st-4th days after transplantation. The cell composition of the zone of growth, its maximal size, and the density of axonal-glial bundles were estimated by the method described previously [4]. The intensity of development of the sympathetic ganglia was determined as the product of the maximal size of the zone of growth (in µ) and the density of the axonal-glial bundles. Density was estimated as the number of intersections of radially directed axonal-glial bundles in the zone of growth in an assigned segment (200µ) at a distance of 250 µ from the edge of the explant, on the 4th day of culture, and was then graded on the following scale: 1) under 10 intersections, 2) 10-30, 3) 30-50, and 4) over 50. The intensity of growth was thus determined in relative units. Axons were identified in the zone of growth by fluorescence-histochemical detection of catecholamines by condensation with glyoxylic acid [6]. The results of the measurements on intravital preparations were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The time course of development of the ganglia during the 1st day and the general pattern of the cell composition and size of the zone of growth by the 4th day in vitro in the control (Fig. 1a) were of the standard character for these conditions of culture and corresponded to processes described in the literature [8] and by the present writers previously [4-6]. In this

Department of Cellular Biology, Academy of Medical Sciences of the USSR, Moscow. Department of Biologically Active Substances, Research Institute of Physical and Organic Chemistry, Kuban State University, Krasnodar. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Meditsiny, Vol. 106, No. 12, pp. 720-722, December, 1988. Original article submitted August 25, 1987.

0007-4888/88/0012-1761$12.50 © 1989 Plenum Publishing Corporation

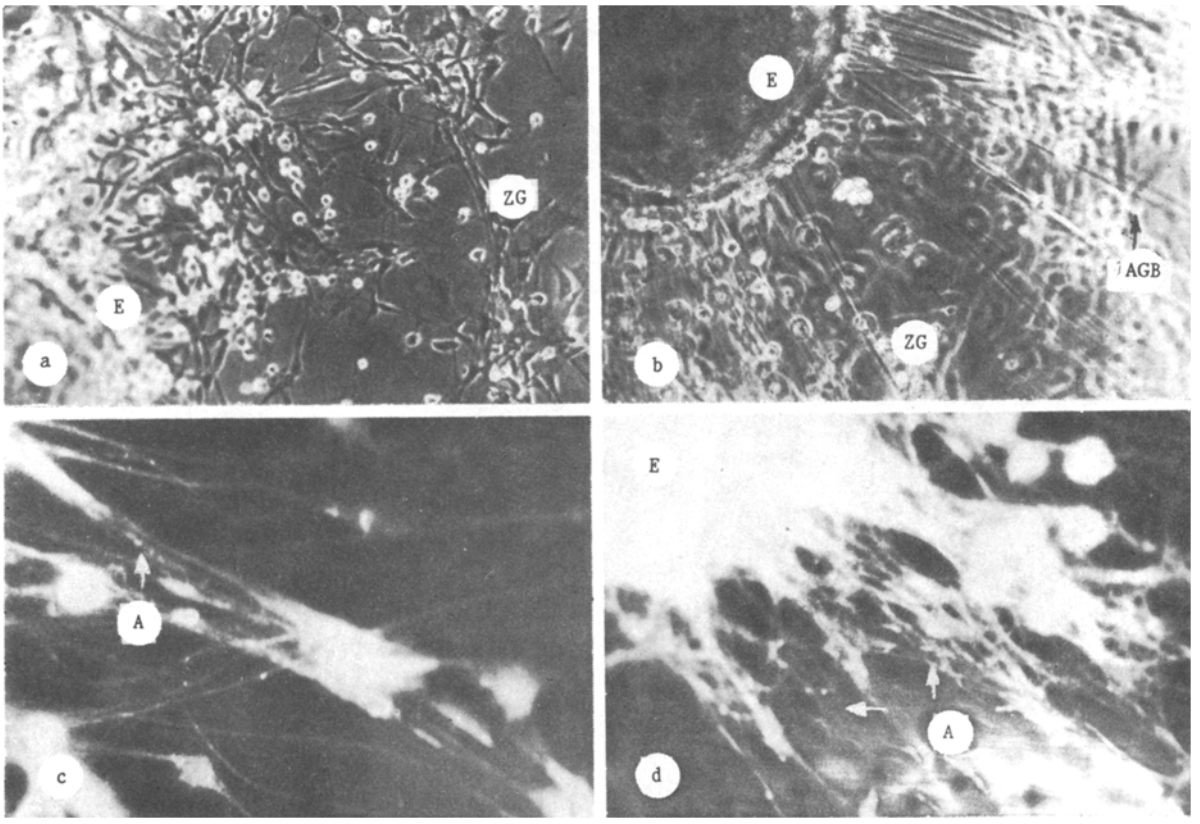

Fig. 1. Effect of Baliz-2 on rat sympathetic ganglia (4th day in culture). a, c) Control; b, d) Baliz-2 in concentration of 0.001%; a, b) intravital microscopy, phase contrast; c, d) fluorescence microscopy. E) Explant, ZG) zone of growth, AGB) axonal-glial bundles, A) axons. Magnification: 250 (a, b); 400 (c, d).

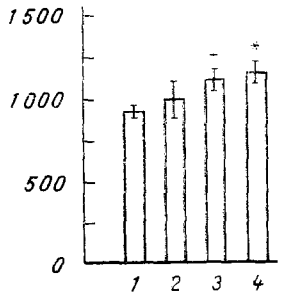

Fig. 2

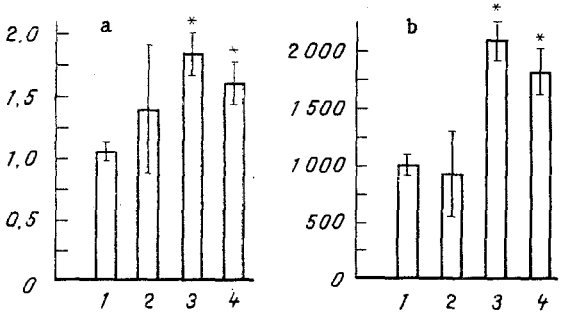

Fig. 3

Fig. 2. Dependence of maximal size of zone of growth of explant on Baliz-2 concentration. Abscissa, concentration: 1) control (n = 52), 2-4) 0.01% (n = 5), 0.001% (n = 44), and 0.0001% (n = 23) respectively; ordinate: maximal size of zone of growth (in μ); *) difference from control statistically significant ($p \leqslant 0.05$).

Fig. 3. Dependence of density of axonal-glial bundles (a) and intensity of reaction (b) on Baliz-2 concentration. Abscissa, concentration: 1) control (n = 52), 2-4) 0.01% (n = 5), 0.001% (n = 44), 0.0001% (n = 23); ordinate: density of axonal-glial bundles (in relative units, a); intensity of reaction (in relative units, b). *)Difference from control statistically significant ($p \leqslant 0.05$).

period the zone of growth was formed mainly by glial and fibroblast-like cells. Their network surrounded the explant evenly, and separate axonal-glial bundles stretched out in the radial direction. Histochemical analysis shows that the number of axons with characteristic small granules giving emerald green fluorescence in the zone of growth of the control cultures was very small. In the zone of growth, besides characteristic catecholamine-containing fibers, other fibers and cells (macrophages, glial cells) with nonspecific green and yellowish fluorescence also were observed (Fig. 1c).

Addition of Baliz-2 (0.001 and 0.0001%) to the growth medium changed the character of development of the cultures. The principal change visible was an increase in density of the axonal-glial bundles (Fig. 1b) and also in the area of the zone of growth. Histochemical preparations clearly revealed an increase in the number of fibers in the zone of growth with characteristic varicose thickenings of emerald green color (Fig. 1d). Dependence of the reaction to Baliz-2 on its concentration in the growth medium was studied in a series of experiments in which this agent was used in concentrations of 0.01, 0.001, and 0.0001%. Statistical analysis showed that the maximal size of the zone of growth, lying between 900 and 1000 μ, was significantly increased only when the preparation was used in concentrations of 0.001 and 0.0001% (Fig. 2). This reaction, however, was not so conspicuous as the increase in density of axonal-glial bundles in the zone of growth (Fig. 3a). This parameter was shown to be more variable: Its value was increased by 1.5-1.7 times compared with the control. The intensity of the reaction, considering the maximal size of the zone of growth and the density of axonal growth, shows (Fig. 3b) that Baliz-2 exhibits its growth-stimulating effect in two concentrations, namely 0.001 and 0.0001%, at which the intensity increases by 2.0 and 1.8 times respectively compared with the control.

The investigation thus showed that Baliz-2, a product of microbiological synthesis, possesses neurotrophic properties and activates growth of explants of sympathetic ganglia in culture. Enhancement of axonal growth may be attributable not only to intensification of differentiation and regeneration, but also to an increase in the survival rate of the neurons in culture. There are as yet no data in the literature to indicate that organic keto acids, the main components of the preparation, have any neurotrophic action. Further analysis of the action of Baliz-2 and its fractions on nerve tissue cultures could therefore serve as a model for the study of regenerative processes in both the peripheral and the central nervous system.

LITERATURE CITED

1. Yu. F. Goncharov, A. Ya. Shurygin, É. Sayadova, et al., Izv. Sev.-Kavkaz. Nauch. Tsent. Vyssh. Shkoly: Estestv. Nauki, No. 2, 84 (1985).
2. R. P. Zubarev, E. M. Sergenyuk, V. N. Zagvozkin, et al., Khirurgiya, No. 1, 19 (1987).
3. O. B. Il'inskii, S. E. Spevak, N. V. Kochetkov, et al., Byull. Éksp. Biol. Med., No. 7, 91 (1985).
4. O. B. Il'inskii, M. V. Kozlova, E. S. Kondrikova, et al., Neirofiziologiya, 17, 550 (1986).
5. M. V. Kozlova, O. B. Il'inskii, V. U. Kalenchuk, et al., Neirofiziologiya, 18, 610 (1986).
6. V. N. Shvalev and N. I. Zhuchkova, Arkh. Anat., No. 6, 114 (1979).
7. R. Adler, M. Manthorpe, S. D. Skaper, et al., Brain Res., 206, 129 (1972).
8. J. H. Chamlly, G. Mark, and G. R. Campbell, Z. Zellforsch., 135, 287 (1972).
9. G. E. Davis, M. Manthorpe, and S. Varon, Devel. Brain Res., 7, 72 (1985).
10. R. Levi-Montalcini, Annu. Rev. Neurosci., 5, 341 (1982).
11. S. Namuri and T. Fujita, Neuropharmacology, 17, 73 (1978).
12. D. L. Needels, M. Nieto-Sompedro, M. Whittemore, et al., Brain Res., 350, 275 (1985).
13. J. E. Turner, Brain Res., 350, 265 (1985).
14. I. S. Zagon and P. J. McLaughlin, Science, 221, 1179 (1983).