The neurite-stimulating effect of peptides from brain in dorsal root ganglion neuron organotypic culture

V. KH. KHAVINSON,[1] N. I. CHALISOVA* and V. B. OKULOV[1]

Laboratory of Physiology of Sensory Receptors, I. P. Pavlov Institute of Physiology, Academy of Sciences of Russia, nab. Makarova 6, St Petersburg 199034, Russia
[1]*Institute of Bioregulation and Gerontology, Wjazowaja 13, St Petersburg 197042, Russia*

Received 20 December 1996; accepted 7 March 1997

Abstract—The effect of natural brain peptides (cortexin and epithalamin) as well as synthetic tetrapeptides (brain peptide and epiphysis peptide) were investigated in organotypic cultures of dorsal root ganglion (DRG) neurons and explants of brain tissue from 10–11 day old chick embryos. Cortexin (2–100 ng/ml), epithalamin (200 ng/ml), brain peptide (2 ng/ml) and epiphysis peptide (20 ng/ml) showed a neurite-stimulating effect in DRG cultures as compared to the control explants. Cortexin at 100 ng/ml and brain peptide at 20 ng/ml showed a stimulating effect in cortical cultures, and epithalamin at 200 ng/ml and epiphysis peptide at 100 ng/ml showed a stimulating effect in cultures of subcortical structures as compared to the control explants.

Keywords: Brain peptides; dorsal root ganglia neuron; cerebral cortex; tissue culture.

1. INTRODUCTION

Results from basic and clinical research have lead to the development of a variety of means to delay the aging process, including the correction of disrupted homeostasis by the aid of regulatory peptides (Khavinson and Morozov, 1983; Khavinson, 1997). This new class of preparation is capable of completely restoring functional impairments and to interfere with the development of pathological processes in those organs and tissues from which they are obtained.

Brain peptides (cortexin and epithalamin) were extracted from brain tissue of large horned cattle (molecular weight about 10 kDa). These peptides can support structural and functional homeostasis of the cell populations which secreted these peptides (Morozov and Khavinson, 1996).

The representation about participation of peptide bioregulators involved in the maintenance of a structural and functional homeostasis of cellular population, which contain and produce these factors, have been given the name cytomedins. Experimental

*To whom correspondence should be addressed. E-mail: krylov@infran.ru

zone. A neurite-stimulating effect was observed in DRG cultures with all brain peptides but in different concentrations. In cultures that showed a neurite-stimulating effect, the neurites were longer, fascicles were denser and the halo was more intense compared to the ones in control explants. These differences were observed in explants cultured for 72 h. The SI of the explants with 20 ng/ml of cortexin increased with $123 \pm 7\%$ ($P < 0.05$), as compared with the control (Fig. 1). The decrease of SI with a concentration of 50 ng/ml cortexin and increase of SI with a concentration of 100 ng/ml may be to two types of polypeptides in cortexin. The neurite-stimulating effect of epithalamin was observed with a concentration of 200 ng/ml when SI increased $28 \pm 8\%$ ($P < 0.05$), as compared to control (Fig. 2).

There was a dose–response relationship with an optimal peak concentration for each of the agents. A cortexin concentration of 2, 10 or 50 ng/ml had the lower neurite-stimulating effect, followed by a concentration of 20 ng/ml. Concentrations of 50 and 200 ng/ml gave less neurite-stimulating effect, as compared to the concentration of 100 ng/ml (Fig. 1). Epithalamin concentrations of 2 and 50 ng /ml have less extensive neurite-stimulating activity, as compared to the concentration 20 ng/ml, and concentrations of 100 and 400 ng/ml have less extensive activity, as compared to the effective concentration of epithalamin, i.e. 200 ng/ml (Fig. 2).

The effective concentration of brain peptide was only 2 ng/ml, but this concentration increased the SI by $40 \pm 5\%$ ($P < 0.01$), as compared to SI of control explants. Concentrations of brain peptide of 0.5 and 1 ng/ml had no neurite-stimulating effect, and concentrations of 10 ng/ml and more inhibited neurite growth (Fig. 1). A con-

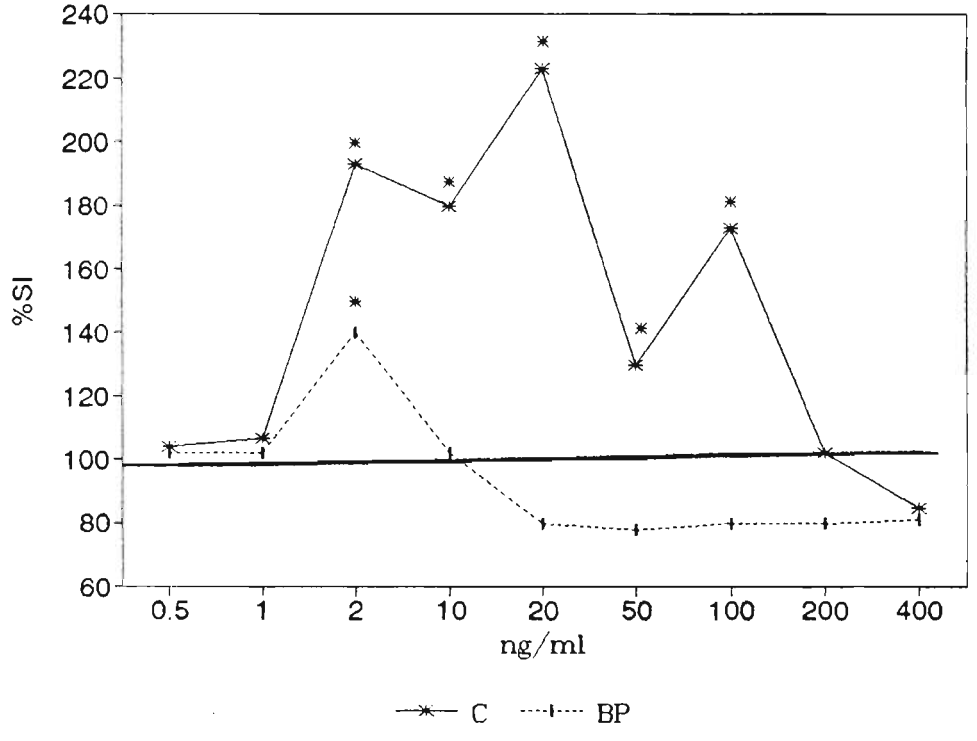

Figure 1. Graph showing the percent SI of chick embryo DRG explants after 3 days *in vitro*: control, 100%. *X*-axis, the concentration of peptides in ng/ml in the culture medium. $^*P < 0.05$. C, cortexin; BP, brain peptide.

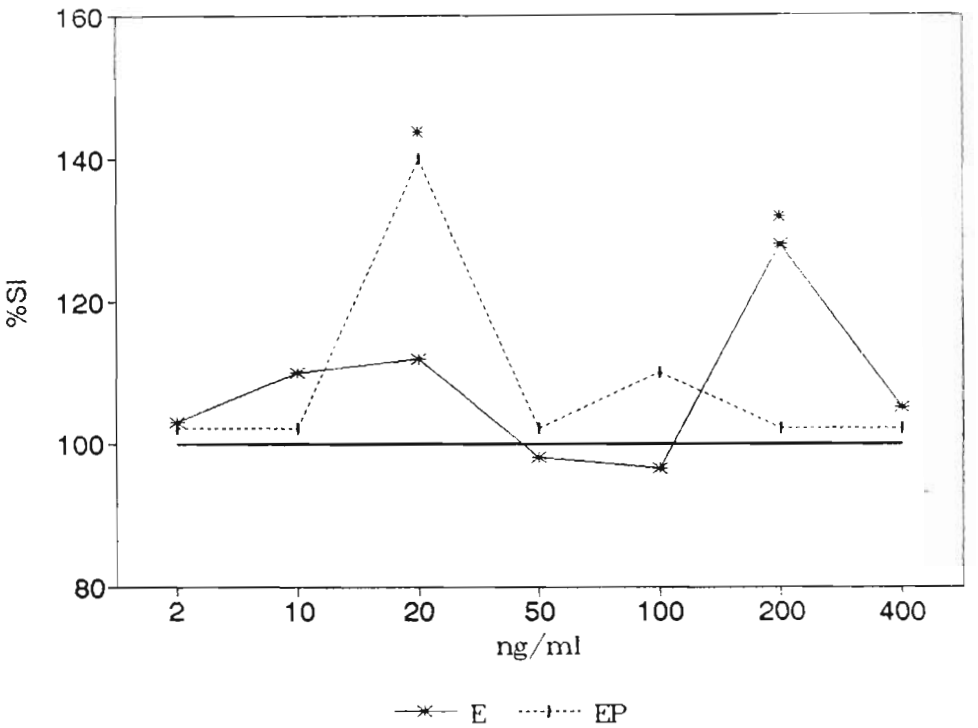

Figure 2. Graph showing the percent SI of chick embryo DRG explants after 3 days *in vitro*: control, 100%. *X*-axis, the concentration of peptides in ng/ml in the culture medium. E, epithalamin; EP, epiphysis peptide. $^{*}P < 0.05$.

centration of 20 ng/ml of epiphysis peptide increased the SI by $40 \pm 9\%$ ($P < 0.05$), as compared to the control. The other concentrations of epiphysis peptide had no neurite-stimulating effect (Fig. 2).

3.2. The effect of peptides in cortical and subcortical structures in tissue culture

A neurite-stimulating effect was observed in cortex culture only with 100 ng/ml of cortexin when the SI increased by $30 \pm 2\%$ ($P < 0.05$), as compared to the control explants. The other concentrations of cortexin had no statistically significant effects on the development of cortex explants (Fig. 3). Brain peptide in a concentration of 20 ng/ml had an intensive neurite-stimulating effect in cortex tissue when SI increased by $40 \pm 7\%$ ($P < 0.05$), as compared to the control cortex explants (Fig. 3). Concentrations of epithalamin and epiphysis peptide from 2 to 400 ng/ml had no stimulating effects in cortex culture, and a concentration of 200 ng/ml of epithalamin to some degree inhibited the development of cortex explants (Fig. 4).

The neurite-stimulating effect in subcortical structure culture was observed only with 200 ng/ml of epithalamin, when SI of explants increased by $27 \pm 7\%$ ($P < 0.05$), as compared to the control explants and with 100 ng/ml of epiphysis peptide, when SI increased by $25 \pm 5\%$ ($P < 0.05$), as compared to the control explants (Fig. 5). A neurite-inhibiting effect was observed with 100 ng/ml cortexin in subcortical structures: SI decreased by $67 \pm 3\%$ ($P < 0.05$), as compared to the SI of control explants (Fig. 6).

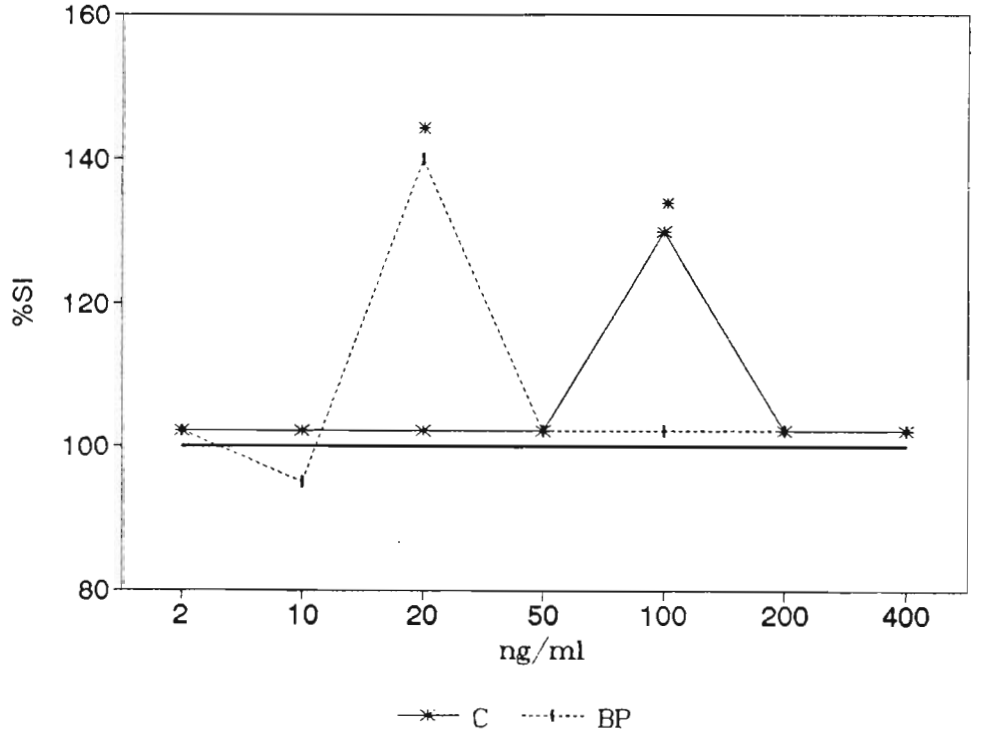

Figure 3. Graph showing the percent SI of chick embryo brain cortex after 3 days *in vitro*: control, 100%. *X*-axis, the concentration of peptides in ng/ml in the culture medium. C, cortexin; BP, brain peptide. $^*P < 0.05$.

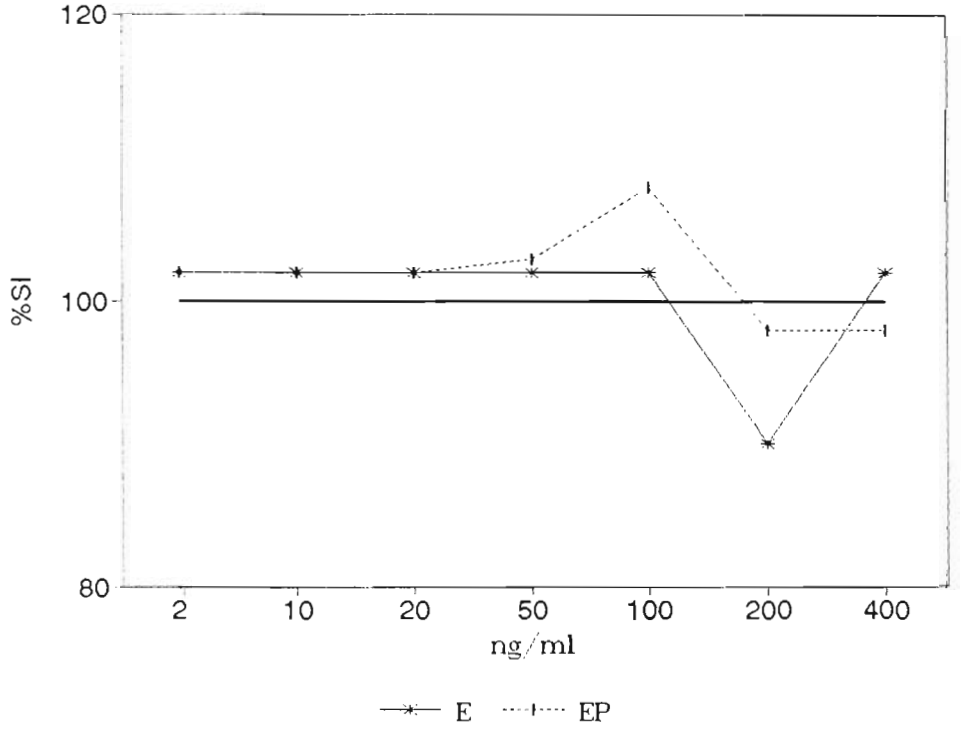

Figure 4. Graph showing the percent SI of chick embryo brain cortex after 3 days *in vitro*: control, 100%. *X*-axis, the concentration of peptides in ng/ml in the culture medium. E, epithalamin; EP, epiphysis peptide. $^*P < 0.05$.

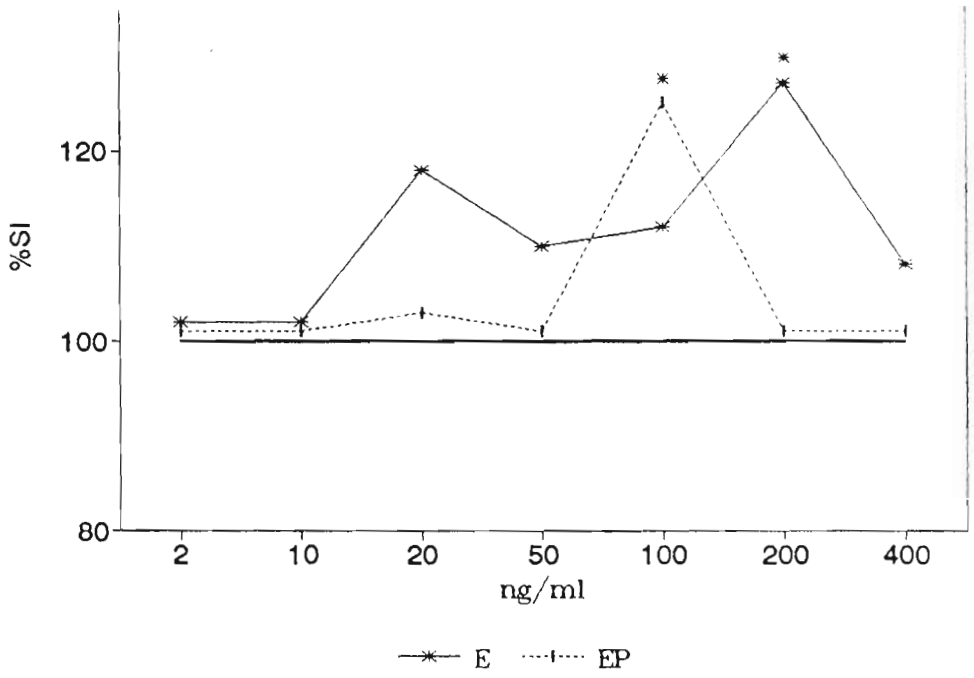

Figure 5. Graph showing the percent SI of chick embryo subcortical structures after 3 days *in vitro*: control, 100%. *X*-axis, the concentration of peptides in ng/ml in the culture medium. E, epithalamin; EP, epiphysis peptide. $^*P < 0.05$.

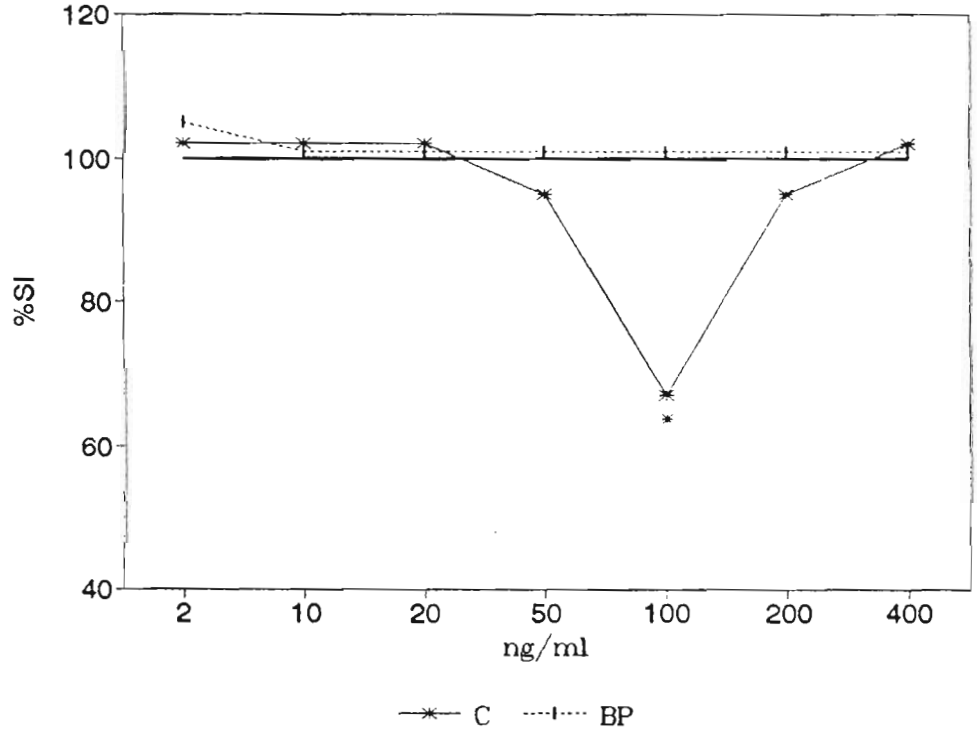

Figure 6. Graph showing the percent SI of chick embryo subcortical structures after 3 days *in vitro*: control, 100%. *X*-axis, the concentration of peptides in ng/ml in the culture medium. C, cortexin; BP, brain peptide. $^*P < 0.05$.

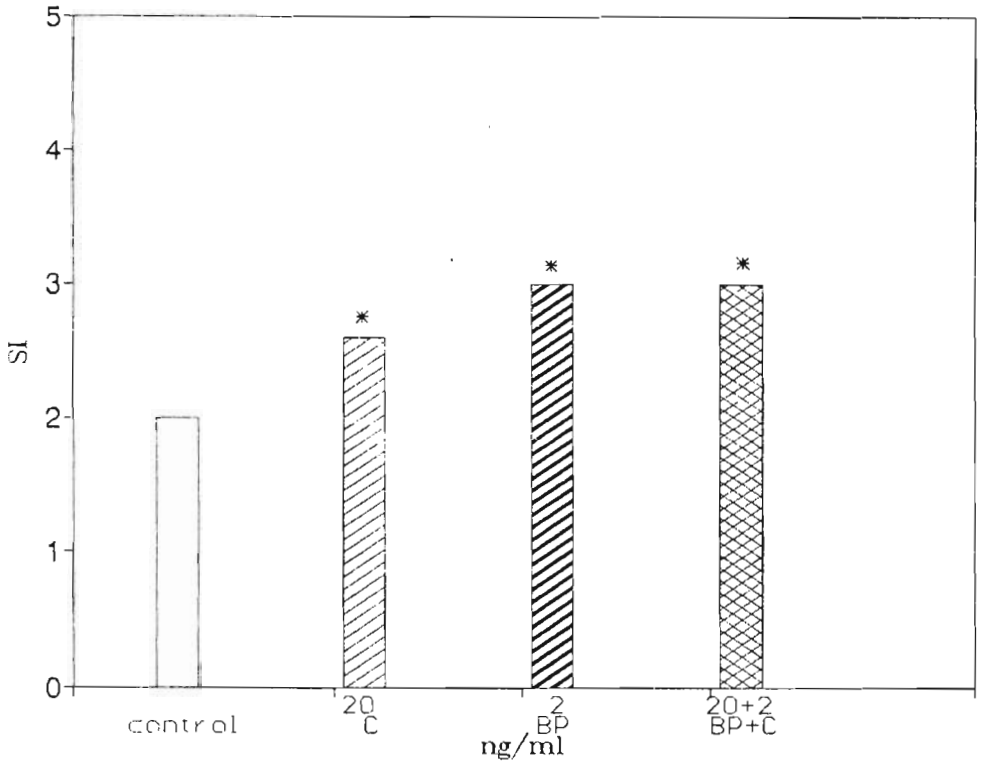

Figure 7. Graph showing the SI (*Y*-axis) of chick embryo DRG explants after 3 days *in vitro*. C, cortexin; BP, brain peptide. $^{*}P < 0.05$.

3.3. Potentiation between peptides

There was no potentiating effect between natural and synthetic peptides. So the simultaneous application of 10 ng/ml cortexin and 2 ng/ml brain peptide had the same neurite-stimulating effect as the application of each agent separately (Fig. 7). In the same manner, there was no effect of potentiation between effective concentrations of epithalamin and epiphysis peptide (Fig. 8). As is showing in Fig. 9, no potentiation was seen in cortex tissue culture when effective concentrations of cortexin and brain peptide were combined.

4. DISCUSSION

Under *in vitro* conditions natural and synthetic peptides from mammalian brain tissue in concentration of 2–200 ng/ml stimulate neurite outgrowth from sensory ganglia. Natural peptides (cortexin and epithalamin) have peaks of neurite-stimulating activity at different concentrations (in the range 2–200 ng/ml). We therefore conclude that these preparations have some peptide fractions with biological activity. It may be that some components of cortexin and epithalamin are the neurotrophic factors. Evidence of this phenomena is the absence of interspecies specificity, as is characteristic of neurotrophic factors (Hefti *et al.*, 1989; Ebendal, 1992; Kurshing *et al.*, 1993); the peptides from mammalian brain have an effect on chick DRG and brain fragments. In contrast to natural peptides, synthetic tetrapeptides have only one effective concentration (brain peptide, 2 ng/ml; epiphysis peptide, 200 ng/ml), but this

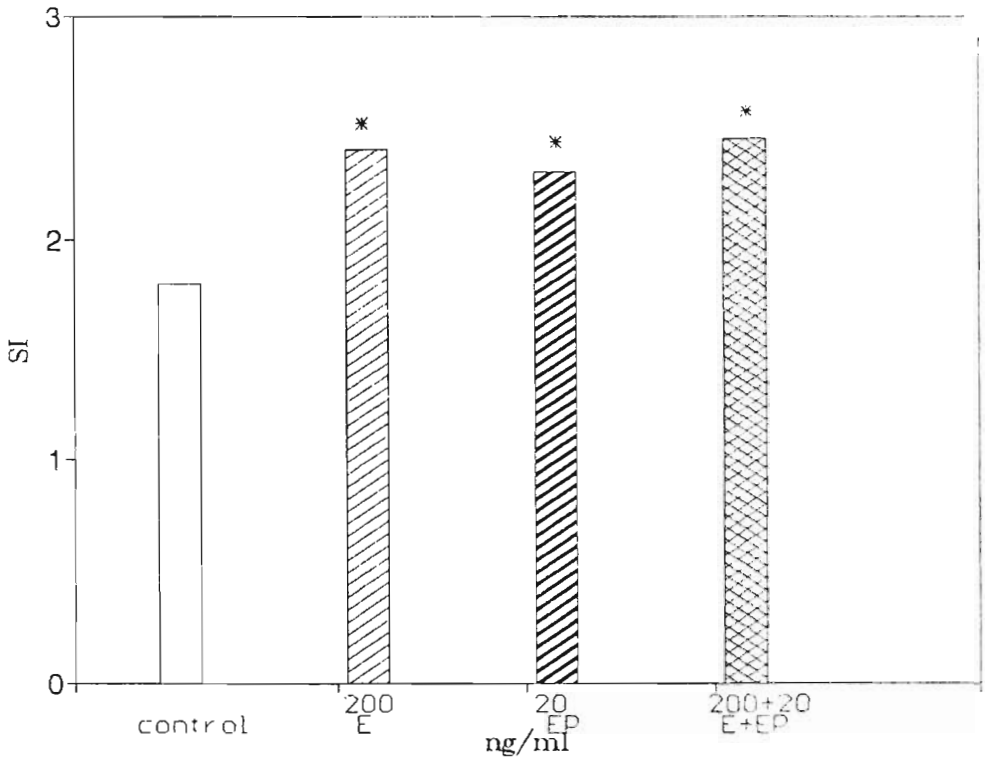

Figure 8. Graph showing the SI (Y-axis) of chick embryo DRG explants after 3 days *in vitro*. E, epithalamin; EP, epiphysis peptide. $^*P < 0.05$.

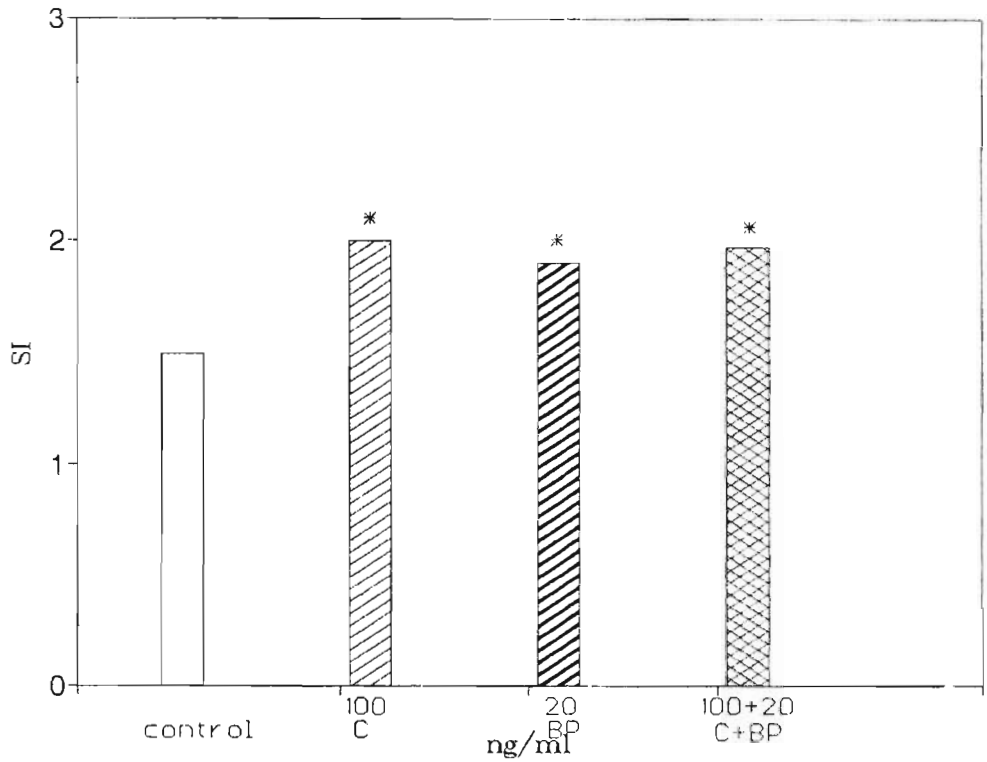

Figure 9. Graph showing the SI (Y-axis) of chick embryo cortex explants after 3 days *in vitro*. C, cortexin; BP, brain peptide. $^*P < 0.05$.

concentration produces extensive neurite-stimulating effect, i.e. the SI increased by 40% as compared to the SI of control explants.

The synthetic tetrapeptides showed a neurite-stimulating effect at lower concentrations in organotypic culture of brain tissue as compared to the concentrations of natural peptides. Thus, the effective concentration of cortexin was 100 ng/ml, but the effective concentration of brain peptide was 20 ng/ml in cortex tissue culture. The effective concentration of epithalamin was 200 ng/ml, but the effective concentration of epiphysis peptide was 20 ng/ml in subcortical structures tissue culture. So we can conclude that artificially constructed tetrapeptides have a more intensive and exact effect on nerve cells as compared to the natural peptides.

There was no effect of potentiation between cortexin and brain peptide or epithalamin and epiphysis peptide, presumably because natural and synthetic peptides act on the same target cells. It is known that there are receptors for many cytyokines on neronal and glial cells (Scott, 1982; Sawada, 1993). The cytomedines may interact with some of these receptors. Also the regulation of NTF content (e.g. nerve growth factor) can be regulated by interleukin-1 or other cytokines from astrocytes and glial cells (Lindsay *et al.*, 1979; Lindholm *et al.*, 1987; Sprangler *et al.*, 1990; Pshenichkin *et al.*, 1992; Aldskogius and Svensson, 1993). Thus, cytomedins may also influence nerve cells by the stimulation of glial cells in the central and peripheral nervous system.

The neurite-stimulating effect of brain peptides can be used in the treatment of neurodegenerative diseases, as has been shown recently for neurotrophic factors (Scott, 1982; Jonson and Yip, 1985; Oppenheim, 1989).

Our data show that like the effects of NTFs the agents examined (natural peptides of the brain and synthetic tetrapeptides) possessed similar biological activity in nerve tissue culture. From a clinical point of view, synthetic tetrapeptides seem to have an advantage, since the very small synthetic molecules should not provoke allergic reaction.

REFERENCES

Akoev, G. N., Babu, K. S., Chalisova, N. I. and Ludino, M. I. (1992). The cerebrospinal fluid from patients with prolaktinoma promotes neurite growth of sensory neurons. *Neuroreport* **4**, 405–406.

Aldskogius, H. and Svensson, M. (1993). Neuronal and glial cell responses to axon injury. *Adv. Struct. Biol.* **2**, 191–223.

Barde, Y.-A., Edgar, D. and Thoenen, H. (1982). Purification of a new neurotrophic factors from mammalian brain. *EMBO J.* **1**, 1549–1553.

Ebendal, T. (1992). Function and evolution in the NGF family and its receptors. *J. Neurosci. Res.* **32**, 461–470.

Hefti, F., Hartikka, J. and Knusel, B. (1983). Function of neurotrophic factors in the adult and aging brain and their possible use in the treatment of neurodegenerative diseases. *Neurobiol. Aging* **10**, 515–533.

Johnson, E. and Yip, H. (1985). CNS and peripheral nerve growth factor provide trophic support critical to nature sensory neuronal survival. *Nature* **314**, 751–766.

Khavinson, V. Kh. (1996). The results of study and application of peptide bioregulators in gerontology. In: *Materials of Int. Symp. 'Gerontological Aspects of Peptide Regulation of Organism Functions'*, St Petersburg, pp. 171–172.

Khavinson, V. Kh. and Morozov, V. G. (1983). The new class of cell systems bioregulators — cytomedins. *Uspehi Sovrem. Biol.* **96**, 339–352.

Korsching, S. (1993). The neurotrophic factor concept: a reexamination. *J. Neurosci.* **13**, 2739–2748.

Levi-Montalcini, R. (1982). Developmental neurobiology and natural history of nerve growth factor. *Annu. Rev. Neurosci.* **5**, 341–356.

Lindholm, D., Heumann, R. and Meyer, M. (1987). Interleukin-1 regulates synthesis of nerve growth factor in non-neuronal cells of rat sciatic nerve. *Nature* **330**, 658–659.

Lindsay, R. (1979). Adult rat brain astrocytes support survival of both NGF-dependent and NGF-insensitive neurons. *Nature* **272**, 80–82.

Morozov, V. G. and Khavinson, V. Kh. (1996). Achievement and prospects in the field of bioregulation and gerontology. In: *Materials of Int. Symp. 'Gerontological Aspects of Peptide Regulation of Organism Functions'*, St Petersburg, pp. 105–107.

Oppenheim, R. W. (1992). The neurotrophic theory and naturally occuring motoneuron death. *Trends Neurosci.* **12**, 252–255.

Pshenichkin, S. P., Szekely, A. M. and Wise, B. C. (1992). Differential regulation of astroglial nerve growth factor mRNA content by interleukin-1, TRA and steroids. *Soc. Neurosci. Abstr.* **1**, 14.

Sawada, M., Itoh, Y. and Suzumura, A. (1993). Expression of cytokine receptors in cultured neuronal and glial cells. *Neurosci. Lett.* **160**, 131–134.

Scott, B. (1982). Adult neurons in cell culture. *Progr. Neurobiol.* **19**, 187–194.

Spranger, M., Lindholm, D. and Bandtlow, C. (1990). Regulation of nerve growth factor (NGF) synthesis in the rat central nervous system: comparison between the effects of interleukin-1 and various growth factors in astrocyte cultures and *in vivo*. *Eur. J. Neurosci.* **2**, 69–76.