

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

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**Abstract**—The effect of natural brain peptides (cortixin 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. Cortixin (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. Cortixin 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 (cortixin 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

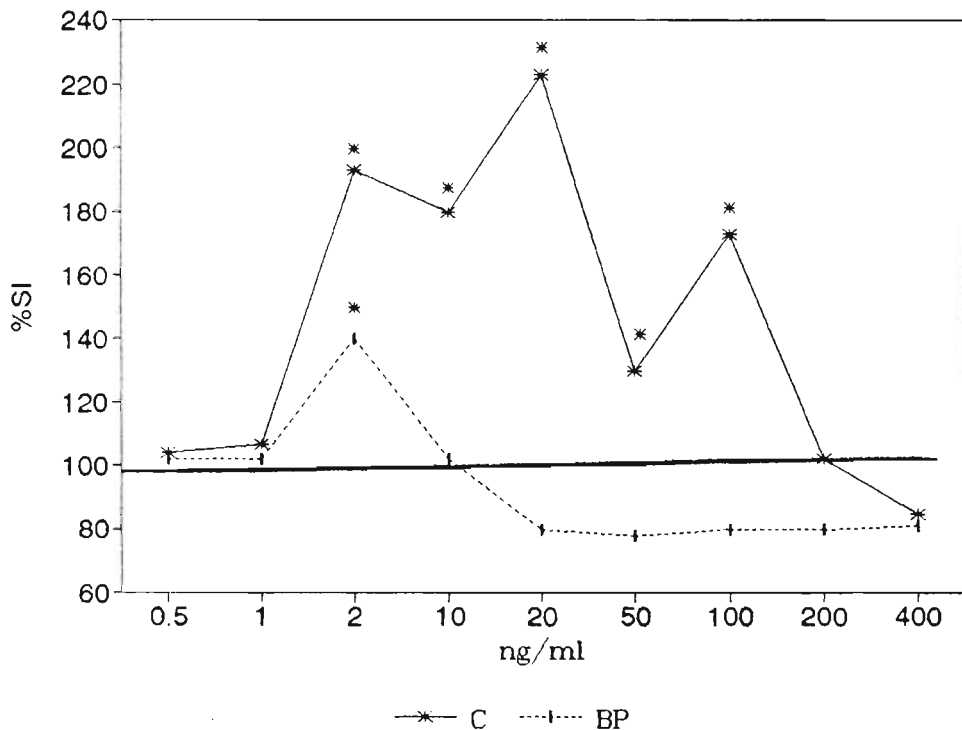
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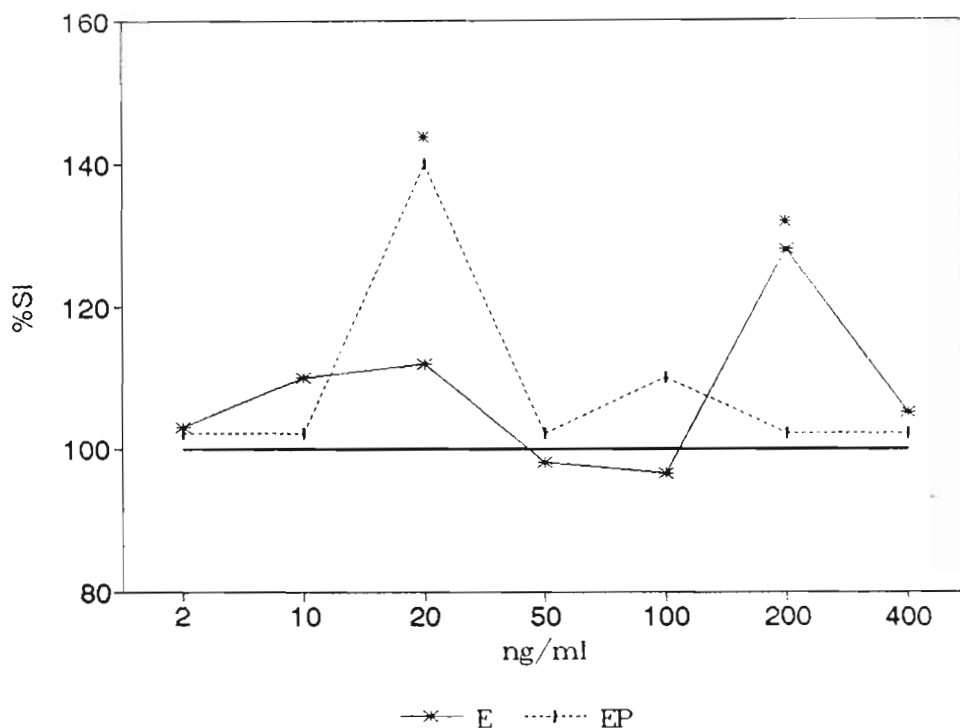
zone. A neurite-stimulating effect was observed in DRG cultures with all brain peptides but in different concentrations. In cultures that showed a neurite-stimulating effect, the neurites were longer, fascicles were denser and the halo was more intense compared to the ones in control explants. These differences were observed in explants cultured for 72 h. The SI of the explants with 20 ng/ml of cortixin 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 cortixin and increase of SI with a concentration of 100 ng/ml may be to two types of polypeptides in cortixin. 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 cortixin 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, cortixin; BP, brain peptide.



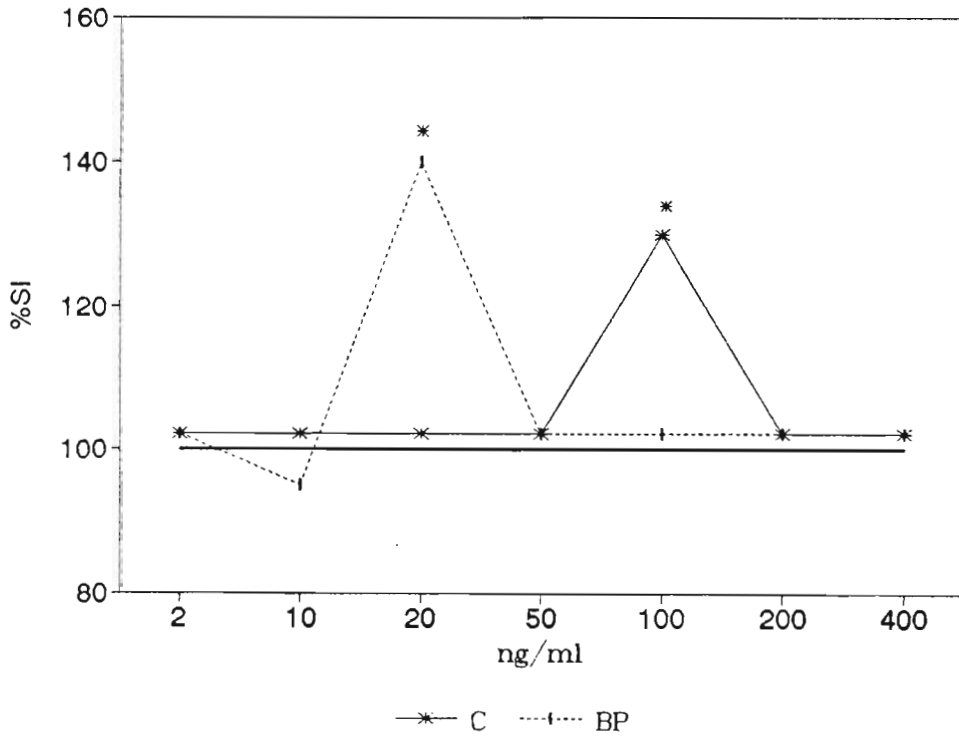
**Figure 2.** Graph showing the percent SI of chick embryo DRG explants after 3 days *in vitro*: control, 100%. X-axis, the concentration of peptides in ng/ml in the culture medium. E, epithalamin; EP, epiphysis peptide. \* $P < 0.05$ .

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).

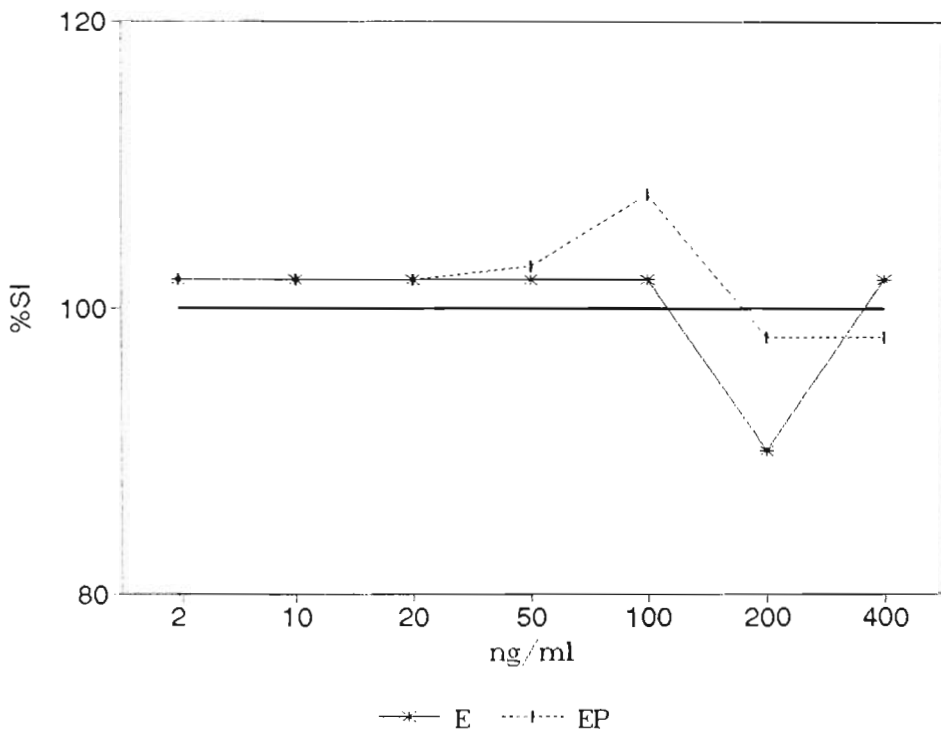
### 3.2. The effect of peptides in cortical and subcortical structures in tissue culture

A neurite-stimulating effect was observed in cortex culture only with 100 ng/ml of cortixin when the SI increased by  $30 \pm 2\%$  ( $P < 0.05$ ), as compared to the control explants. The other concentrations of cortixin 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).

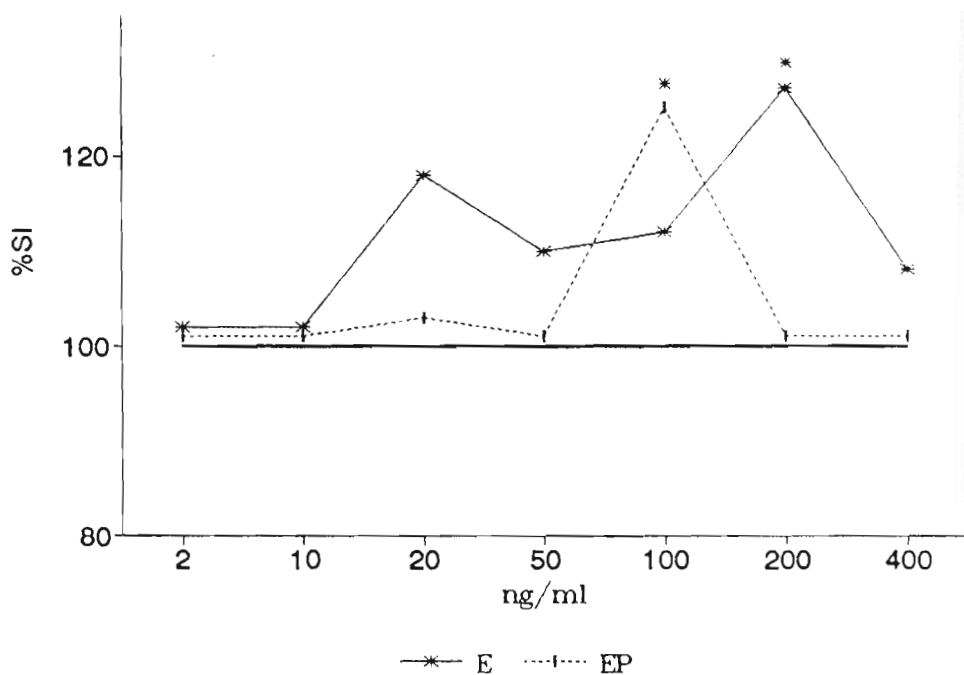
The neurite-stimulating effect in subcortical structure culture was observed only with 200 ng/ml of epithalamin, when SI of explants increased by  $27 \pm 7\%$  ( $P < 0.05$ ), as compared to the control explants and with 100 ng/ml of epiphysis peptide, when SI increased by  $25 \pm 5\%$  ( $P < 0.05$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



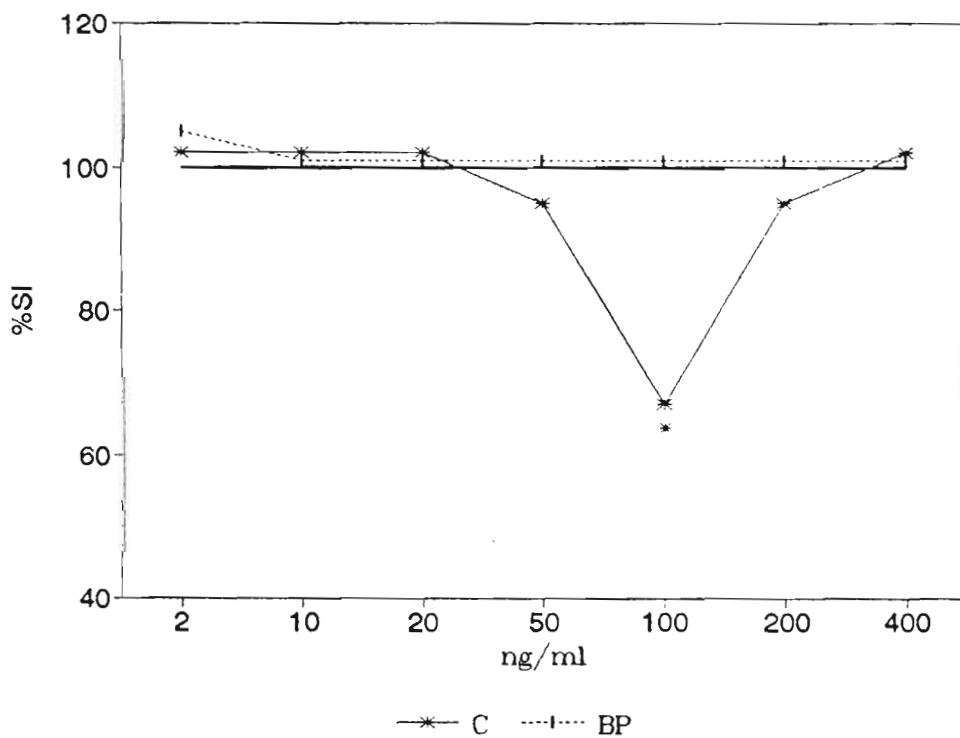
**Figure 3.** Graph showing the percent SI of chick embryo brain cortex after 3 days *in vitro*: control, 100%. X-axis, the concentration of peptides in ng/ml in the culture medium. C, cortixin; BP, brain peptide. \* $P < 0.05$ .



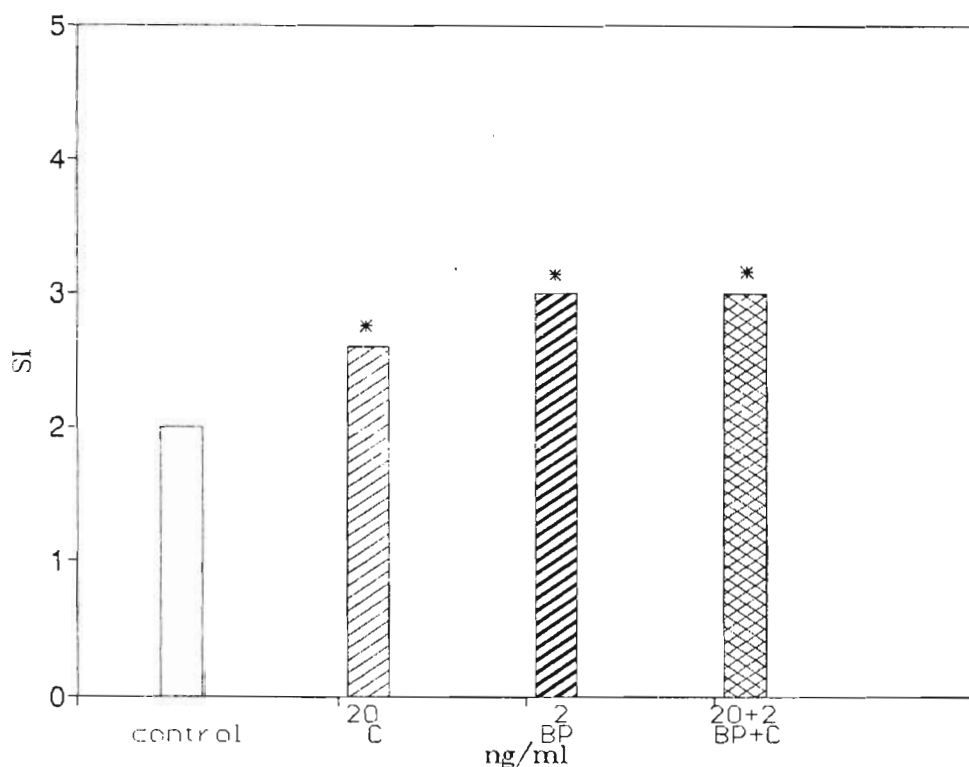
**Figure 4.** Graph showing the percent SI of chick embryo brain cortex after 3 days *in vitro*: control, 100%. X-axis, the concentration of peptides in ng/ml in the culture medium. E, epithalamin; EP, epiphysis peptide. \* $P < 0.05$ .



**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, cortixin; BP, brain peptide. \* $P < 0.05$ .



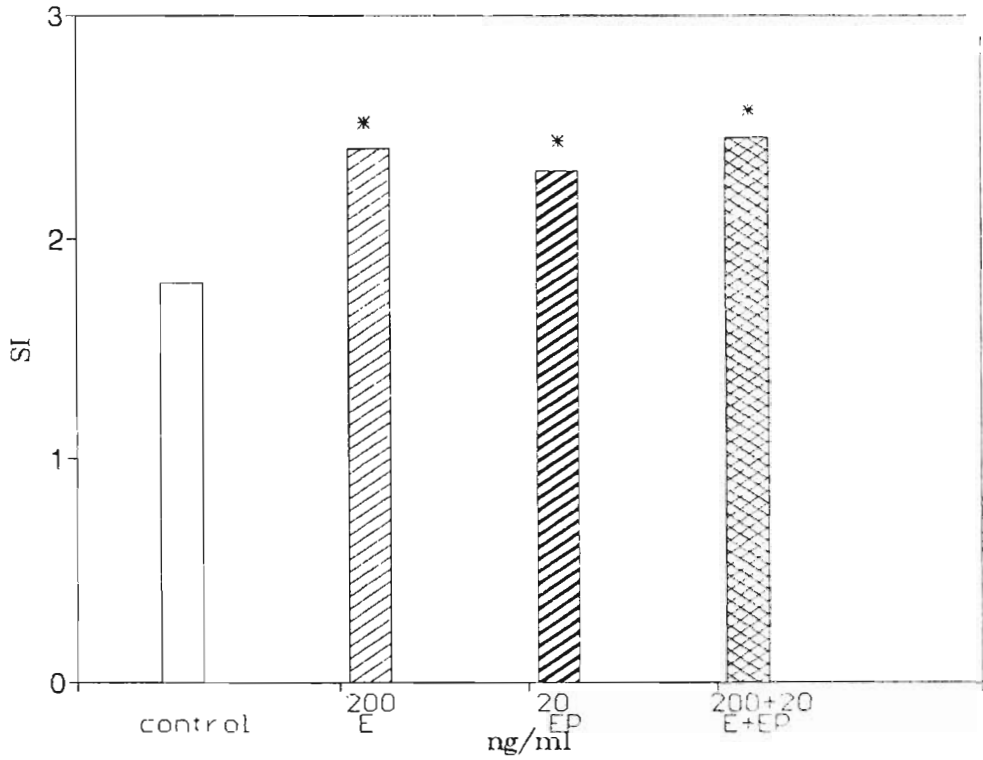
**Figure 7.** Graph showing the SI (Y-axis) of chick embryo DRG explants after 3 days *in vitro*. C, cortixin; BP, brain peptide. \* $P < 0.05$ .

### 3.3. Potentiation between peptides

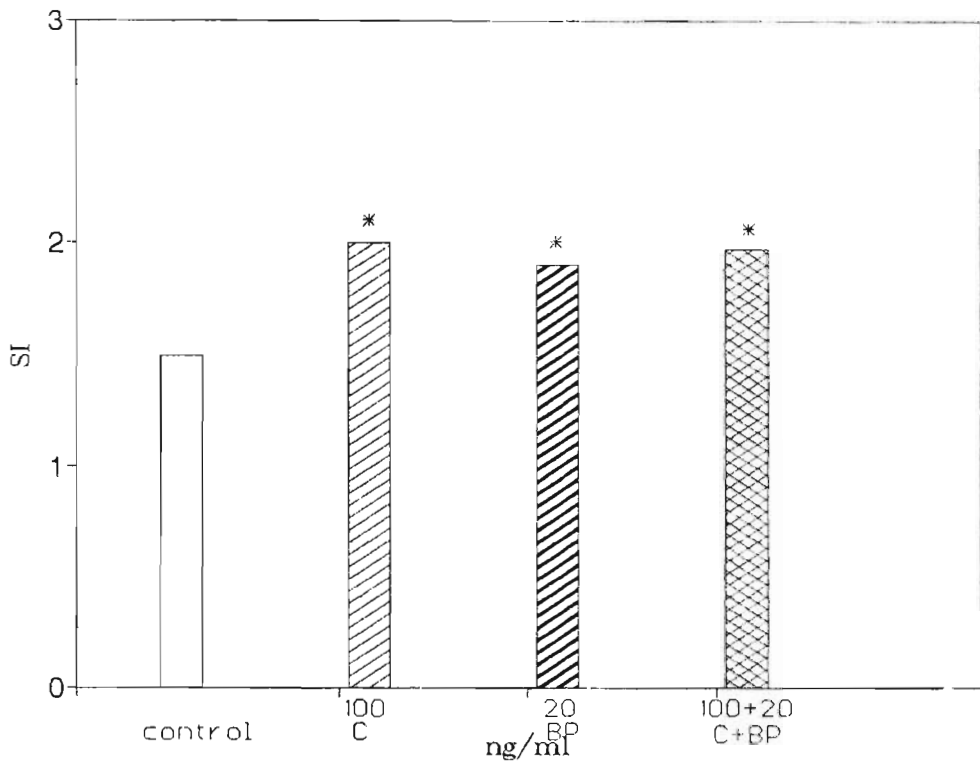
There was no potentiating effect between natural and synthetic peptides. So the simultaneous application of 10 ng/ml cortixin and 2 ng/ml brain peptide had the same neurite-stimulating effect as the application of each agent separately (Fig. 7). In the same manner, there was no effect of potentiation 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 cortixin 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 (cortixin 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 cortixin 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, cortex; 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 cytokines on neuronal 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.

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