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SYNTHETIC PEPTIDE CORRESPONDING TO 30 AMINO ACIDS OF THE C-TERMINAL OF NEURON-SPECIFIC ENOLASE PROMOTES SURVIVAL OF NEOCORTICAL NEURONS IN CULTURE

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Summary: Neuron-specific enolase (NSE), one of the glycolytic enzymes, is a γγ-isozyme of enolase that is specifically expressed in neurons. Our previous studies demonstrated that NSE promotes survival of rat embryonic neocortical neurons in culture but that the αα-isozyme (non-neuronal enolase; NNE) has no effect. In this study, we found that a synthetic peptide corresponding to the C-terminal portion of NSE (404-433) also promotes the survival of neocortical neurons. By contrast, a synthetic peptide of the C-terminal portion of NNE (404-433) has no effect on neuronal survival. These findings would be important for further analysis of the neurotrophic mechanism of NSE.

Neuronal development including differentiation, maturation and survival is considered to be regulated by various neurotrophic factors in the central nervous system (CNS). We previously investigated novel neurotrophic factors in bovine brain extract by using primary cultures of embryonic rat neocortical neurons and found that one of the neurotrophic molecules is identical to neuron specific enolase (NSE), which is a γγ-isozyme of enolase (1).

Within the CNS, NSE is present only in neurons and the αα-isozyme of enolase (non-neuronal enolase; NNE) is present in a wide variety of cells

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including glial cells. Although NSE and NNE show high homology (82%) in amino acid sequence (2), we have found the neurotrophic activity in only NSE. To obtain further information on the neurotrophic mechanism of NSE, we investigated the effects of synthetic peptides corresponding to the C-terminal portion of NSE and NNE on the survival of neocortical neurons in vitro.

Materials and Methods

Materials

Rat NSE and NNE were purified according to the method previously described (3). Synthetic peptides corresponding to 30 amino acids of the C- terminals of rat NSE and NNE were obtained from Nihon Millipore Ltd. The amino acid sequences of the peptides are shown in Fig. 1. ITS Premix and putrescine were obtained from Collaborative Research. Minimum essential medium (MEM) was obtained from GIBCO. Fetal calf serum (FCS) was purchased from Nippon Biosupply. Center. Monoclonal antibody to microtubule-associated protein 2 (MAP-2) and glial fibrillary acidic protein (GFAP) were purchased from Amersham.

Neuronal survival assay

Neurotrophic activity was measured by using a primary culture of embryonic rat neocortical neurons according to the method previously described (4), with slight modification. The neocortical tissues were removed from fetal rats on embryonic day 17. The tissue was minced with scalpels and digested with trypsin (0.1%)/DNase I (0.01%). Cells were seeded into the wells of a poly-L-lysine-coated 24-well plate at a density of $5X10^4$cells/cm^2 and cultured in MEM containing 1% FCS, 0.5 mg/ml sodium bicarbonate, 5 mg/ml glucose, 10μg/ml insulin, 10μg/ml transferrin, 10μg/ml selenium (ITS premix), 100μM putrescine, 50 units/ml penicillin, and 100 μg/ml streptomycin. Synthetic peptides or other factors were added 24 h after seeding. Culture was continued for another 4 days and the surviving neurons were counted under a microscope.

Immunocytochemical staining of the neurons or the glial cells with mouse monoclonal antibody to MAP-2 or GFAP was performed according to the method described elsewhere (4).

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          404                                  413
s-γ   AKYNQLMRIEEELGEEARFAGHNFRNPSVL
s-α   -----IL-------SK-K---RS----LAK
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Fig. 1. Amino acid sequences of synthetic peptides corresponding to the C-terminal (404-443) of NSE (s-γ) and of NNE (s-α).

Results

As shown in Fig. 2, the synthetic peptide corresponding to 30 amino acids of the C terminal of NSE (s-γ; residues 404-433) was found to have promoted the survival of primary cultured neocortical neurons on the fifth day of culture. This neurotrophic effect was detected at a relatively low dose and was maximum at about 20 nM. The half-maximal concentration of s-γ is about 2 nM. As reported previously, purified NSE also promoted the survival of primary cultured neurons at a lower concentration than that of s-γ, and its half-maximal concentration is about 0.3 nM. By contrast, the synthetic peptide corresponding to 30 amino acids of the C-terminal of NNE (s-α; residues 404-433) and purified NNE did not promote the survival of primary cultured neurons.

Typical photographs of immunostained cells with MAP-2 monoclonal antibody are shown in Fig. 3. The number of MAP-2-positive cells in the culture containing purified rat NSE (2 nM) and s-γ (20 nM) was significantly higher than that in the control culture (Fig. 3A). The neuronal processes were well developed in these cultures. However,

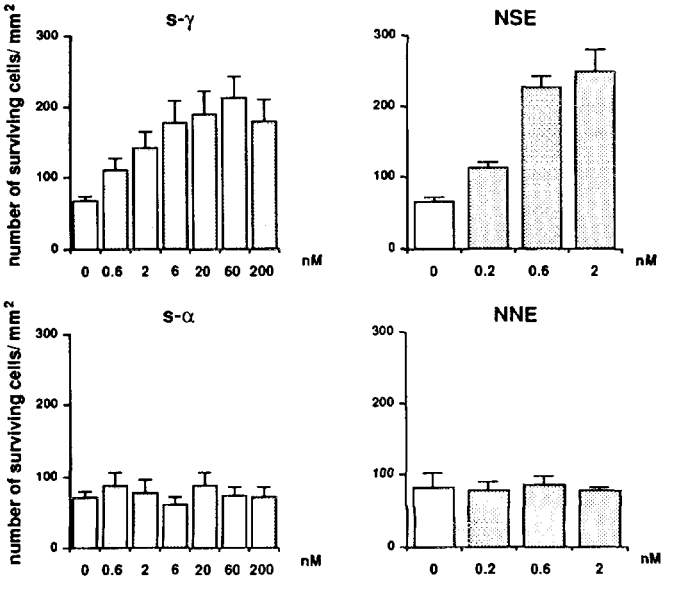

Fig. 2. Effects of rat NSE, NNE and their synthetic peptides of the C-terminal portion. Twenty-four hours after seeding, s-γ, s-α , NSE or NNE was added to the medium and culture was continued for a further 4 days. Values indicate means±SD (n=4).

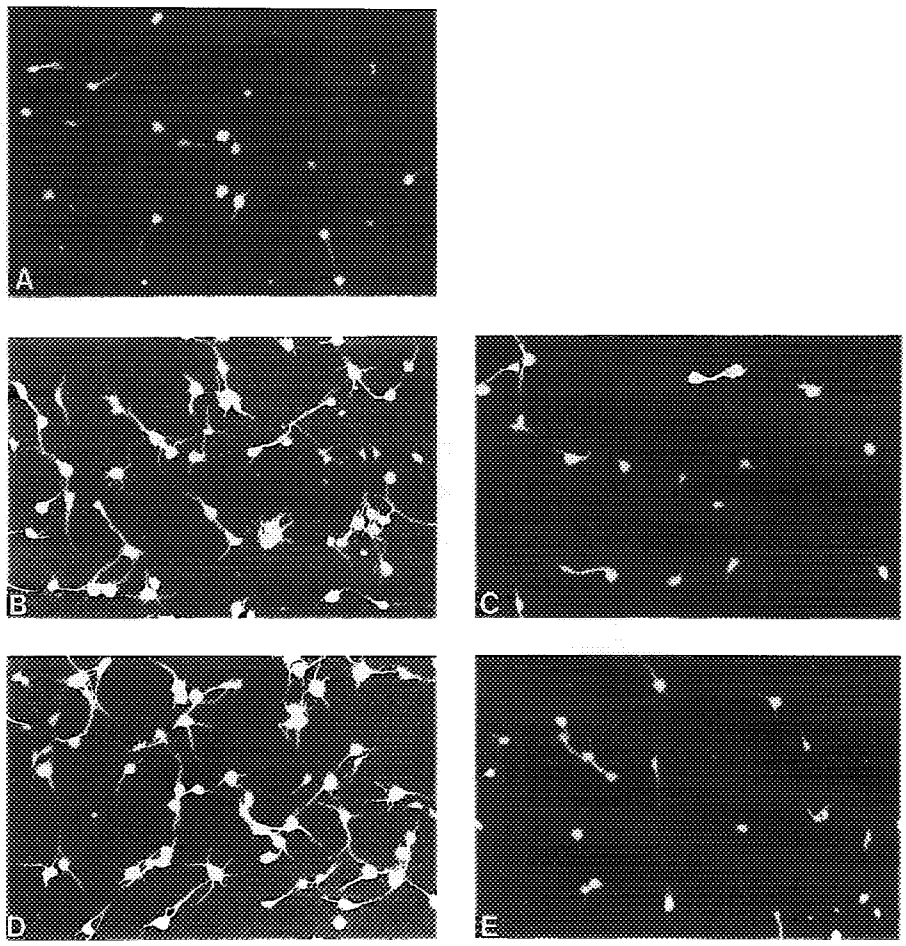

Fig. 3. Typical photomicrographs of cultured neocortical neurons immunocytochemically stained with anti-MAP-2 monoclonal antibody taken on the 5th day of culture. A, control; B, cultured with purified NSE (2 nM); C, with purified NNE (2 nM); D, with s-γ (20 nM); and E, with s-α (20 nM).

purified rat NNE (2 nM) and s-α (20 nM) did not alter the number of MAP-2-positive cells. Because almost all the cells were MAP-2 positive and hardly any GFAP-positive cells were detected (data not shown) in our culture system, it seems that NSE and s-γ act directly on neurons.

Discussion

We reported previously that NSE promotes the survival of rat embryonic neocortical neurons in culture (1). The glycolytic enzyme,

enolase, is present in the CNS as two isozymes, NSE ($\gamma\gamma$) and NNE ($\alpha\alpha$). NNE is found in most neural cells and NSE appears in only differentiated and mature neurons (5, 6, 7). Although the amino acid sequences of these isozymes are highly homologous, only NSE shows neurotrophic activity (1). To clarify the neurotrophic mechanism of NSE, we directed our attention to the 30 residues of the C-terminal portion of NSE and NNE, since in this portion there is a significant difference in amino acid sequence between the two isozymes (8). Interestingly, we found that s-γ (NSE 404-433) but not s-α (NNE 404-433) promotes neuronal survival. This fact led to the idea that this C-terminal region of NSE is a biologically active domain for neurotrophic activity. However, the EC_{50} of s-γ is higher than that of native NSE. One plausible explanation is that higher dimensional structure of NSE may be important for expressing neurotrophic activity.

Recently the physiological significance of the C-terminal portion of NSE has been investigated. Human autoantibodies against the C-terminal region of amyloid β/A4-protein (residues 29-40) have been found in Alzheimer's disease patients (9). It was reported that an antibody against a synthetic C-terminal peptide of amyloid β/A4-protein (residues 28-40) cross-reacted with NSE but not with NNE. The cross-reacting epitope was considered to be residues 402-423 of NSE (10). This domain is mainly hydrophilic in character and is located on the surface of the three-dimensional structure of NSE (2). These residues seem to represent one of the antigenic determinant regions among enolase isozymes (8).

Most discussions of the role of NSE in neurons include its function as a metabolic enzyme (11). However, our previous findings (1) together with the present results also raise the possibility that NSE plays certain roles in the CNS for regulating neuronal development and maintenance. The present findings could be an important clue to clarify the neurotrophic mechanism of NSE in the CNS.

Acknowledgments

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