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[Lab. of Molecular Biology]

An Improved Solid-Phase Bioassay for Evaluation of Neurotrophic Factors.MITSUNARI NAKAJIMA, SHOEI FURUKAWA*, KYOZO HAYASHI,
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A bioassay system was developed to evaluate the biological activities of neurotrophic factor (NTFs) and is referred to as a solid-phase bioassay (SPB). The principle and an outline of the SPB are as follows: (a) Test samples containing NTFs are applied to polyacrylamide gel electrophoresis (PAGE) of the SDS or two-dimensional type and then transferred onto nitrocellulose membranes, (b) neurons are cultured on the protein-blotted membranes, and (c) the distribution of the surviving neurons are estimated following fixation and clarification of the nitrocellulose membranes. The rationale is that neuron survival will be restricted to the migration positions of the NTF(s). Using this system, we found active entities, with molecular masses of ~100-200 kD, different from nerve growth factor (NGF) in crude extracts of mouse submaxillary glands. The SPB is considered to be a useful tool for obtaining information on the physicochemical and/or biological properties of putative NTFs in crude samples.

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[Lab. of Molecular Biology]

Interleukin-4 and -5 as Modulators of Nerve Growth Factor Synthesis/Secretion in Astrocytes.HIROFUMI AWATSUJI, YOSHIKO FURUKAWA*, MASAO HIROTA, YUTAKA MURAKAMI,
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To examine the regulation of nerve growth factor (NGF) gene expression with respect to neural trauma, we examined the effects of T cell-derived lymphokines on NGF synthesis/secretion in cultured mouse astrocytes. Interleukin (IL)-4 and IL-5 significantly increased the amounts of NGF secreted by astrocytes, whereas IL-2, IL-3, and IL-6 had no significant effect. IL-4 and IL-5 produced marked increases in NGF mRNA levels in astrocytes as demonstrated by the reverse transcription-polymerase chain reaction (RT-PCR) method. The effect of IL-4 and IL-5 was greater in quiescent astrocytes than in growing cells. Neither increase in thymidine incorporation nor any morphological change was observed during the treatment with IL-4 and IL-5. The stimulatory effect of IL-4 and IL-5 on NGF synthesis was completely inhibited by the addition of anti-IL-4 monoclonal antibody and anti-IL-5 monoclonal antibody, respectively. The results indicate that IL-4 and IL-5 specially trigger a cascade of events to regulate NGF synthesis in astrocytes, independent of cell growth.

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[Lab. of Molecular Biology]

Age-Dependent Survival-Promoting Activity of Vitamin K on Cultured CNS Neurons.MITSUNARI NAKAJIMA, SHOEI FURUKAWA*, KYOZO HAYASHI, AKIO YAMADA,
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Neurons from the central nervous system (CNS) of rat embryos die within several days when seeded at a low density of 10^4 cells/cm² and cultured in a serum-free defined medium. A fat-soluble vitamin, vitamin K, was found to possess activity to promote the survival of these neurons: more than 50 % of the cortical neurons from 19-day-old rat embryos could survive for 4 days in the presence of vitamin K. The survival-promoting effect of vitamin K₁ was found on neurons from not only cortex, but also hippocampus, striatum, and septum. In addition to vitamin K₁, vitamin K₂ and K₃ also showed the same effect on cortical neurons. The effect of vitamin K₁ and K₂ was observed at concentrations from 10^{-8} to 10^{-6} M, and that of vitamin K₃ was slightly detected at 10^{-6} M. The activity of vitamin K₁ was weaker toward the neurons from 21-day-old embryos compared with that toward 19-day-old ones, and was not recognized toward 16-day-old ones. These results suggest the potential role of the K vitamins on the maintenance of the survival of CNS neurons during the later stages of embryogenesis *in vivo*.