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Immunohistochemical Study on the Distribution of Insulin-like Growth Factor Binding Protein 7 (IGFBP7) in the Central Nervous System of Adult Rats

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Abstract : In the present study, we performed immunohistochemical studies to investigate the detailed distribution of insulin-like growth factor binding protein 7 (IGFBP7) in the central nervous system of adult rats.

Twelve adult (4 ~ 6 month old) Sprague-Dawley rats were examined in this study. Immunohistochemistry using specific antibodies against IGFBP7 was performed in accordance with the free-floating method.

In the present study, IGFBP7 immunoreactivity was observed in the cerebral cortex, hippocampus, brainstem, cerebellum and spinal cord. In the cerebral cortex, heavily stained neurons were seen in layers II-VI. In the hippocampus, pyramidal cells in CA1-3 region were strongly immunoreactive for IGFBP7. Strong immunoreactive neurons were also found in the supraoptic nucleus, paraventricular nucleus, periaqueductal gray and oculomotor nucleus. In the cerebellum, IGFBP7 immunoreactivity was prominent in the Purkinje cells and cerebellar output neurons. IGFBP7-immunoreactive neurons were prominent in the superior vestibular nucleus, cochlear nucleus, trigeminal motor nucleus, nucleus of the trapezoid, and facial nucleus. IGFBP7-immunoreactive neurons were also observed mainly in the anterior horn of the spinal cord.

The first demonstration of IGFBP7 localization in the whole brain may provide useful data for the future investigations on the structural and functional properties of IGFBP7.

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Key words : Insulin-like growth factor binding protein 7 (IGFBP7), Cerebral cortex, Hippocampus, Brainstem, Cerebellum, Spinal cord

Introduction

Insulin-like growth factor binding proteins (IGFBPs) classically comprise 6 isoforms (IGFBP1-IGFBP6) (Hwa et al. 1999). IGFBPs bind to IGF and thereby modify its metabolism, distribution, and ability to bind to the IGF receptor. Recently, IGFBPs that exhibit a low affinity for IGF were reclassified as IGFBP-related proteins (IGFBPrP) (Hwa et al. 1999). One of these IGFBPrPs is IGFBP7, which was originally identified as a gene that shows reduced mRNA expression in meningioma cell lines as compared to normal

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cells (Murphy et al. 1993). IGFBP7 shares high homology with IGFBPs and binds to IGF-I/II and insulin, but its binding affinity for IGFs is considerably lower than those of IGFBPs 1～6 (Collet and Candy 1998). It is possible that the IGFBPrP family may have unique physiological activities that do not involve regulating insulin and IGF1 activity (Nayak and Giudice 2003).

IGFBP7 has been shown to be expressed in all human tissues using a cDNA probe and RNA blot analysis (Oh et al. 1996). By immunohistochemistry, the previous study indicated that IGFBP7 localization was not universal; rather, its distribution in each of the organs was restricted to certain cell types. Immunostaining was absent or weak in several tissues, including the ovary, placenta, and prostate secretory epithelium, despite high levels of mRNA (Oh et al. 1996). Staining of peripheral nerves in all tissue specimens was always intense, thereby serving as a suitable internal positive control for immunoreactivity. Furthermore, the high level of IGFBP7 production in nerves is a likely explanation for the ubiquitous mRNA expression found in human tissue samples by Northern blotting (Oh et al. 1996). Our previous research demonstrated that IGF-I receptor immunoreactivity was increased in the CNS of $SOD1^{G93A}$ transgenic mice (Chung et al. 2003). Although The IGF-I signaling system is complex and regulated by IGF-I receptor and seven IGFBPs, little information is available on the distribution of IGFBP7 in the CNS. Therefore, we performed immunohistochemical studies to investigate the distribution of IGFBP7 in rat CNS.

Materials and Methods

Twelve adult (4～6 month old) Sprague-Dawley rats were examined in this study. The rats used in this study were treated in accordance with the 'Principles of Laboratory Animal Care' (NIH publication No. 86-23). The animals were perfused transcardially with cold phosphate buffered saline (PBS, 0.02M, pH 7.4), and then with ice-cold 4% paraformaldehyde. Brains were cryoprotected in a series of cold sucrose solutions, and were cut at 40 μm in the coronal plane. The sections were incubated using the free-floating method for 48～72 hrs at 4°C in primary antiserum containing Triton X-100 (0.3%), bovine serum albumin (0.5 mg/mL) and normal goat serum (3 drops/10 mL), and goat polyclonal anti-IGFBP7 antibody (sc-34795, Santa Cruz Biotechnology Inc.) Sections were visualized according to the avidin-biotin complex (ABC) method, using an ABC kit (Vectastain™, Vector Laboratories, Berlingame, CA), and then developed for peroxidase reactivity with 3,3′-diaminobenzidine (DAB) (Sigma, St. Louis, MO).

A sample of sections was reacted without any primary antiserum, whereas a different sample was reacted with a primary antiserum that had been preincubated for 24 hours with control antigen peptides. No sections from both groups exhibited any of the immunoreactivity described in this report (Fig. 1C). To observe the stained cells, a microscope (Leica DM4500B; Leica Microsystems, Germany) with a computer-driven digital camera (DFC320; Leica Microsystems) was used.

Results

In the present study, IGFBP7 immunoreactivity was obvious in the cerebral cortex, hippocampal regions, several nuclei of brainstem, cerebellum and spinal cord. In the cerebral cortex and hippocampus, heavily stained neurons were seen in layers II-VI in the parietal association cortex (Fig. 1A) and infralimbic cortex (Fig. 1B). There was IGFBP7 immunoreactivity in the cell bodies and apical dendrites of the pyramidal cells in several regions, including cingulate cortex and piriform cortex. It was noted that the pyramidal cells

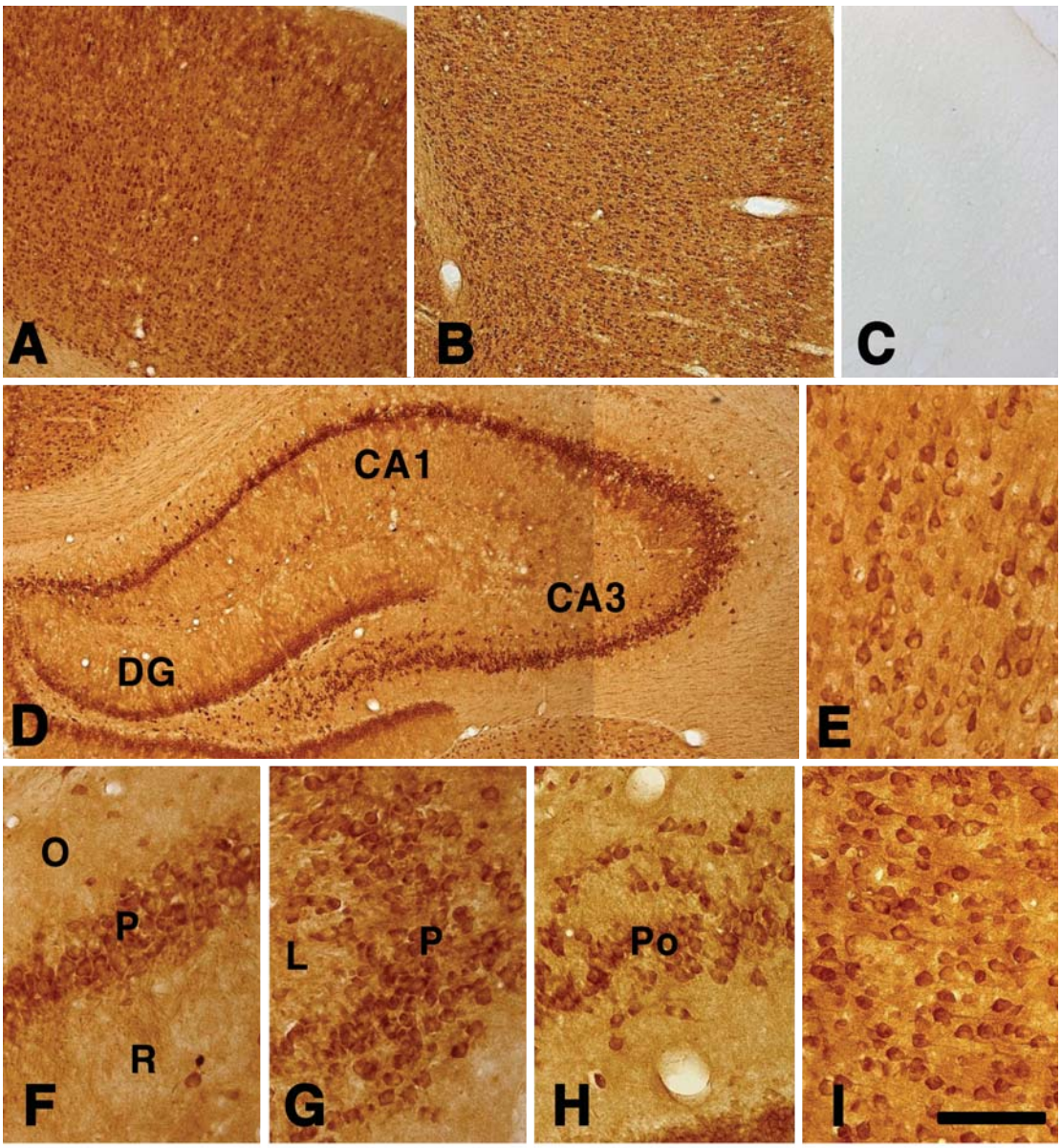

Fig. 1. Cellular localizations of IGFBP7-immunoreactive neurons in the cerebral cortex (A, B, C, E, I) and hippocampus (D, F-H). Heavily stained neurons were seen in the parietal association cortex (A) and infralimbic cortex (B). No sections reacted with a primary antiserum that had been preincubated for 24 hours with control antigen peptides exhibited any of the immunoreactivity described in this report (C). At a higher magnification, IGFBP7-immunoreactive cells illustrated typical morphology of pyramidal neurons in layer V (E, I). In the hippocampus, the pyramidal cells in CA1-3 region and granule cells in the dentate gyrus were strongly immunoreactive for IGFBP7 (D, F, G, H). CA1-3, fields CA1-3 of Ammon's horn; DG, dentate gyrus; L, stratum lucidum; O, stratum oriens; P, pyramidal cell layer; Po, polymorphic layer, R, stratum radiatum. Scale bars=100 μm (A-D); 50 μm (E-I).

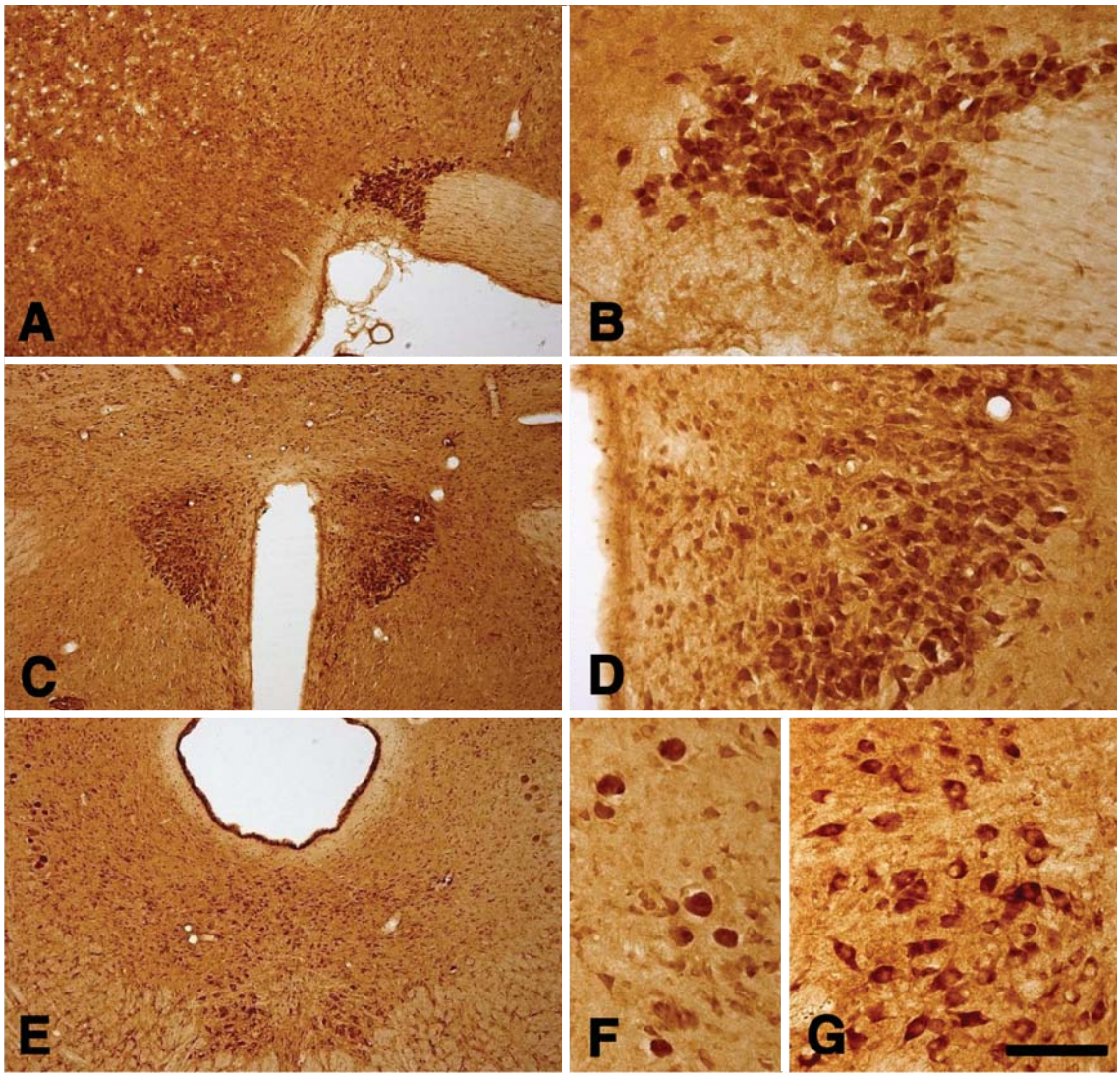

Fig. 2. Localizations of IGFBP7-immunoreactive neurons in the supraoptic nucleus (A, B), paraventricular nucleus (C, D), periaqueductal gray (E, F) and oculomotor nucleus (G). In several nuclei of hypothalamus and midbrain, strongly stained cell bodies and processes of some neurons were observed. Scale bars=100 μm (A, C, E); 50 μm (B, D, F, G).

in CA1-3 region were strongly immunoreactive for IGFBP7 in the hippocampus (Fig. 1D, F, G). In the dentate gyrus, immunoreactivity for IGFBP7 was also observed in the granule cell layers and polymorphic layers (Fig. 1D, H). High magnification of IGFBP7-immunoreactive cells illustrated typical morphology of pyramidal neurons in layer V (Fig. 1E, I). Strong immunoreactivity was also found in the cell bodies and processes of some medium to large-sized neurons in the supraoptic nucleus (Fig. 2A, B), paraventricular nucleus (Fig. 2C, D), periaqueductal gray (Fig. 2E, F) and oculomotor nucleus (Fig. 2G). In the cerebellar

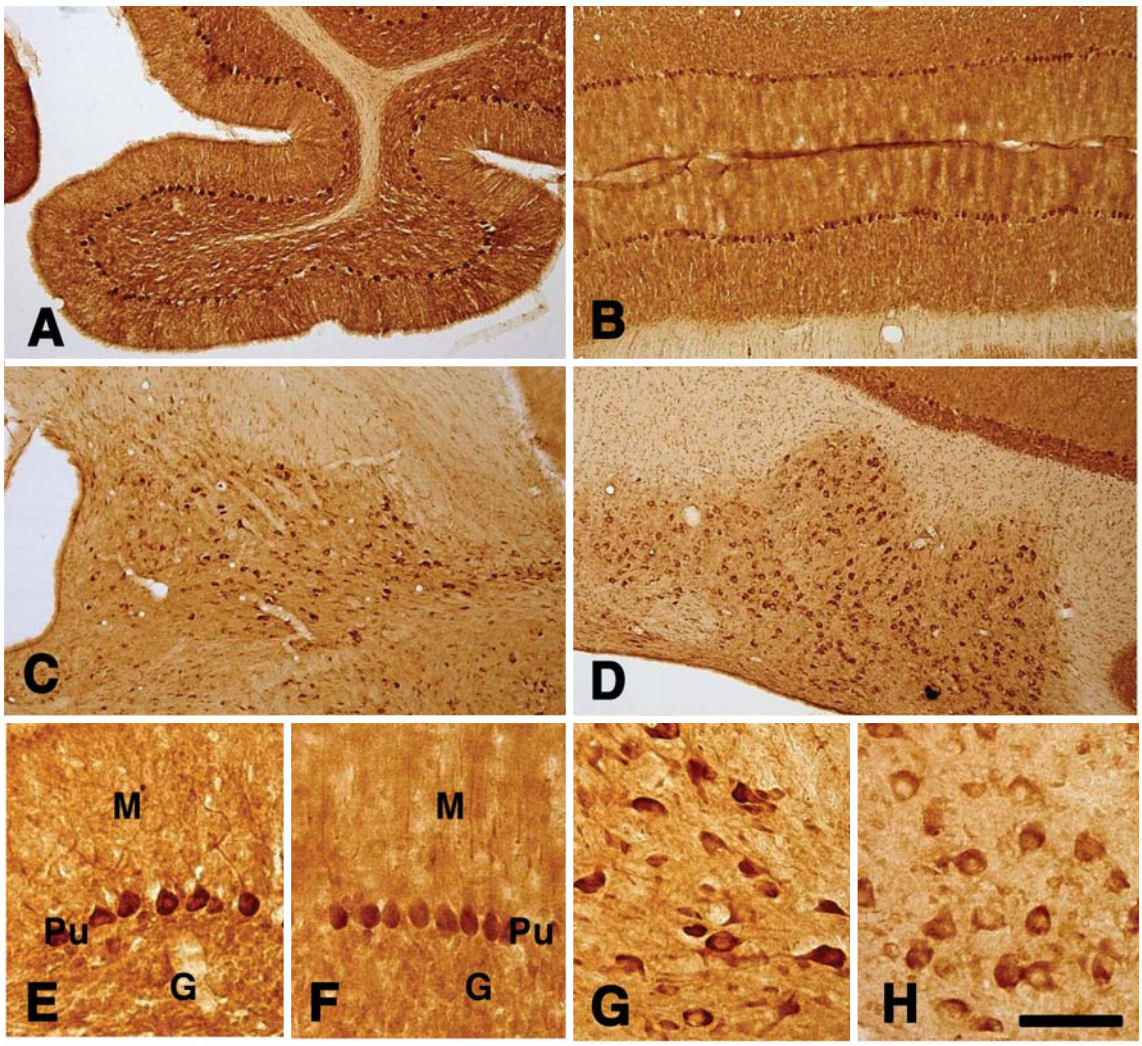

Fig. 3. Localizations of IGFBP7-immunoreactive cells in the cerebellar cortex (A, B, E, F), superior vestibular nucleus (C, G) and interposed nucleus (D, H). In the cerebellar cortex, IGFBP7 immunoreactivity was prominent in the Purkinje cell bodies and dendrites (A, B, E, F). The cell bodies of cerebellar output neurons showed moderate immunoreactivity for IGFBP7 in the nucleus medialis, interpositus and lateralis (D, H). G, granular layer; M, molecular layer; Pu, Purkinje cell layer. Scale bars=100 μm (A-D); 50 μm (E-H).

cortex, IGFBP7 immunoreactivity was prominent in the Purkinje cell bodies and dendrites (Fig. 3A, B, E, F). The cell bodies of cerebellar output neurons showed moderate immunoreactivity for IGFBP7 in the nucleus medialis, interpositus and lateralis (Fig. 3D, H). In the brainstem, IGFBP7-immunoreactive neurons were prominent in the superior vestibular nucleus (Fig. 3C, G), cochlear nucleus (Fig. 4A, C), trigeminal motor nucleus (Fig. 4B, D), nucleus of the trapezoid (Fig. 4E), and facial nucleus (Fig. 4F). IGFBP7-immunoreactive neurons were also observed mainly in the anterior horn of the spinal cord (Fig. 4G-I).

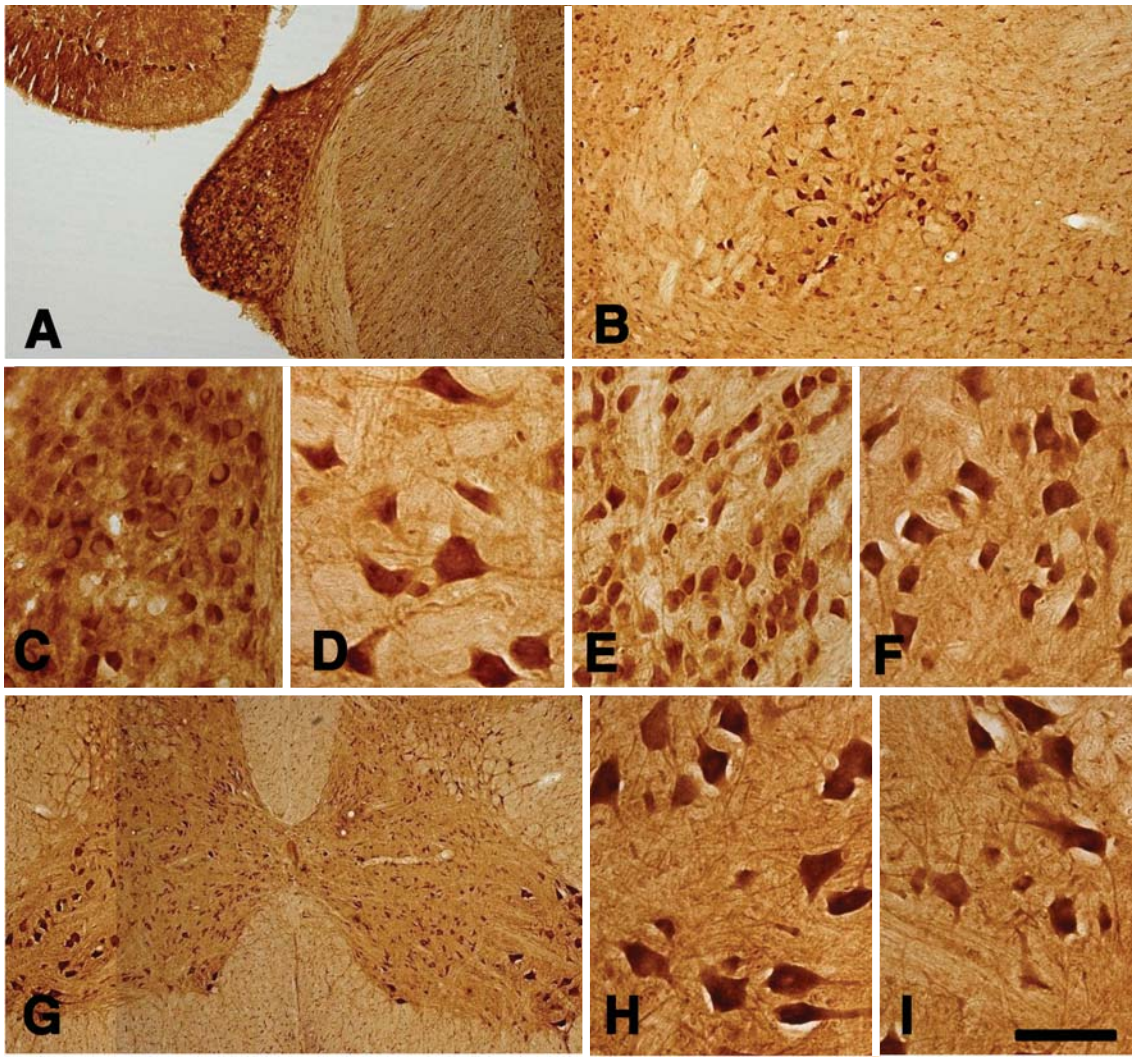

Fig. 4. Localizations of IGFBP7-immunoreactive neurons in the brainstem (A-F) and spinal cord (G-I). It was noteworthy that IGFBP7-immunoreactive neurons were prominent in the several nuclei of brainstem, including the cochlear nucleus (A, C), trigeminal motor nucleus (B, D), nucleus of the trapezoid (E), and facial nucleus (F). IGFBP7-immunoreactive neurons were observed mainly in the anterior horn of the spinal cord (G-I). Scale bars=100 mm (A, B, G); 50 mm (C-F, H, I).

Discussion

Tightly regulating the actions of IGFs are structurally distinct IGFBPs that can inhibit or promote IGF effects by preventing receptor interaction or by transporting IGF to target cells. Regulatory molecules of the IGF-system are known to be differentially expressed in the course of neurodegenerative diseases. The present results showed that IGFBP7-immunoreactive neurons were observed in the cerebral cortex, hippocamus, brainstem, cerebellum and spinal cord. As

shown for the other IGFBPs in the CNS, the immunostaining of IGFs is co-localized with staining for IGFBPs, suggesting a mechanism to locally increase the amount and bioavailability of IGFs (D'Ercole et al. 1996). On the other hand, strong staining of glial cells was observed in the brain (astrocytes and oligodendrocytes) and spinal cord of normal human tissue (Degeorges et al. 2000). A previous report suggested that IGF-I is involved in an autocrine proliferation loop in the growth of astrocytes (Han et al. 1992). Saneto et al. (1988) showed that IGF-I also regulates the differentiated function of oligodendrocytes by increasing the synthesis of myelin basic protein. These previous results on the localization of IGFs and IGFBPs in glial cells were not in agreement with ours, which may be due to differences in the relative ages and the animal strains used.

IGFBP7 has also been implicated in cell senescence and tumor suppression (Sprenger et al. 1999, 2002), although it has also been shown to enhance the growth of fibroblastic cells (Akaogi et al. 1996). IGFBP7 has the ability to stimulate prostacyclin production in vascular endothelial cells (Yamauchi et al. 1994), thereby controlling vascular permeability. Furthermore, IGFBP7 accumulates in small blood vessels from tumor tissue (Akaogi et al. 1996), providing a mechanistic link to tumor survival by increasing vessel size and permeability. Recently, Girard et al. (1999) showed that IGFBP7 is a marker of high endothelial venules in an area of cell junctions that might be involved in the control of lymphocyte emigration. Therefore, IGFBP7 may be directly involved in the regulation of cell growth, differentiation, or migration in an IGF-independent manner.

The wide variety of IGF-I actions during CNS development and maintenance are dependent on a number of factors including developmental stage, microenvironmental conditions, tissue specific properties and proper regulation in concert with other growth factors (Anlar et al. 1999, Russo et al. 2005). A dominating role in IGF regulation is exerted by IGFBPs which govern and co-ordinate tissue specificity of IGF-I receptor signaling. Although each of the IGF system components bears the capacity to dictate IGF actions, IGFBPs appear to be key factors in IGF regulation in the CNS and during several pathological conditions of the CNS. IGFBP7 appeared to be related with IGF-I during cerebellar development. IGF-I is transiently expressed in large projection neurons and Purkinje cells in the cerebellum (Lee et al. 1992), which was consistent with the localization of IGFBP7. Because IGF-I is essential for proper CNS development, this mechanism may possibly illustrate an attempt to maintain normal CNS development under conditions of undernutrition.

IGFBP activity is generally controlled in part by IGFBP-degrading proteases, including matrix metalloproteinases (Hwa et al. 1999, Wang and Chard 1999, Nayak and Giudice 2003); however, IGFBP7 activity may also be controlled by other mechanisms. The release of free IGFBP7 either activates it or makes it accessible to posttranslational modifications that influence its activity. Supporting this notion is the study by Ahmed et al. (2006), who reported that a membrane-bound serine proteinase, matriptase, cleaves IGFBP7 to produce a 25-kDa short form that has a different character when compared to one of 31 kDa. Finally, IGFBP7 might undergo proteolysis, as shown for IGFBP3, for which proteolytic fragments can have IGF-independent actions through specific receptors (Oh et al. 1993, Leal et al. 1997). Obviously, many of the biological roles of IGFBP7 remain to be elucidated, including an investigation of its proteolysis.

In conclusion, this study provides the first immunohistochemical results concerning the differential regulation of IGFBP7 in the CNS. Neurodegenerative diseases, malignancies and injuries to the CNS display alterations in levels of a variety of cytokines and growth factors including IGFs and their IGFBPs. Although expression patterns for IGFBP7 were not reported

under certain pathological states, conditions of the CNS often display regulation of IGFBPs in conjunction with alterations of IGF-I or IGF-II expression, indicating a pivotal role for this binding protein in directing IGF actions in damaged tissue. Therefore, these detailed morphological data may provide a framework for further studies that examine its actions in the normal and degenerated nervous system.

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흰쥐 중추신경계에서 IGFBP7의 분포에 관한 면역조직화학적 연구

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간추림 : 본 연구에서는 흰쥐 중추신경계에서 insulin-like growth factor binding protein 7 (IGFBP7) 의 분포를 규명하고자 면역조직화학적 연구를 시행하였다.

실험에는 4 ~ 6개월 된 성숙 흰쥐가 사용되었고 IGFBP7에 대한 항체를 이용하여 면역조직염색을 실시하였다.

연구결과, IGFBP7 면역염색성은 대뇌 겉질, 해마, 뇌줄기, 소뇌 및 척수에서 관찰이 되었다. 대뇌 겉질에서는 겉질의 II ~ VI층에서, 해마에서는 CA1-3 부위의 피라미드 세포들에서 IGFBP7에 대해 강한 면역염색성을 나타내는 신경세포들이 관찰되었다. 시각로위핵, 뇌실곁핵, 중간뇌수도관주위회색질 및 눈돌림신경핵에서도 강한 면역염색성을 나타내는 신경세포들이 발견되었다. 소뇌에서는 조롱박세포와 소뇌핵 신경세포에서, 뇌줄기에서는 위안뜰핵, 달팽이핵, 삼차신경 운동핵, 마름체핵 및 얼굴신경핵에서 IGFBP7 면역염색성을 나타내는 신경세포들이 관찰되었다. 척수에서는 주로 앞뿔에 위치하는 운동신경세포에서 강한 면역염색성을 나타냈다.

본 연구에서 밝혀진 IGFBP7의 중추신경계 분포는 앞으로의 구조적, 기능적 연구에 유용한 자료가 될 것이라 사료된다.

찾아보기 낱말 : Insulin-like growth factor binding protein 7 (IGFBP7), 대뇌 겉질, 해마, 뇌줄기, 소뇌, 척수

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