Synectin in the nervous system: expression pattern and potential as a binding partner of neurotrophin receptors

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Abstract To assess the potential for functional interaction between synectin and neurotrophin receptors (Trk receptors) in the nervous system, we characterized synectin expression in the rat brain. Synectin is widely expressed in the brain and its expression levels are regulated both temporally and spatially, correlating with those of Trk receptors. Biochemical studies indicated that synectin interacts with TrkB but not with TrkC in the developing brain. We also found that axotomized motoneurons upregulate synectin mRNA expression as well as TrkB mRNA. These data suggest that synectin plays a role in neural development and regeneration in association with TrkB.

Keywords: GIPC; PDZ protein; Axotomy; Development

1. Introduction

Synectin (also designated GIPC, SEMCAP-1 or GLUT1-CBP) is a PDZ protein that contains a central PDZ domain and a C-terminal acyl carrier domain, and has been identified as an interaction partner of syndecan-4, RGS-GAIP, SemaF, and glucose 1 transporter [1–4]. Synectin is widely expressed in mammalian tissues, including the brain [1–4]. Electron microscopic studies have defined the localization of synectin on clathrin-coated vesicles, suggesting a role of synectin in the process of endocytosis [2,5]. However, intracellular functions of synectin are still poorly understood.

Synectin has been reported to have the ability to interact with neurotrophin receptors, which are receptor tyrosine kinases (Trk receptors) [6]. Neurotrophins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3), and neurotrophin 4/5 (NT4/5), act to promote the growth, differentiation, and survival of neurons in the peripheral nervous system (PNS) and central nervous system (CNS) [7]. In addition, recent studies have shown a role of neurotrophins in synapse development and plasticity [8]. Neurotrophins exert their functions by interacting with different subsets of Trk receptors: NGF binds to TrkA, BDNF and NT4 to TrkB, and NT3 to TrkC. Interaction of neurotrophins with Trk receptors triggers multiple signal pathways and initiates internalization of the ligand–receptor complexes. Internalization of the ligand–Trk receptor complex is critical for some biological functions of neurotrophins [9,10]. The molecular mechanisms governing the process of internalization are not well understood. However, clathrin-dependent endocytosis is likely involved in this process [10,11]. It is therefore conceivable that synectin may associate with internalized ligand–Trk complex in clathrin-coated vesicles.

To better understand the functional relevance of interactions between Trk receptors and synectin, we characterize here synectin expression in developing and mature rat brain and study the interaction between synectin and Trk receptors in the CNS. Furthermore, to investigate functional relevance of Trk receptors and synectin in the PNS, we use an animal model with facial nerve injury, which is known to cause upregulation of BDNF and TrkB [12], and examine gene regulation of synectin mRNA in this model.

2. Materials and methods

2.1. Animals

For the developmental study, Wistar male rats of postnatal day one, three, seven, 14, 21 and 28 days, and 8 week ($n = 3$ at each time point) were used. For the regeneration study, male Wistar rats (150 g) were subjected to the operation described in the next section.

2.2. Motor nerve transection

The left facial nerve was transected under pentobarbital anesthesia. The facial nerve was transected at a point just distal to the posterior auricular branch and about 5 mm of the distal nerve segment was removed. Following a postoperative time of 1, 3, 7, 14, 21 or 28 days, the animals ($n = 5$ to 6 at each time point) were subjected to analysis by in situ hybridization histochemistry.

2.3. Immunohistochemistry

Wistar male rats (200 g) were deeply anesthetized and intracardially perfused with 4% paraformaldehyde in PBS. Brains were postfixed in the same fixative overnight and cryoprotected in 30% sucrose in PBS. Frozen sections (18 μm thick) were cut on a cryostat, blocked 3 h at room temperature in PBS containing 0.3% Triton-X, 1% goat serum, and 1% albumin, and then incubated in the following solutions at 4 °C: anti-synectin antibody (1:1000) in blocking solution for 2 days, 0.1 M PBS for 30 min, biotinylated goat anti-rabbit antiserum (1:500, Vector Laboratories) in blocking buffer overnight, washed in PBS for 30 min, VECTASTAIN ABC reagent (Vector Laboratories) for 1 h, and finally visualized with diaminobenzidine (DAB) as a substrate. Control sections that underwent the same procedure except for the incubation with anti-synectin antibody did not show any immunoreactivity (data not shown).

2.4. In situ hybridization histochemistry

Animals were deeply anesthetized with diethylether and sacrificed. The brains were immediately removed, embedded in OCT, and quickly

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frozen with powdered dry ice. Frozen sections (18 μm thick) were cut on a cryostat and kept at −20 °C until use. The synectin mRNA-specific oligonucleotide probe, corresponding to nucleotide positions 86–125 of the rat synectin cDNA (Accession No. AF089817), was labeled with [^{35}S]dATP using terminal deoxynucleotidyl transferase. Subsequent procedures were described in detail elsewhere [13]. To compare the data for different animals at each time point, hybridization for all animals was done at the same time and with the same probe. The sections were exposed to BIOMAX MR film (Kodak) for 7 days and thereafter dipped in NBT2 nuclear track emulsion (Kodak) followed by exposure in a dark box for three weeks. Some slides were then stained with Thionine. In competition experiments in which sections were hybridized with a 300-fold excess of a non-labeled probe, hybridization signals were virtually absent. For quantitative analysis of the results, we counted silver grains located on typical five facial motoneurons in the control and operated sides and performed statistical analysis.

2.5. Immunoblotting, subcellular fractionation and coimmunoprecipitation

Brain samples were homogenized in lysis buffer [50 mM Tris, pH 7.4, 150 mM NaCl, 1 mM DTT, 1.5 mM MgCl2, 4 mM EDTA, 10% (v/v) glycerol and complete protease inhibitor cocktail (Roche Diagnostics), containing 1% (v/v) Triton X-100] using a Teflon Potter-Elvehjem homogenizer. Homogenates were kept on ice for 60 min and cleared by centrifugation at 15 000 rpm for 30 min. The supernatant was collected and its protein concentration was measured by the Bradford method. The sample was separated by SDS–PAGE and transferred to a nitrocellulose membrane (Schleicher & Schüell). The membrane was blocked with 5% (w/v) non-fat dried milk powder in PBS for 60 min followed by a 12 h incubation with primary antibodies. After washing, bound Igs were visualized with horseradish peroxidase-conjugated secondary antibodies using the ECL system (Pierce).

Subcellular fractionations of rat brains were prepared as described by Li et al. [14]. In brief, whole brains were homogenized in 4 mM HEPES, pH 7.4, containing 0.32 M sucrose and proteinase inhibitors, using a Teflon Potter-Elvehjem homogenizer. Homogenates were centrifuged at 800×*g* for 10 min to yield pellet P1. The supernatant was collected and centrifuged at 9200×*g* for 15 min, yielding a pellet (P2) and supernatant (S2). The S2 fraction was further centrifuged at 165 000×g for 60 min. The resulting supernatant represents the soluble fraction (S3). The P2 pellet was resuspended in homogenization buffer and represents a crude synaptosome fraction. The crude synaptosome fraction was lysed by adding 10 vol. of ice-cold water containing protease inhibitors followed by homogenization with a glass-Teflon homogenizer. The synaptic membrane fraction (LP1) was collected from this homogenate by centrifugation at 25 000×*g* for 20 min, and the supernatant was further centrifuged at 165 000×g for 60 min to give crude synaptic vesicle pellet (LP2) and supernatant (LS2) fractions. For coimmunoprecipitation, LP1 and LP2 fractions were collected from the brain of a P14 rat and homogenized in TNE buffer [10 mM Tris, pH 8.0, 150 mM NaCl, 1mM EDTA, 1% NP-40 and complete protease inhibitor cocktail (Roche Diagnostics)]. The homogenates were cleared by centrifugation at 15 000 rpm for 30 min and the supernatant was incubated for 1 h at 4 °C with anti-Trk antibody or non-immunized rabbit whole IgG fraction (control) followed by incubation with protein A–Sepharose (Amersham) for an additional 1 h at 4 °C. The beads were washed extensively with TNE buffer and proteins retained on the beads were eluted with SDS–PAGE sample buffer. Anti-Trk antibody (C-14) that recognizes full-length TrkA, TrkB, and TrkC was purchased from Santa Cruz Biotechnology. Anti-synectin antibody was kindly provided by Prof. M. Simons [1].

2.6. Yeast two-hybrid analyses

The DupLexA yeast two-hybrid system (Origene) was used for interaction analyses. The cDNA fragment encoding the juxtamembrane region of TrkB (residues 458–543) or TrkC (residues 459–545) was amplified by polymerase chain reaction from a rat brain cDNA library (Origene). The products were subcloned in frame into the inducible pGilda bait plasmid to generate pGilda-TrkB and pGilda-TrkC, and verified by DNA sequencing. The pJG4-5 prey plasmid including a cDNA fragment encoding synectin (residues 119–333) or PSD-95 (residues 33–724) was obtained by yeast two-hybrid screening which we performed previously [15].

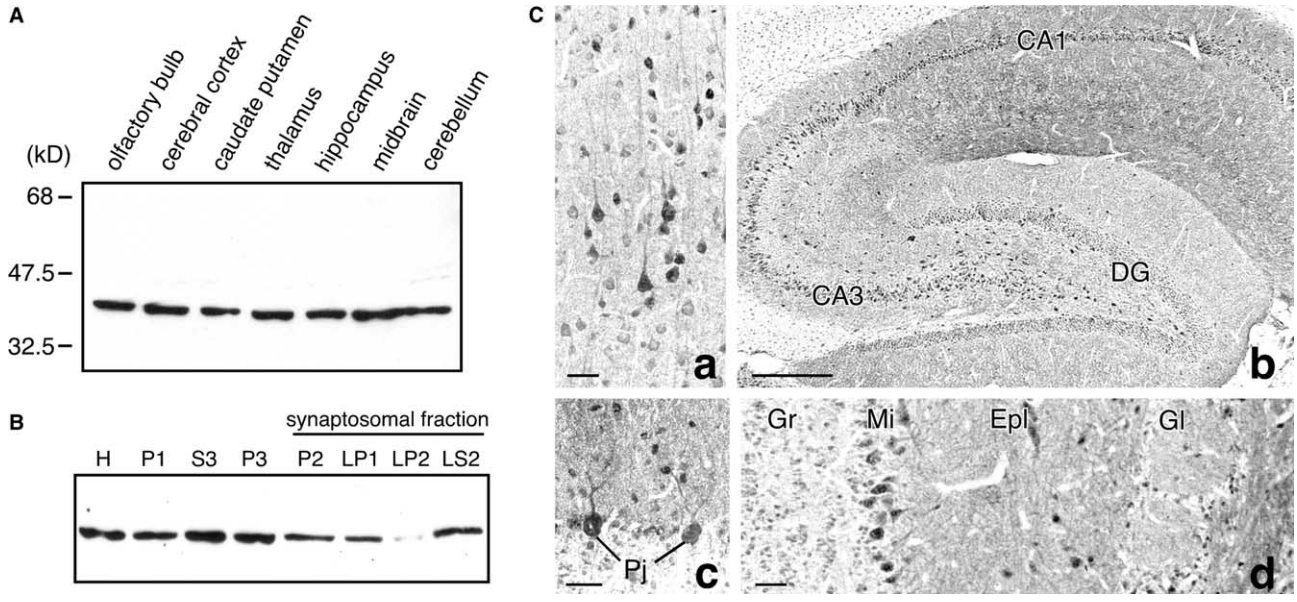


Fig. 1. Synectin expression in the adult CNS. (A) Western blot analysis showing a wide-spread expression of synectin in the CNS. 30 μg of the respective protein extracts from brain was loaded per lane. The anti-synectin antibody recognized a band corresponding to 40 kDa. (B) Subcellular fractionations of the adult rat brain were performed as described in Section 2. 8 μg of protein was loaded in each lane. Synectin is abundant in the cytoplasmic soluble fraction (S3). In the synaptosomal fraction, synectin is enriched in the high-speed supernatant of synaptosomal fraction (LS2) but barely detectable in the synaptic vesicle fraction (LP2). (C) Immunohistochemical localization of synectin. Strong immunoreactivity for synectin was observed in the pyramidal cells of cerebral cortex (a), hippocampal pyramidal cells (b), Purkinje cells in the cerebellum (c), and mitral cells in the olfactory bulb (d). Scale bar = 50 μm (a, c, d), 400 μm (b). Abbreviations: DG, dentate gyrus; Epl, external plexiform layer; Gl, glomerular layer; Gr, granule cell layer; Mi, mitral cell layer; Pj, Purkinje cell.

3. Results and discussion

3.1. Regional distribution of synectin protein in the adult brain

Several studies have documented the wide tissue distribution of synectin mRNA and protein, with highest levels of expression being found in the brain, lung, and heart [1–4]. To determine the regional distribution of synectin in the adult rat CNS, 1% Triton-X soluble proteins were collected from the olfactory bulb, caudate putamen, cerebral cortex, thalamus, hippocampus, midbrain, and cerebellum, and subjected to Western blot analysis (Fig. 1A). On Western blots, anti-synectin antibody reacted with a ca. 40 kDa protein that corresponds to the apparent molecular mass of synectin determined in previous studies [1,3,4,6]. As indicated in Fig. 1A, synectin was widely expressed in the adult brain and we did not find significant differences in the levels of synectin expression among brain regions.

To characterize the subcellular localization of synectin, we fractionated homogenates of adult brain according to a standard procedure (Section 2.5) [14,16] and analyzed the fractions with the same antibody (Fig. 1B). Synectin was abundant in the soluble S3 fraction and the high-speed supernatant (LS2), and in the crude synaptosomal fraction (P2), suggesting the presence of large cytosolic pools of synectin in the brain. In the P2 fraction (9200 g pellet), synectin was also present in the membrane fractions, but primarily associated with synaptic membranes (LP1) and barely detected in the small vesicle fraction (LP2).

To better define the cells that contain synectin in the brain, we performed immunohistochemistry (Fig. 1C). On tissue sections from the adult rat brain, synectin immunoreactivity was observed in neuron populations throughout the brain. Strong immunoreactivity for synectin was detected in the cytoplasm of neurons and this was in agreement with the data showing the abundance of synectin in the soluble S3 fraction. The most prominent expression was observed in the mitral cells of olfactory bulb (Fig. 1C-d), pyramidal neurons of cerebral cortex (Fig. 1C-a), pyramidal cells in the hippocampus (Fig. 1C-b), Purkinje cells in the cerebellum (Fig. 1C-c), and neurons in the cerebellar nuclei (data not shown). Dendrites of cerebral pyramidal neurons and Purkinje cells were clearly labeled.

3.2. Developmental expression of synectin in the rat brain

To examine developmental changes in synectin expression, total brain homogenates of several developmental stages were analyzed by Western blotting (Fig. 2). Synectin expression was low in the neonatal brain, but markedly increased during the first three days. High levels of synectin expression were observed at P3, P7, and P14. Thereafter, synectin expression decreased and moderate levels of expression were observed in adulthood. Subsequent in situ hybridization histochemistry confirmed and extended these findings. Synectin mRNA was ubiquitously expressed in the brain, while expression levels were significantly changed postnatally (Fig. 3). At P0, synectin mRNA expression was very weak in all brain regions examined, which contrasted to intense signals in the olfactory

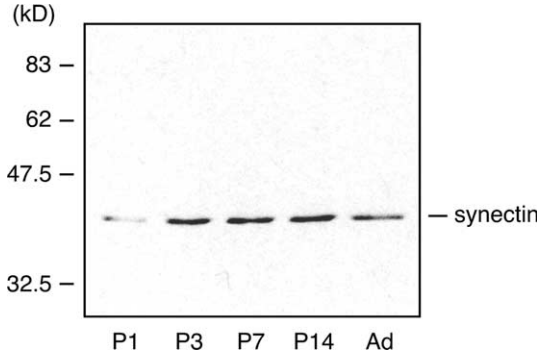


Fig. 2. Synectin expression during postnatal CNS development. Western blot analysis showing relative protein levels for synectin in the developing brain. 30 μg of total brain extract was separated on 7.5% SDS–PAGE and blotted on a nitrocellulose membrane. Lanes correspond to postnatal days (P1, P3, P7, P14, and adulthood).

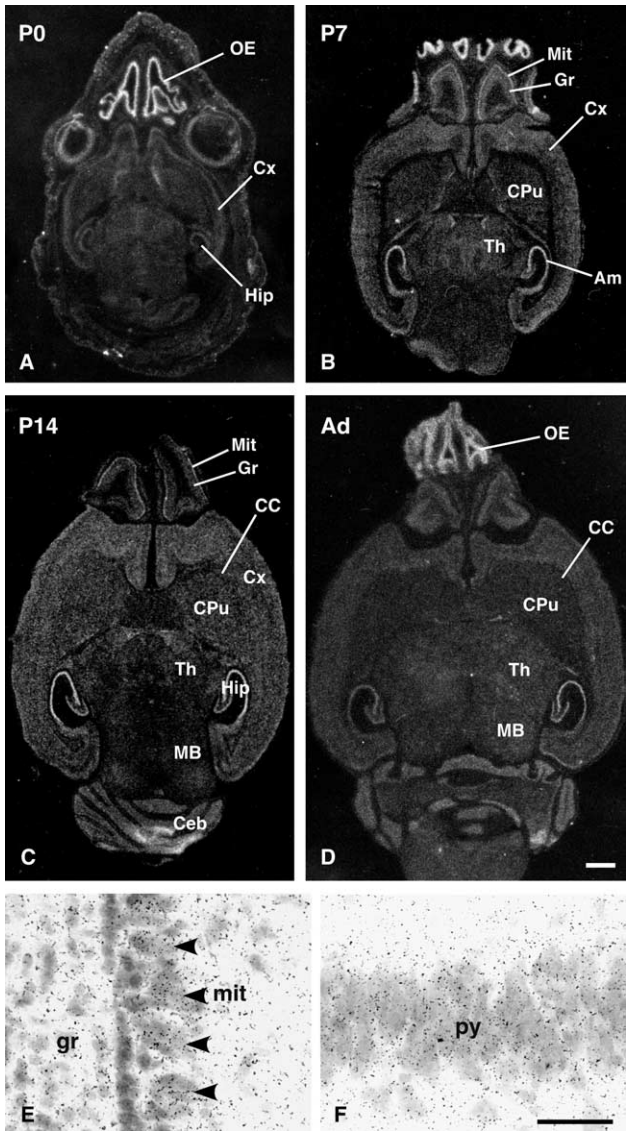


Fig. 3. Synectin mRNA expression in the postnatal CNS. (A–D) Horizontal brain sections at P0 (A), P7 (B), P14 (C), and in adult (D) showing in situ hybridization signals for synectin mRNA. Scale bar = 1 mm. (E,F) Bright-field micrographs showing accumulation of hybridization signals on the mitral cells (mit) in the olfactory bulb (E) and pyramidal cells (py) in the hippocampus (F). Scale bar = 50 μm. Abbreviations: Am, Ammon's horn of hippocampus; CC, corpus callosum; Ceb, cerebellum; CPu, caudate putamen; Cx, cerebral cortex; Gr, granule cell layer of olfactory bulb; gr, granule cell; Hip, hippocampus; MB, midbrain; Mit, mitral cell layer of olfactory bulb; olfactory bulb; OE, olfactory epithelia; Th, thalamus.

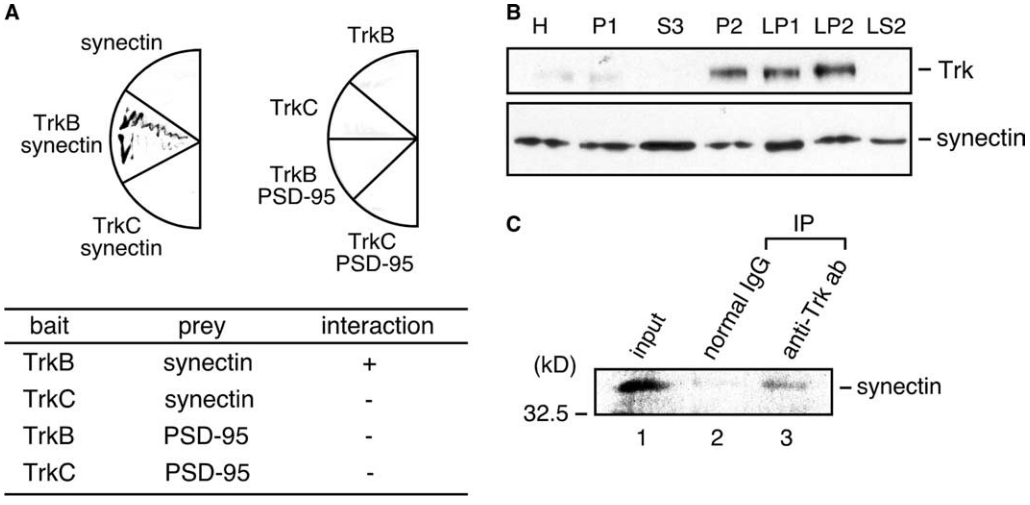
epithelia (OE) (Fig. 3A). During the first postnatal week, synectin expression was dramatically upregulated with regional differences in the signal intensity. At P7, high signal levels were detected in the hippocampus and olfactory bulb. In particular, Ammon's horn of the hippocampus (Am) and the mitral cell layer (Mit) of the olfactory bulb were intensely labeled with the probe. Moderate signal levels were detected in the granule cell layer (Gr) of olfactory bulb, cerebral cortex (Cx), and thalamus (Th) (Fig. 3B). Signal levels reached their postnatal maximum at P14. At this time, significant levels of synectin mRNA expression were also observed in the caudate putamen (CPu) (Fig. 3C). Hybridization signals were distributed over the neuronal population throughout the brain (Fig. 3E and F), in good agreement with the immunohistochemistry analysis. Thereafter, synectin mRNA expression was reduced in most brain regions. In the adult brain, intense signals remained only in the Ammon's horn of the hippocampus (Fig. 3D). Synaptogenesis takes place extensively in the first few weeks after birth [17], and adult synaptic plasticity has been well studied in the cerebral cortex and hippocampus [18]. Thus, expression patterns of synectin in the developing and matured brains suggest that synectin might be involved in synapse development and synaptic plasticity.

3.3. *In vivo association of Trk and synectin*

We are interested in the fact that synectin has the ability to interact with neurotrophin receptors, TrkA and TrkB [6]. However, it is still unclear whether Trk receptors interact with synectin in the CNS. To consider this, we first asked whether these two molecules are present in the relevant locations. The expression of Trk receptors in the CNS has been studied [19]. In contrast to the very restricted localization of TrkA, TrkB and TrkC are widely distributed in the adult CNS. In particular, TrkB and TrkC are highly expressed in the hippocampus, cerebellum, and cerebral cortex. In addition, expression of TrkB and TrkC mRNA is developmentally regulated, and transiently exhibits peak levels during the first two postnatal weeks [20]. Spatio-temporal expression patterns for TrkB and TrkC were thus seemingly correlated and synchronized with those of synectin (Fig. 3).

Lou et al. [6] have demonstrated that synectin (GIPC) is a binding partner of TrkA and TrkB, and have shown that the synectin binding site is located in the juxtamembrane region of these Trk receptors. However, it has not yet been determined whether TrkC also has the ability to interact with synectin. To test this, we generated bait constructs encoding a juxtamembrane region of TrkB or TrkC, and performed yeast two-hybrid analyses (Fig. 4A). As expected, we could detect the interaction between TrkB and synectin. However, despite structural homology in the juxtamembrane region between TrkB and TrkC, no interaction was found between TrkC and synectin. Synectin has a central PDZ domain through which it binds to TrkB [6]. To further investigate the specificity of binding between synectin's PDZ domain and TrkB, we also tested the binding ability of TrkB to PSD-95, which involves three PDZ domains within the PSD-95 structure. As shown in Fig. 4A, no interaction could be detected between TrkB and PSD-95, suggesting that the interaction between TrkB and synectin's PDZ domain is highly specific.

In the adult brain, we detected only a small amount of synectin in the LP2 fraction, where small vesicles in the synapse are primarily recovered [16]. We speculated that if synectin associates with internalized ligand–Trk complex in clathrin-coated vesicles, synectin and Trk receptors should be enriched in the LP2 fraction of the developing brain. In fact, we found that synectin is present mainly as a membrane-associated form (LP1 and LP2) in the developing synapses (Fig. 4B), in contrast to the adult brain where lesser amounts of synectin are found in these fractions (Fig. 1B). To examine in vivo interaction between synectin and Trk receptors, coimmunoprecip-



bait	prey	interaction
TrkB	synectin	+
TrkC	synectin	-
TrkB	PSD-95	-
TrkC	PSD-95	-

Fig. 4. Biochemical interaction between synectin and Trk. (A) Yeast two-hybrid analyses of Trk receptor (TrkB and TrkC) interaction with synectin and PSD-95. Positive interactions were determined by β-galactosidase assay and cell growth on the selection plates (–Ura, –Trp, –His, –Leu). Only TrkB and synectin show an interaction. (B) Subcellular localizations of Trk receptors and synectin in P14 rat brain. 10 μg of protein was loaded in each lane. Note that significant levels of synectin were recovered in the LP2 fraction. (C) Coimmunoprecipitation of synectin by anti-Trk antibody. LP1 and LP2 fractions were prepared from P14 rat brain, incubated with anti-Trk Ab or non-immunized whole rabbit IgG fraction (control) and precipitated with protein A–Sepharose. Proteins retained on the beads were eluted, separated on 10% SDS–PAGE and immunoblotted with anti-synectin Ab. Synectin and Trk were coprecipitated with anti-Trk Ab (lane 3), but not with non-immunized rabbit IgG (lane 2). Lane 1 shows synectin immunoreactivity in the input sample.

itation was carried out on LP1 and LP2 fractions of P14 rat brain. Anti-Trk antibody precipitated synectin (Fig. 4C), while non-immunized rabbit whole IgG fraction did not, supporting the idea that synectin interacts with Trk receptors in vivo. Taken together, our results suggest that synectin interacts with TrkB in the clathrin-coated vesicles and that this interaction may be necessary for BDNF signaling and functions in synapse development and plasticity.

3.4. Upregulation of synectin mRNA following motor nerve transection

Kobayashi et al. [12] have documented that BDNF and TrkB are upregulated by facial nerve transection. Given that synectin is functionally associated with TrkB in the PNS, synectin mRNA may also be upregulated after motor nerve transection. Based on this hypothesis, we examined gene regulation of synectin mRNA following motor nerve transection (Fig. 5). After nerve transection, synectin mRNA was promptly upregulated in the facial nucleus on the operated side and enhanced hybridization signals were already observed at postoperative day 1 (Fig. 5A). Expression levels of synectin mRNA were increased during the first postoperative week and reached a maximum around postoperative day 7, with about 4-fold greater hybridization signals compared to the control side. In the second postoperative week, synectin mRNA expression began to decline and returned to the control level at postoperative day 28. Nerve transection did not affect the levels of synectin mRNA expression in the facial nucleus on the contralateral side. Enhanced signals for synectin mRNA were exclusively accumulated on the facial motoneurons (Fig. 5B). Fig. 5C summarizes the changes in expression levels of synectin mRNA after facial nerve transection. To verify that the upregulation of synectin gene expression is also induced in another peripheral motor system, we performed the same examination using a model of masseteric nerve transection, and found that synectin mRNA was also upregulated in the axotomized trigeminal motoneurons (data not shown). These results indicated that upregulation of synectin gene is possibly

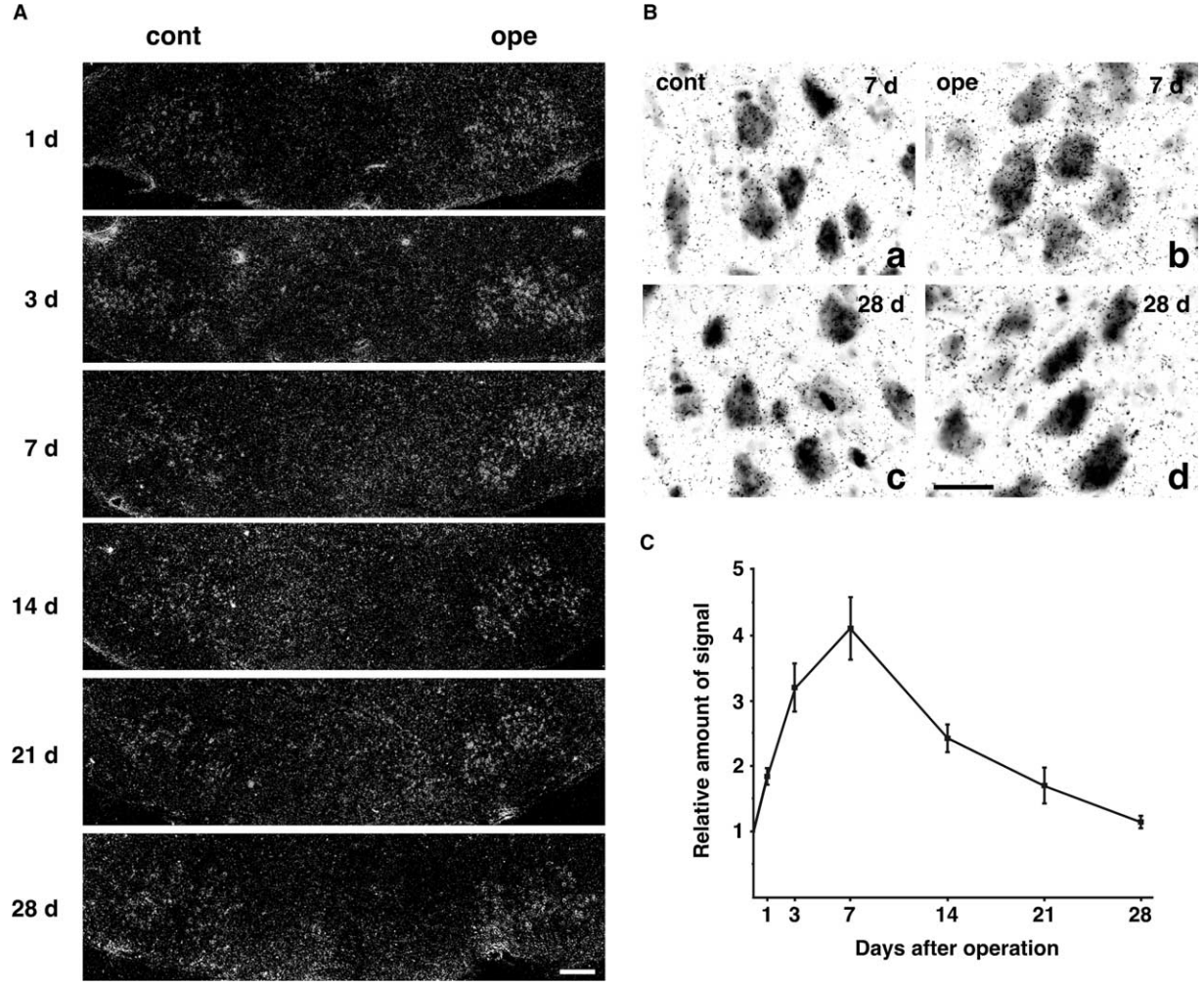


Fig. 5. Upregulation of synectin mRNA expression in facial motoneurons following facial nerve transection. (A) Dark-field micrographs showing changes in synectin mRNA expression in facial nucleus at postoperative day 1, 3, 7, 14, 21, and 28. Nerve transection induced transient enhancement of synectin mRNA expression in the facial nucleus on the operated side (right), but did not affect expression levels on the control side (left). Scale bar = 500 μm. (B) Bright-field micrographs showing the localization of enhanced hybridization signals on the axotomized facial motoneurons. Scale bar = 50 μm. (C) Changes in the levels of hybridization signals after nerve transection. The number of silver grains on a facial motoneuron was counted on each side and the ratio of counts between an axotomized and a control facial motoneuron was calculated as relative amount of signal. Each data point represents the mean values from five trials using different neurons ± S.E.M.

a general feature in the regenerating motoneurons and that synectin may play an important role in the process of nerve regeneration. In facial motoneurons, trkB mRNA starts to increase 2 days after axotomy and persists for 2–3 weeks [12]. The time-course of synectin mRNA expression after axotomy was largely synchronized with gene regulation of trkB mRNA expression in the axotomized motoneurons, which support the notion that synectin may functionally associate with TrkB in the PNS.

The Ras–Raf–Erk pathway is critical to regenerative growth of peripheral nerves [21]. In fact, previous studies have documented that Erk1/2 are activated in the axotomized motoneurons [22,23], although it has not yet been defined how Erk1/2 is activated. Interestingly, overexpression of synectin (GIPC) in PC12 cells selectively decreases NGF-induced Erk1/2 activation with no effect on Shc, Akt, or PLC-γ1 [6]. This suggests that increased synectin expression after axotomy may reflect a need for synectin in the activation of Erk1/2. Interaction of neurotrophins with Trk receptors initiates clathrin-dependent internalization of the ligand-receptor complexes, which is critical for some biological functions of neurotrophins including neurite outgrowth in PC12 cells [10]. Indeed, Howe et al. (2001) have demonstrated that Erk1/2 activation occurs at clathrin-coated vesicles in NGF-treated PC12 cells [11]. These findings are reminiscent of electron microscopic observations showing the localization of synectin in clathrin-coated vesicles [2,5] and also subcellular fractionations shown in Fig. 4B. It has been suggested that BDNF interacts with TrkB at the proximal stump of lesioned motor nerves [12]. We speculate that internalized BDNF-TrkB associates with synectin in clathrin-coated vesicles and this association may provide a platform to recruit additional signaling components for the activation of Erk1/2. Alternatively, synectin may be involved in the clathrin-mediated endocytosis machinery governing the internalization of the BDNF-TrkB complexes.

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References

[1] Gao, Y., Li, M., Chen, W. and Simons, M. (2000) J. Cell Physiol. 184, 373–379.
[2] De Vries, L., Lou, X., Zhao, G., Zheng, B. and Farquhar, M.G. (1998) Proc. Natl. Acad. Sci. USA 95, 12340–12345.
[3] Wang, L.H., Kalb, R.G. and Strittmatter, S.M. (1999) J. Biol. Chem. 274, 14137–14146.
[4] Bunn, R.C., Jensen, M.A. and Reed, B.C. (1999) Mol. Biol. Cell 10, 819–832.
[5] Lou, X., Mcquistan, T., Orlando, R.A. and Farquhar, M.G. (2002) J. Am. Soc. Nephrol. 13, 918–927.
[6] Lou, X., Yano, H., Lee, F., Chao, M.V. and Farquhar, M.G. (2001) Mol. Biol. Cell 12, 615–627.
[7] Huang, E.J. and Reichardt, L.F. (2001) Annu. Rev. Neurosci. 24, 677–736.
[8] Martinez, A., Alcantara, S., Borrell, V., Del Rio, J.A., Blasi, J., Otal, R., Campos, N., Boronat, A., Barbacid, M., Silos-Santiago, I. and Soriano, E. (1998) J. Neurosci. 18, 7336–7350.
[9] Riccio, A., Pierchala, B.A., Ciarallo, C.L. and Ginty, D.D. (1997) Science 277, 1097–1100.
[10] Zhang, Y., Moheban, D.B., Conway, B.R., Bhattacharyya, A. and Segal, R.A. (2000) J. Neurosci. 20, 5671–5678.
[11] Howe, C.L., Valletta, J.S., Rusnak, A.S. and Mobley, W.C. (2001) Neuron 32, 801–814.
[12] Kobayashi, N.R., Bedard, A.M., Hincke, M.T. and Tetzlaff, W. (1996) Eur. J. Neurosci. 8, 1018–1029.
[13] Ohno, K., Kitahara, T., Takeda, N., Kubo, T. and Kiyama, H. (1994) Neuroscience 63, 1101–1109.
[14] Li, H.J., Sharp, A.H., Li, S.H., Dawson, T.M., Snyder, S.H. and Ross, C.A. (1996) Proc. Natl. Acad. Sci. USA 93, 4839–4844.
[15] Ohno, K., Koroll, M., El Far, O., Scholze, P., Gomeza, J. and Betz, H. (2004) Mol. Cell. Neurosci. (in press).
[16] Huttner, W.B., Schiebler, P., Greengard, P. and De Camilli, P. (1983) J. Cell Biol. 96, 1374–1388.
[17] Sanes, D.H., Reh, T.A. and Harris, W.A. (2000) In: Development of the Nervous System, pp. 289–348, Academic Press, San Diego.
[18] Malenka, R.C. and Siegelbaum, S.A. (2001) in: Synapse (Cowan, W.C., Südhof, T.C. and Stevens, C., Eds.), pp. 393–454, Johns Hopkins University Press, Baltimore.
[19] Lindsay, R.M., Wiegand, S.J., Altar, C.A. and DiStefano, P.S. (1994) Trends Neurosci. 17, 182–190.
[20] Ringstedt, T., Lagercrantz, H. and Persson, H. (1993) Brain Res. Dev. Brain Res. 72, 119–131.
[21] Markus, A., Patel, T.D. and Snider, W.D. (2002) Curr. Opin. Neurobiol. 12, 523–531.
[22] Kiryu, S., Morita, N., Ohno, K., Maeno, H. and Kiyama, H. (1995) Mol. Brain Res. 29, 147–156.
[23] Kitahara, T., Kiryu, S., Ohno, K., Morita, N., Kubo, T. and Kiyama, H. (1994) Neurosci. Res. 20, 275–280.