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Chordin and noggin expression in the adult rat trigeminal nuclei

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ABSTRACT

Bone morphogenetic proteins (BMP) exert its biological functions by interacting with membrane bound receptors. However, functions of BMPs are also regulated in the extracellular space by secreted antagonistic regulators, such as chordin and noggin. Although the deep involvement of BMP signaling in the development and functions of the trigeminal nuclei has been postulated, little information is available for its expression in the trigeminal nuclei. We, thus, investigated chordin and noggin expression in the adult rat trigeminal nuclei using immunohistochemistry. Chordin and noggin were intensely expressed throughout the trigeminal nuclei. In addition, interesting differences are observed between chordin expression and noggin expression. For example, chordin prefers dendritic expression than noggin, suggesting that chordin is involved in the regulation of dendritic morphology and synaptic homeostasis. Furthermore, chordin and noggin were differentially expressed in the neuropil of the trigeminal nuclei. Since BMP signaling is known to play a pivotal role to make precise neural network, theses differences might be important to keep precise interneuronal connections by regulating local BMP signaling intensity in each region. Interestingly, we also detected chordin and noggin expression in axons of the trigeminal nerves. These data indicate that chordin and noggin play pivotal roles also in the adult trigeminal system.

1. Introduction

Bone morphogenetic proteins (BMP) were initially founded by their ability to promote ectopic bone formation, are now shown to play pivotal roles in many biological events (Bragdon et al., 2011). The activities of the BMPs are mediated by a heterodimeric complex of type I and type II BMP serine/threonine kinase receptors, including BMP receptor type I (BMPRIA, BMPRIB) and type II (BMPRII) (Bragdon et al., 2011). On BMP binding, the type I BMPRs activate the receptor activated Smads (R-Smads; Smad1/5/8) which oligomerize with common-mediator Smad (Co-Smad; Smad4). The Smad complex then moves to the nucleus and plays as a transcription regulator (Moustakas and Heldin, 2009). Although many of the biological effects of BMPs have been related to the Smad-dependent pathways, it has been also reported that activated BMPRIA complexes are able to initiate other downstream signaling pathways such as ERK, MAPK p38, JNK, ERK and NFkB via Smad-independent pathways (Bragdon et al., 2011; Massague, 2003). Functions of BMPs are also regulated in the extracellular space by secreted antagonistic regulators such as chordin and noggin, which are reported to bind BMPs and prevent their interaction with their receptors (Cho and Blitz, 1998).

Chordin plays major roles in dorsoventral axis formation and in the induction, maintenance, and differentiation of neural tissues during gastrulation, and is secreted by the Spemann organizer of xenopus and zebrafish, and by the node of chick and mouse embryos (Miller-Bertoglio et al., 1997; Sasai and De Robertis, 1997; Streit et al., 1998). Noggin is also an extracellular BMP antagonist that binds to BMP-2/4 with high affinity and thus interferes with binding to BMP receptors. Lim et al. have reported that, in the adult subventricular zone, BMP2 and 4 expressed in the type B/C cells potently inhibit neurogenesis, and its antagonist noggin secreted from the ependymal cells makes a niche for adult neurogenesis (Lim et al., 2000). Above-mentioned data strongly indicate that chordin and noggin play pivotal roles in many biological events.

Abbreviations: BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; CNS, central nervous system; GFAP, glial fibrillary acidic protein; IR, like immunoreactivity; PB, phosphate buffer.

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Also in the trigeminal system, Hodge et al. have shown that retrograde BMP signaling regulates trigeminal sensory neuron identities and the formation of precise face maps (Hodge et al., 2007), suggesting that BMP signaling are deeply involved in the development and functions of trigeminal system. However, little information is available for chordin and noggin expression in the adult trigeminal nuclei. It is, thus, necessary to perform detailed investigations of the expression pattern of chordin and noggin in the adult trigeminal nuclei.

2. Materials and methods

2.1. Animals and section preparation

Under deep diethylether anesthesia, brain samples were isolated from male Wistar rats (n = 6, 7 weeks old; Japan SLC Inc., Shizuoka Japan). For immunohistochemistry, the rats were perfused transcardially with saline followed by 0.1 M phosphate buffer (PB, pH 7.4) containing 4% paraformaldehyde and 0.2% picric acid. The brains were removed rapidly, and then postfixed in the same fixative for 2 h at 4 °C. All brains were immersed in 10%, 20%, 25% buffered sucrose each, for overnight at 4 °C respectively. Frozen sections (20 μm for immunoperoxidase staining, 10 μm for double immunofluorescence staining in thickness) were cut on a cryostat. All animal experiments in this study were carried out in accordance with the UK animals Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, and the National Institutes of Health guide for the care and use of Laboratory animals.

2.2. Immunohistochemistry

For immunoperoxidase staining, the sections were treated with 10% normal goat serum and 0.2% Triton X-100 in 0.1 M PB for 2 h at room temperature, and incubated further with the primary antibodies overnight at 4 °C. After being washed with 0.1 M PB, sections were incubated with the secondary antibody for 2 h at room temperature. After being washed with 0.1 M PB, immunoreaction was visualized with 3,3′-diaminobenzidine (Wako, Osaka, Japan).

A rabbit anti-chordin (diluted 1:50, the final concentration, 2 μg/ml; ABGENT Inc., San Diego, CA, USA), and a rabbit anti-noggin (diluted 1:100, the final concentration, 2 μg/ml; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) were used as primary antibodies. And a goat anti-rabbit IgG with peroxidase complex (no dilution, ready-to-use; EnVision™ System, K4002; DAKO, Tokyo, Japan) was used as a secondary antibody.

For double immunofluorescence staining, sections were treated with 10% normal donkey serum and 0.2% Triton X-100 in 0.1 M PB for 20 min at room temperature, and incubated further in both mouse anti-glial fibrillary acidic protein (GFAP) antibody (diluted 1:1000; the initial concentration is not available; Merck Millipore, Temecula, CA, USA) and rabbit anti-chordin antibody (diluted 1:200) or rabbit anti-noggin antibody (diluted 1:300) for 1.5 h at room temperature. After being washed with 0.1 M PB, sections were incubated in both Alexa Fluor 488 donkey anti-mouse IgG (diluted 1:500; the final concentration, 4 μg/ml; Molecular Probes, Inc, Eugene, OR, USA) and Alexa Fluor 594 donkey anti-rabbit IgG (diluted 1:250; the final concentration, 8 μg/ml; Molecular Probes) for 1.5 h at room temperature.

Bright-field images were obtained using a microscope (Eclipse 80i; Nikon, Tokyo, Japan). Fluorescence images were obtained using a fluorescence microscope (Eclipse E-600; Nikon). They were further processed in image analysis software (Photoshop; Adobe, Tokyo, Japan).

3. Results

3.1. General expression patterns

The specificity for the antibodies used in this study has been evaluated in our previous studies (Mikawa and Sato, 2011, 2014). Fig. 1 shows the overview of chordin and noggin expressions in the adult trigeminal nuclei. Chordin-like immunoreactivity (IR) and noggin-IR were observed throughout the trigeminal nuclei. Both proteins were abundantly expressed in the mesencephalic trigeminal nucleus, motor trigeminal nucleus, principal trigeminal nucleus (Fig. 1A and B), oral part of the spinal trigeminal nucleus (Fig. 1C and D), and caudal part of the spinal trigeminal nucleus (Fig. 1E and F). The relative intensity of chordin-IR and noggin-IR in the rat trigeminal nuclei is summarized in Table 1.

3.2. Mesencephalic trigeminal nucleus

Chordin and noggin-IRs were abundantly seen in the mesencephalic trigeminal nucleus (Fig. 2A–F). In the mesencephalic trigeminal nucleus, the majority of the neurons are large primary afferent neurons, which transmit information from muscle spindles of jaw muscles and periodontal receptors of both maxillary and mandibular teeth (Waite, 2004). Interestingly, chordin-IR was strongly observed in the cell bodies of large primary afferent neurons (arrowheads in Fig. 2D). In addition, these neurons also very strongly expressed noggin proteins (arrowheads in Fig. 2F). Furthermore, weak neuropil staining was observed for both proteins (Fig. 2C–F).

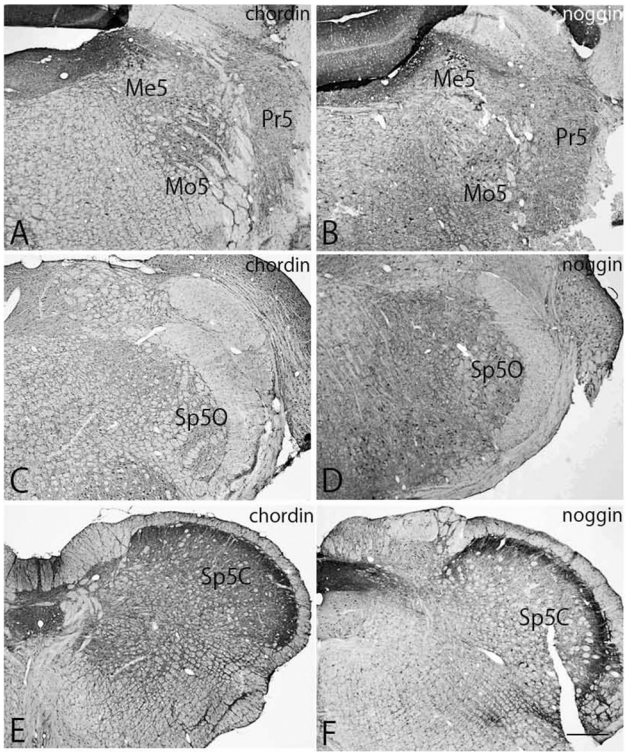

Fig. 1. Chordin and noggin expression in the adult rat trigeminal nuclei. Me5, mesencephalic trigeminal nucleus; Mo5, motor trigeminal nucleus; Pr5, principal trigeminal nucleus; Sp5C, caudal part of spinal trigeminal nucleus; Sp5O, oral part of spinal trigeminal nucleus. Scale bar = 400 μm.