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The Roles of Brain-derived Neurotrophic Factor in the Development of Nasal Chemoreceptor Neurons

Shigeru Takami, Rumi Hasegawa and Fumiaki Nishiyama

Department of Anatomy, School of Health Sciences, Kyorin University, Hachioji, Tokyo 192-8508, Japan

Correspondence to be sent to: Shigeru Takami, e-mail: takamis@kyorin-u.ac.jp

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Introduction

Brain-derived neurotrophic factor (BDNF) is one of the neurotrophins, and known to facilitate differentiation, growth and maturation of neurons. BDNF binds to the high-affinity receptor, tyrosine kinase receptor B (TrkB) to initiate signal transduction (Korsching, 1993; Lindsay *et al.*, 1994).

Olfactory and vomeronasal receptor neurons (ORNs and VRNs, respectively) are chemoreceptors located in the nasal cavity of most mammalian species (Graziadei, 1977). In the olfactory epithelium (OE) lining the olfactory mucosa (OM), progenitor cells differentiate into immature and mature ORNs; a single dendrite extending from the apical pole of each soma reaches the surface of the OE to form a dendritic ending, and a single axon from the base of the soma runs downward to pass through the basement membrane of the OE to reach the brain. Similar bipolar neurons are also contained in the vomeronasal sensory epithelium (VSE) of the vomeronasal organ (VNO), and designated as VRNs or vomeronasal sensory cells/neurons (Graziadei, 1977; Takami, 2002). Although several neurotrophic factors including neurotrophins are thought to be involved in the development, maturation, and regeneration of ORNs (Carter and Roskams, 2002; Schwob, 2002), the functional roles of BDNF remain to be understood. In the case of the VRNs, we were the first to report the distribution of BDNF and TrkB at both domestic (Takami *et al.*, 2001) and international (Takami and Nishiyama, 1997b) meetings. In this paper, we present summary of our recent studies concerning BDNF and TrkB in the rat OM and VNO. Our overall research goal is to clarify functional roles of BDNF in the differentiation and maturation of ORNs and VRNs.

Expression of BDNF in ORNs and VRNs

Using immunohistochemical methods, we demonstrated that ORNs and VRNs of rats (Sprague–Dawley strain) were BDNF-immunoreactive. Western blot analyses demonstrated that OM and VNO, as well as several brain regions, contained 27 kDa bands (Takami *et al.*, 1997, 1999). Double- and triple-labeling fluorescence methods confirmed the co-localization of BDNF and protein gene product 9.5 (PGP) in ORNs (Figure 1A,B). PGP was expressed in both mature and immature ORNs and VRNs (Taniguchi *et al.*, 1993; Takami *et al.*, 1995). Similar findings were obtained from the VSE; VRNs contained both BDNF and PGP (Takami *et al.*, 2001).

In situ hybridization (ISH) methods in which anti-sense and sense probes to cRNA BDNF demonstrated that ORNs and VRNs at postnatal day 1 (P1) and at 6 weeks old (6W) contained BDNFmRNA. RT–PCR analyses confirmed that BDNF was expressed in the OM and VNO as well as in the olfactory bulbs, cerebrum, and cerebellum, but not in the liver (Takami *et al.*, 2000, 2001).

Developmental study for BDNF expression in the OE and VSE

We identified and localized the protein and mRNA of BDNF in the OE and VSE at embryonic day 20 (E20), P1, P7, P14, P28, 6W and

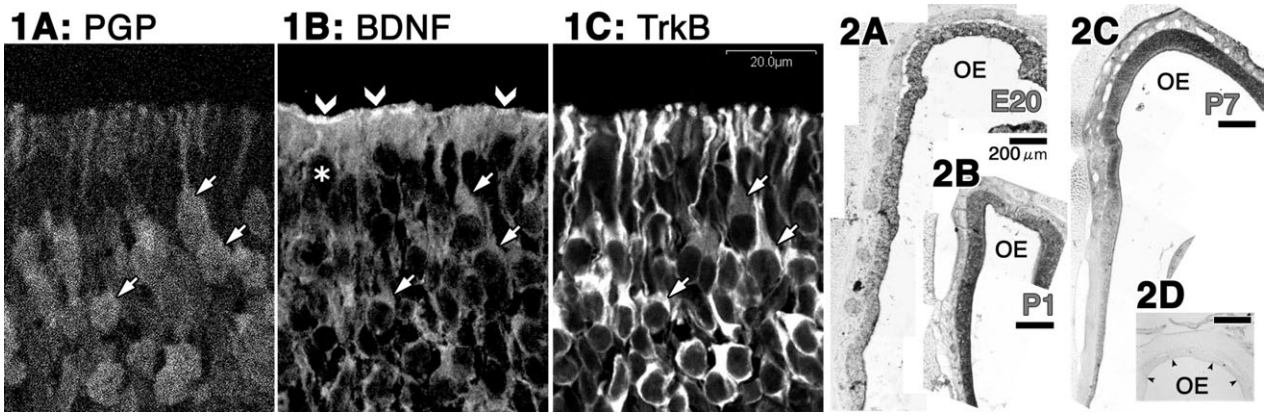

Figure 1 Simultaneous demonstration of immunofluorescence for PGP **(A)**, BDNF **(B)** and TrkB **(C)** in the olfactory epithelium at postnatal day-1 (P1), obtained by a confocal laser-scanning fluorescence microscope (FLUOVIEW FV500-IX-UV, Olympus Tokyo, Japan). Arrows indicate olfactory receptor neurons that exhibit immunofluorescence for PGP, BDNF, and TrkB. Sustentacular cells (asterisk indicates a nucleus of a sustentacular cell) also exhibit immunofluorescence for BDNF (arrowheads in B).

Figure 2 The presence of BDNFmRNA in the olfactory epithelium (OE) at E20 **(A)**, P1 **(B)** and P7 **(C)**. Although the anti-sense probe for BDNFmRNA resulted in intense staining (A–C), no specific staining was observed when the sense probe was used **(D)**.

10W; both ORNs and VRNs in all ages expressed BDNF (Figure 2). Furthermore, detailed analyses for the OE at 6W demonstrated that the OE in the septal region of the nasal cavity contained much lower amounts of BDNFmRNA, when compared to the OE in the laterally located turbinates (Hasegawa *et al.*, 2002; Takami *et al.*, 2002). The biological significance of this regional difference remains to be understood.

TrkB protein in the OM and VNO

Immunoreactivity for TrkB was reported to be present in ORNs, VRNs and their axon bundles in the lamina propria (Takami and Nishiyama, 1997a,b; Takami *et al.*, 1997). Furthermore, our quantitative analysis for the intensity of TrkB immunofluorescence in ORNs suggests that immature ORNs contain larger amounts of TrkB protein when compared to mature ORNs that are immunoreactive for olfactory marker protein (OMP; Takami *et al.*, 2004).

A post-embedding immunogold method demonstrated that gold particles were present on the membranes of olfactory cilia as well as olfactory axons (Takami *et al.*, 1998). Thus, it is likely that ciliary and axonal membranes are the actual sites where the binding of BDNF and TrkB does take place.

Co-localization of BDNF and TrkB

Double- and triple-labeling fluorescence methods and a combined method of ISH and immunofluorescence microscopy demonstrated that subpopulations of BDNF-expressing ORNs and VRNs contained TrkB (Figure 1A–C). Further analysis for these subpopulations is underway in our laboratory.

Possible modes of BDNF actions within ORNs and VRNs

Our resent studies in albino rats indicate that (i) BDNF is produced by ORNs and VRNs during pre- and post-natal development as well as in adult stages; (ii) autocrine and paracrine modes of BDNF exist in the OE and VSE; and (iii) TrkB protein is localized at least in the ciliary and axonal membranes of ORNs. These findings suggest that BDNF is an important factor for developing and maintaining ORNs and VRNs. It is likely that BDNF plays a crucial role in the growth of axons, dendrites, and cilia/microvilli of ORNs and VRNs.

Except for ORNs and VRNs, sustentacular cells within the OE and VSE were another source for BDNF (Figure 1B). However, TrkB immunoreactivity was not detected in sustentacular cells (Figure 1C), suggesting that BDNF secreted by them may have biological effects exclusively on ORNs and VRNs.

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