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Research report

BMP3 expression in the adult rat CNS

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ABSTRACT

Bone morphogenetic protein-3 (BMP3) is a very unique member of the TGF-β superfamily, because it functions as an antagonist to both the canonical BMP and activin pathways and plays important roles in multiple biological events. Although BMP3 expression has been described in the early development of the kidney, intestine and bone, little information is available for BMP3 expression in the central nervous system (CNS). We, thus, investigated BMP3 expression in the adult rat CNS using immunohistochemistry. BMP3 was intensely expressed in most neurons and their axons. Furthermore, we found that astrocytes and ependymal cells also express BMP3 protein. These data indicate that BMP3 is widely expressed throughout the adult CNS, and its abundant expression in the adult brain strongly supports the idea that BMP3 plays important roles in the adult brain.

1. Introduction

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor β (TGF- β) superfamily (Bragdon et al., 2011). Although BMPs were initially reported to promote ectopic bone formation, now it is well known to be involved in several pivotal biological events (Bragdon et al., 2011). BMP signals are mediated by a complex of type I and type II BMP receptors (Bragdon et al., 2011). By binding to the type I BMPRs, BMPs activate the receptor-activated Smads (R-Smads; Smad1/5/8) which oligomerize with common-mediator Smad (Co-Smad; Smad4) in the cytoplasm. Then, the Smad complex enters the nucleus and initiates transcription (Moustakas and Heldin, 2009). Although many of the biological effects of BMPs have been related to the Smad-dependent pathways, Smad-independent pathways have been also reported (Massague, 2003). Interestingly, secreted antagonistic regulators such as chordin, noggin, follistatin, neurogenesin-1 are reported to bind BMPs and prevent their interaction with their receptors (Cho and Blitz, 1998; Ueki et al., 2003).

Bone morphogenetic protein-3 (BMP3) is a very unique member of the TGF-β superfamily, because it functions as an antagonist to both the canonical BMP and activin pathways (Lowery et al., 2013). Interestingly, BMP3 shares only 40% amino acid identity with the BMP2/4 and BMP5/6/7 groups (Takao et al., 1996), and is highly divergent from other BMP ligands, and it is situated at an intermediate phylogenetic position between TGF-β/activin and the other BMP ligands (Katoh and Katoh, 2006; Lowery and de Caestecker, 2010). BMP3 is reported to show antagonistic effects in many different situations. For example, while other BMPs promote osteogenesis, *Bmp3* knockout mice have high bone mass, indicating that BMP3 acts as a negative regulator of osteogenesis in vivo (Daluiski et al., 2001). In addition, BMP3 inhibits differentiation of osteoprogenitors into osteoblasts (Bahamonde and Lyons, 2001; Kokabu et al., 2012). Furthermore, overexpression studies in chick (Gamer et al., 2008), xenopus (Gamer et al., 2005; Hino et al., 2003), and mouse (Gamer et al., 2009) also indicate that BMP3 negatively regulates the BMP and activin pathways. While the precise mechanism for this inhibition remains unclear, BMP3 has been demonstrated to sequester BMP receptors into inactive signaling complexes (Daluiski et al., 2001; Gamer et al., 2005) through high affinity interaction with activin receptor 2B (ACVR2B) (Kokabu et al., 2012; Allendorph et al., 2006; Stewart et al., 2010).

BMP3 expression has been described in the early development of the kidney, intestine and bone (Vukicevic et al., 1994; Daluiski et al., 2001). However, there is no report about BMP3 expression in the adult central nervous system (CNS). Furthermore, BMP ligands, such as BMP2 and 4 (Mikawa et al., 2006; Sato et al., 2010), BMP receptor IA, IB and II (Miyagi et al., 2011, 2012), and antagonists, noggin and chordin (Mikawa and Sato, 2011, 2014), are also reported to be abundantly expressed in the adult rat CNS. Thus, it is necessary to investigate the expression pattern of BMP3 in the

Abbreviations: ACVR2B, activin receptor 2B; BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; BSA, bovine serum albumin; CNS, central nervous system; IR, like immunoreactivity; LTD, long term depression; LTP, long term potentiation; PB, phosphate buffer; SVZ, subventricular zone; TGF-β, transforming growth factor β.

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adult rat brain. In the present study, we show that BMP3 protein is widely expressed throughout the adult CNS, and that neurons, astrocytes and ependymal cells express BMP3 protein.

2. Results

To certify the specificity of the antibody (rabbit anti-BMP3), we performed pre-absorption of the antibody with BMP3-peptide. It completely abolished the immunostainings (Fig. 1(A) and (B)). Furthermore, since BMP3 has been reported to be abundantly expressed in bone-forming cells (Kloen et al., 2003), we performed immunostaining using this antibody for embryonic day 20 fetuses. Fig. 1(C) clearly shows that this antibody can also recognize bone-forming cells in vertebral bones (arrows in Fig. 1(C)). These data indicate that this antibody specifically recognize the BMP3 protein.

2.1. General expression patterns

In Fig. 2, we show the overview of BMP3 expression in the adult rat brain. BMP3-like immunoreactivity (IR) was seen throughout the adult rat brain. Abundant BMP3-IR was seen in the olfactory bulb (Fig. 2(A)), olfactory tubercle (Fig. 2(B)), cerebral cortex (Fig. 2 (B)-(G)), nucleus accumbens (Fig. 2(C)), caudate putamem (Fig. 2 (D) and (E)), hippocampus (Fig. 2(E)-(G)), thalamus (Fig. 2(E) and (F)), hypothalamus (Fig. 2(E) and (F)), midbrain (Fig. 2(G) and (H)), pontine nucleus (Fig. 2(H)), cerebellum (Fig. 2(I)), brainstem (Fig. 2 (I) and (J)), and spinal cord (Fig. 2(K)). The relative intensity of BMP3-IR in the adult rat CNS is summarized in Table 1.

2.2. Telencephalon

2.2.1. Olfactory bulb and related areas

The olfactory bulb abundantly expressed BMP3 protein (Fig. 3(A)). Interestingly, in the outer half of the external plexiform layer, many large-sized tufted neurons were strongly stained (arrows in Fig. 3(B)). In the glomerular layer, many periglomerular neurons showed very strong BMP3-IR (arrows in Fig. 3(C)). In the mitral cell layer, the cell bodies of mitral cells were very strongly labeled (arrows in Fig. 3(D)). In the granular layer, granular neurons also exhibited very strong BMP3-IR (arrowheads in Fig. 3(D)). Interestingly, the olfactory tubercle exhibited very strong BMP3-IR (arrows in Fig. 3(E)), in addition, the anterior olfactory nucleus also showed moderate BMP3-IR (Fig. 3(E)).

2.2.2. Septum and nuclei of the diagonal band of Broca

The lateral septal nucleus contained many strongly-positive cells (Fig. 3(F)). In contrast, BMP3-IR in the medial septal nucleus and diagonal band was weak (Fig. 3(F)).

2.2.3. Islands of Calleja and subfornical organ

The islands of Calleja contained many cells showing strong BMP3-IR (Fig. 3(G) and (H)). In addition, BMP3-IR positive cells were scattered in the subfornical organ (Fig. 3(I)).

2.2.4. Piriform cortex

Many pyramidal neurons in the layers II and III showed strong BMP3-IR (arrowheads in Fig. 3(J)).

2.2.5. Cerebral cortex

BMP3-IR positive cells were observed in the layers I-VI of the cerebral cortex (Fig. 4(A)). In the layer I, weakly-stained cells were sparsely observed, while in the layer II, many neurons were intensely stained (Fig. 4(B)). In the layer V, many pyramidal neurons were very strongly stained (Fig. 4(C)). Closer observation showed that BMP3-IR was detected in the cytoplasm and around the nuclei of pyramidal neurons (arrows in Fig. 4(D)).

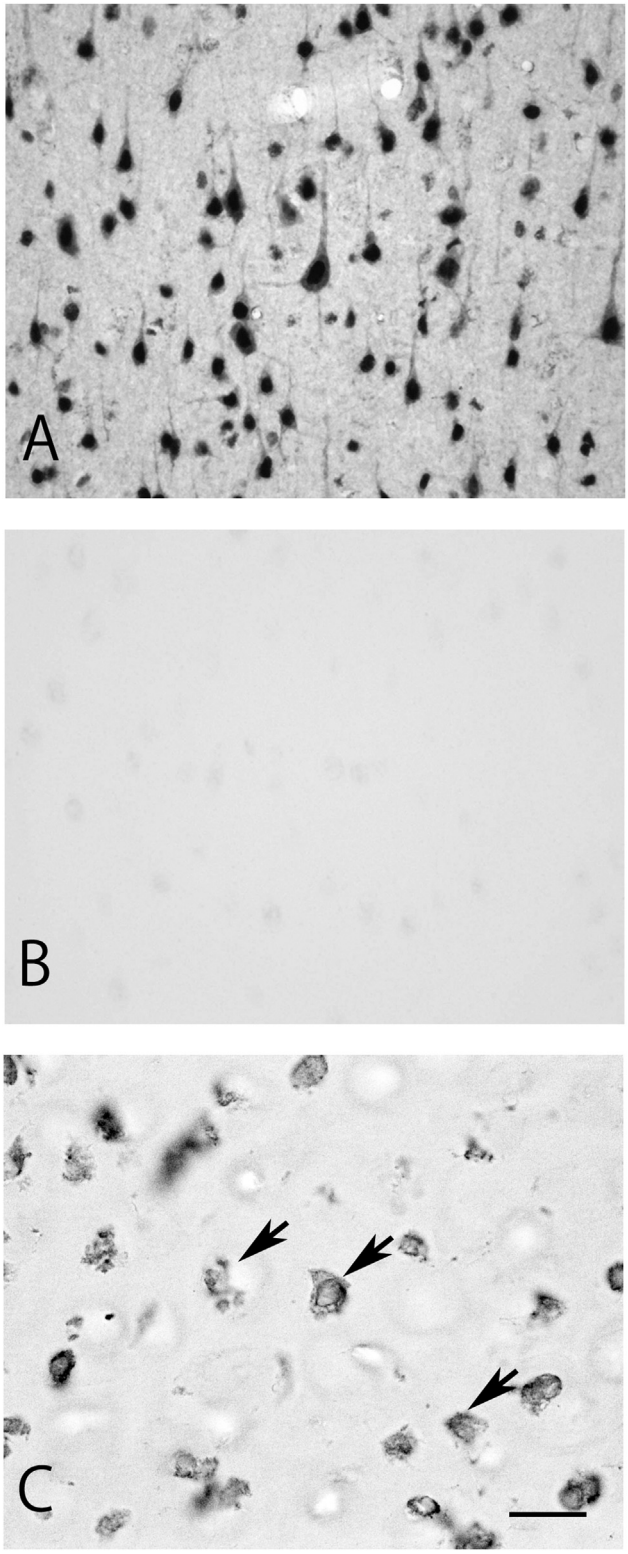

Fig. 1. Photomicrographs of pre-absorption test; control (**A**), pre-absorbed (**B**), and BMP3 immunostaining in vertebral bones of embryonic day 20 fetus (**C**). Note that immunoreactivities in the layer V of the cortex are completely abolished by pre-absorption test. Scale Bar=40 μm for A, B; 16 μm for C.

2.2.6. Hippocampus

BMP3-IR was abundantly detected throughout the hippocampus (Fig. 4(E)). Cell bodies of pyramidal cells of the Ammon's horn were very strongly stained (Fig. 4(E) and (F)). In the dentate