Strong Immunoreactivity of Platelet-Derived Growth Factor and Its Receptor at Human and Mouse Neuromuscular Junctions

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¹*Department of Pediatrics, Tohoku University School of Medicine, Sendai 980–8574, and* ²*Department of Pediatrics, Second Clinical College of China Medical University, Shenyang, China*

ZHAO, Y., HAGINOYA, K. and IINUMA, K. *Strong Immunoreactivity of Platelet-Derived Growth Factor and Its Receptor at Human and Mouse Neuromuscular Junctions*. Tohoku J. Exp. Med., 1999, **189** (4), 239–244 — Platelet-derived growth factor (PDGF) and its α -receptor were localized at human and mouse neuromuscular junctions (NMJs) using specific polyclonal antibodies against each, anti-PDGF-A and anti-PDGF α -receptor, respectively. By applying double fluorescence labeling, immunoreactivity for PDGF and its receptor was closely co-localized with acetylcholine receptors, which were identified with α -bungarotoxin. PDGF might be involved in the interaction between the presynaptic and postsynaptic components. This is the first demonstration of PDGF and its receptor concentrated at human and mouse NMJs. ——— platelet-derived growth factor; platelet-derived growth factor receptor; neuromuscular junction; immunohistochemistry © 1999 Tohoku University Medical Press

Platelet-derived growth factor (PDGF) consists of two disulfide-bonded peptide chains, A and B, and occurs naturally as three isoforms, PDGF-AA, PDGF-AB and PDGF-BB (Heldin and Westermark 1990). PDGF is a growth stimulant and chemoattractant principally for mesenchymal connective tissue-forming cells (Ross et al. 1986; Heldin and Westermark 1990). PDGF induces cell proliferation, as well as a number of other critical functions, by binding to specific high-affinity cell surface receptors. The PDGF α -receptor binds PDGF A- and B-chains, while the β -receptor recognizes only PDGF B-chains (Heldin et al. 1988). There is evidence that PDGF and its receptors are present in the central and peripheral nervous system, as well as in the muscle cells, and are synthesized in specific spatial and temporal patterns. They have been shown to be involved in regulating neuronal, glial and muscle cell development and

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differentiation (Sejersen et al. 1986; Jin et al. 1990, 1991; Pringle et al. 1991; Sasahara et al. 1991; Yeh et al. 1991; Tidball et al. 1992). In this study, we present evidence that PDGF and its receptor are strongly concentrated at the human and mouse neuromuscular junctions (NMJs).

MATERIALS AND METHODS

Human muscle

The diagnostic muscle biopsies were screened with hematoxylin and eosin, modified Gomori-trichrome and a battery of histochemical reactions. Five specimens that showed no abnormality and contained pan-esterase-positive NMJs were selected for study. Two hundred sixty five NMJs in total were examined.

Mouse muscle

Mature C57BL/10 mice were sacrificed by cervical dislocation. The tibialis anterior muscles were isolated quickly and frozen immediately in liquid-nitrogen-cooled isopentane. One hundred fifty two NMJs in total were examined.

Immunohistochemistry

Immunohistochemistry was performed on cold acetone-fixed 10 μ m transverse sections of fresh-frozen human and mice muscle biopsies. The following antibodies against PDGF and PDGF receptor were used: 1) rabbit polyclonal antibodies against human PDGF-A from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA), diluted 1:500, 2) rabbit polyclonal antibodies against human PDGF α -receptor from Santa Cruz Biotechnology Inc., diluted 1:500. The specificity of PDGF and its receptor immunoreactivity was determined by a) omitting the primary antibody, b) replacing the primary antibody by the same concentration of non-immune rabbit IgG (Zymed Laboratories, Inc., San Francisco, CA, USA). In all experiments, NMJs were identified by binding of rhodamine-labeled α -bungarotoxin (α -BT) (Molecular Probes, Inc., Eugene, OR, USA) to the nicotinic acetylcholine receptors. In order to colocalize PDGF or PDGF receptor with α -BT, indirect immunofluorescence staining with Cy2-conjugated secondary antibody (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) was utilized.

RESULTS

Antibodies against PDGF-A and PDGF α -receptor strongly immunostained human and mouse NMJs that were identified by pan-esterase staining (Fig. 1). Double immunolabeling with α -BT and PDGF-A or its α -receptor demonstrated that more than 95% of NMJs were closely associated with PDGF-A or its α -receptor in both human and mouse specimens (Fig. 2). The muscle non-junctional sarcolemma was not immunoreactive in humans; however weak staining of non-junctional sarcolemma was seen with both antibodies in some muscle

Fig. 1. Pan-esterase-staining (a) and PDGF-A immunolabeling (b) on serial sections of the human muscle. Immunoreactivities for PDGF-A were closely associated with acetylcholine receptors. (bar = 20 μ m)

fibers in mouse muscles. When the primary antibody was omitted or replaced by non-immune rabbit IgG, no immunoreaction was evident.

DISCUSSION

In vivo, PDGF-A is constitutively produced by neurons within the developing and mature central and peripheral nervous systems of the rodent (Yeh et al. 1991). Large motor neurons of the ventral gray matter in the spinal cord and dorsal root ganglia in the peripheral nerve system were positive for PDGF-A, as demonstrated by in situ hybridization and immunohistochemistry (Yeh et al. 1991; Eccleston et al. 1993). It has been suggested that PDGF is an important regulatory factor in the differentiation of oligodendrocytes, since the expression of PDGF α -receptors is seen in oligodendrocyte progenitors in the rodent brain during the late embryonic and postnatal periods and decreases markedly after a certain postnatal day (Yeh et al. 1991; Oumesmar et al. 1997). Moreover, recent analysis has shown that neurons of various central nervous system regions also express PDGF α -receptor transcripts and protein as early as postnatal day 1, and this is maintained at all ages (Oumesmar et al. 1997). In the mature rodent brain, PDGF α -receptor transcripts and protein were localized in the neurons of many structures, including the cerebral cortex, hippocampus, and brainstem nuclei, and in motor neurons of the ventral horn of the spinal cord (Oumesmar et al. 1997). The expression of PDGF α -receptors in mature neurons could play a physiological role in the normal functioning of neurons in vivo (Oumesmar et al. 1997). PDGF-A transcripts are also expressed in developing rat muscle tissue and cultured rat myoblasts (Sejersen et al. 1986; Jin et al. 1990). However, there are no reports on the immunolocalization of PDGF and its receptors at NMJs. At NMJs, the molecular compositions of the extracellular matrix, plasmalemma, and subsynaptic cytoplasmic domain of muscle fibers are different from those in non-synaptic regions (Froehner 1991). More than 50 proteins have been localized to the synaptic region of muscle fibers (Hall and Sanes 1993; Askanas et al. 1998). The mechanisms responsible for maintaining the high concentration of certain proteins postsynaptically are largely unknown. Some growth factors, such as

Fig. 2. Double immunolabeling with PDGF-A (a, c) or PDGF α -receptor (e, g) and α -bungarotoxin (b, d, f, h) at human (a, b, e, f) and mouse (c, d, g, h) neuromuscular junctions. PDGF-A and PDGF α -receptor immunoreactivity closely co-localized with acetylcholine receptors identified with α -bungarotoxin. (bar = 20 μ m)

fibroblast growth factor (Bilak et al. 1994) and transforming growth factor (McLennan and Koishi 1994; Toepfer et al. 1999), have been identified at the NMJs, suggesting that they function either as a trophic factor for mature motoneurons or as an autocrine regulator of synaptic protein production (McLennan and Koishi 1994). In demonstrating that PDGF-A and PDGF α -receptors are strongly concentrated at the NMJs, our study raises the question of their normal function at this site. PDGF-A and its α -receptor may be involved in: (a) maintaining NMJs by regulating the activities of muscle jun-

ctional (postsynaptic) nuclei that produce junctional-proteins (Askanas et al. 1998); (b) serving as a trophic or proliferating factor influencing terminal Schwann cells and fibroblasts close to the NMJ, since PDGF has a mitogenic effect on Schwann cells and fibroblasts (Davis and Stroobant 1990); or (c) serving as a trophic factor for mature motoneurons (Oppenheim et al. 1993; Yin et al. 1994).

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