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Drebrin (developmentally regulated brain protein) is associated with axo-somatic synapses and neuronal gap junctions in rat mesencephalic trigeminal nucleus

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ABSTRACT

Drebrin (developmentally regulated brain protein)-like immunoreactivity was investigated in the adult rat mesencephalic trigeminal nucleus (MTN) using light and electron microscope. Intense immunoreactive puncta were observed on the cytoplasmic membrane and within the cytoplasm. The cytoplasm was also faintly immunopositive for drebrin, and thus MTN somata other than multipolar cells were distinguishable from non-MTN somata. These immunoreactive cell bodies were localized from the level of the superior colliculus to the pons. Electron microscopic observation showed that the post-synaptic cytoplasmic membrane at axo-somatic synapses was immunoreactive for drebrin. Drebrin-like immunoreactivity was also observed on spine-like processes emanating from MTN somata. In addition, the post-synaptic cytoplasmic membrane at axo-somatic synapses was also immunopositive for drebrin. Within the cytoplasm of MTN cell bodies, a part of the rough endoplasmic reticulum and neighboring structures were also immunopositive. Further, both ends of the somato-somatic close appositions that contained neuronal gap junctions harbored immunoreactive structures. We can infer from the results that drebrin is an ideal marker protein for MTN cell bodies. The abundance of drebrin-like immunoreactivity in the MTN neurons suggests that the MTN has highly flexible synaptogenesis.

The primary afferents in the sensory systems have soma in the peripheral ganglia. However, the trigeminal nervous system has primary afferent somata in both the peripheral trigeminal ganglion and the central mesencephalic trigeminal nucleus (MTN). The sensory ganglia of MTN contain various-sized cell bodies that express size-specific marker proteins [20]. Although some markers, such as substance P and calcitonin gene-related protein, are expressed only in the peripheral sensory ganglia, some of these markers, such as calcium-binding proteins and carbonic anhydrase [4–6], are also expressed in the MTN cell bodies [12,24]. Unlike trigeminal and dorsal root ganglia, MTN somata receive inputs from nerve terminals containing various types of neurotransmitters and/or neuromodulators [20,21]. These synapses on MTN neurons may have a crucial role in modulating and coordinating masticatory muscle movement [2,8].

Drebrin (developmentally regulated brain protein) is a developmentally regulated brain protein first described in the embryonic chicken brain [32,33]. It is an actin-binding protein and has two isoforms, namely, adult (drebrin A) and embryonic (drebrin E) forms. The adult brain contains only drebrin A, which is a neuron-specific isoform [19] and abundantly found within post-synaptic dendritic spines of excitatory synapses [1]. Over-expression of drebrin A induces the elongation of dendritic spines, and suppression reduces spine density, resulting in the formation of thin immature spines [10,36]. These findings indicate that the drebrin–actin complex plays a pivotal role in the regulation of spine morphology. In this study, we investigated the distribution of drebrin, probably drebrin A-like immunoreactivity, in the MTN. The results indicated that drebrin was an ideal marker protein of MTN neurons.

Male Wistar rats ($n = 7$) were used in this study. All surgical procedures were approved by the Ethics Committee of Kanagawa Dental College, and were performed in accordance with the guidelines of this committee. The animals were deeply anesthetized with pentobarbital sodium (Wako Pure Chemical Industries, Ltd., Osaka, Japan). They were then perfused with 0.9% NaCl and subsequently with 4% formaldehyde and 0.2% picric acid in 0.1 M sodium phosphate buffer (PB, pH 6.9). The brain was rapidly dissected out and

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further fixed in the same fixative for 1 or 2 days at 4 °C. After washing in PB and immersing in 20% sucrose, samples were cut into 20-μm-thick transverse sections using a sliding microtome equipped with a freezing stage, and free-floating sections were immunostained.

Immunohistochemistry was performed mainly according to our routine method [42]. Briefly, the sections were washed overnight in 0.1 M PB (pH 7.4) containing 0.9% saline (PBS), and incubated with guinea pig anti-drebrin serum (Progen Biotechnik GmbH, Heidelberg, Germany) diluted 1:2000 in PBS containing 1% bovine serum albumin (BSA) and 0.3% Triton X-100 (PBS-BSAT) for 24 h at 4 °C. After washing in PBS, the sections were incubated with a secondary antibody (biotinylated goat anti-guinea pig IgG, Vector Laboratories, Burlingame, CA) diluted 1:200 in PBS-BSAT for 1 h at room temperature. The sections were then washed again in PBS and incubated with avidin–biotin–horseradish peroxidase complex (ABC; Vector Laboratories) diluted 1:200 in PBS-BSAT for 30 min at room temperature. After a final wash in PBS, the sections were reacted with 0.02% 3,3′-diaminobenzidine tetrahydrochloride (DAB) and 0.005% hydrogen peroxide in 0.05 M Tris–HCl buffer solution (pH 7.4). Thereafter, sections were counterstained with thionin and coverslipped with Malinol (Muto Pure Chemicals, Tokyo, Japan). Some sections were coverslipped without counterstaining. Controls for anti-drebrin serum were prepared by omitting the antiserum in the first incubation step, or by using antiserum preabsorbed with recombinant drebrin (1 μg/ml; Abnova Corporation, Taipei, Taiwan). Immunoreactive somata were measured using a software program, Flovel Filling System, with an Olympus light microscope (U-TVO, 35XC-2, Tokyo, Japan). For the size analysis of drebrin-like immunoreactive cells, we measured 489 somata from 5 animals.

For electron microscopic preparations by the pre-embedding method, 20-μm-thick sections were cut using a sliding microtome as described above. Drebrin-like immunoreactivity was detected by the above method except for the elimination of Triton-X 100 from all solutions. After the DAB reaction, the sections were fixed with 1% OsO_4, dehydrated, and embedded in Quetol 812 (Nissin EM, Tokyo, Japan). Following embedding, ultrathin sections were cut and mounted on nickel grids. After staining with uranyl acetate and lead citrate for 1 min each, ultrathin sections were examined with an electron microscope (JEM 1220; JEOL, Tokyo, Japan).

Drebrin-immunoreactive cell bodies were observed in the whole rostrocaudal extension of the MTN (Figs. 1 and 2). At the level of the superior colliculus, scattered immunoreactive somata were observed on the lateral area of the periaqueductal gray matter (Fig. 1A and B). In the inferior collicules, immunoreactive somata were localized near the lateral border of the periaqueductal gray (Fig. 1C). At the levels of pons-midbrain junction and pons, immunoreactive somata tended to group in a triangle at the lateral corner of the periaqueductal gray (Fig. 2A and B), and the most abundant cell bodies were located in the pons among the rostrocaudal extension of the nucleus. Fine immunoreactive puncta were found along the cytoplasmic membrane of immunoreactive somata. The cytoplasm of MTN somata was faintly immunoreactive, and several intensely immunoreactive puncta were scattered within the cytoplasm. No immunoreactivity was observed after either omission of the primary antiserum or preabsorption with recombinant drebrin (not shown).

At the electron microscopic level, immunoreactive DAB deposition was seen in spine-like processes protruding from the somata (Fig. 3A). Most of these immunoreactive spine-like processes were associated with synapses (Fig. 3). In addition to immunoreactive DAB deposition in the spine-like processes that tended to envelope synapses, post-synaptic membranes and their adjacent cytoplasm were also immunopositive (Fig. 3B and C). The sizes of the synapses with an immunopositive post-synaptic membrane varied from 20 to 50 nm in diameter. Post-synaptic membranes of most terminals forming axo-somatic synapses were immunopositive for drebrin, although there were synapses whose post-synaptic membrane was free from immunoreactivity in the surrounding neuropils (not shown). Drebrin-like immunoreactivity was also seen in cytoplasm located on both sides of the somato-somatic junctional complex (Fig. 4). Between these immunoreactive areas, neuronal gap junction-like structures were observed (Fig. 4B). Within the cytoplasm, DAB deposition was seen on part of the rough endoplasmic reticulum (rER) where neurofilaments and neurotubules were associated with these immunoreactive rER (not shown).

The diameters of the immunoreactive somata were between 20 and 50 μm (Fig. 5), and the population under 24 μm and over 43 μm was minor. The mean size of drebrin-immunoreactive somata was 31.9 μm in diameter [(long axis + short axis)/2]. There was no remarkable difference in somata size between the midbrain

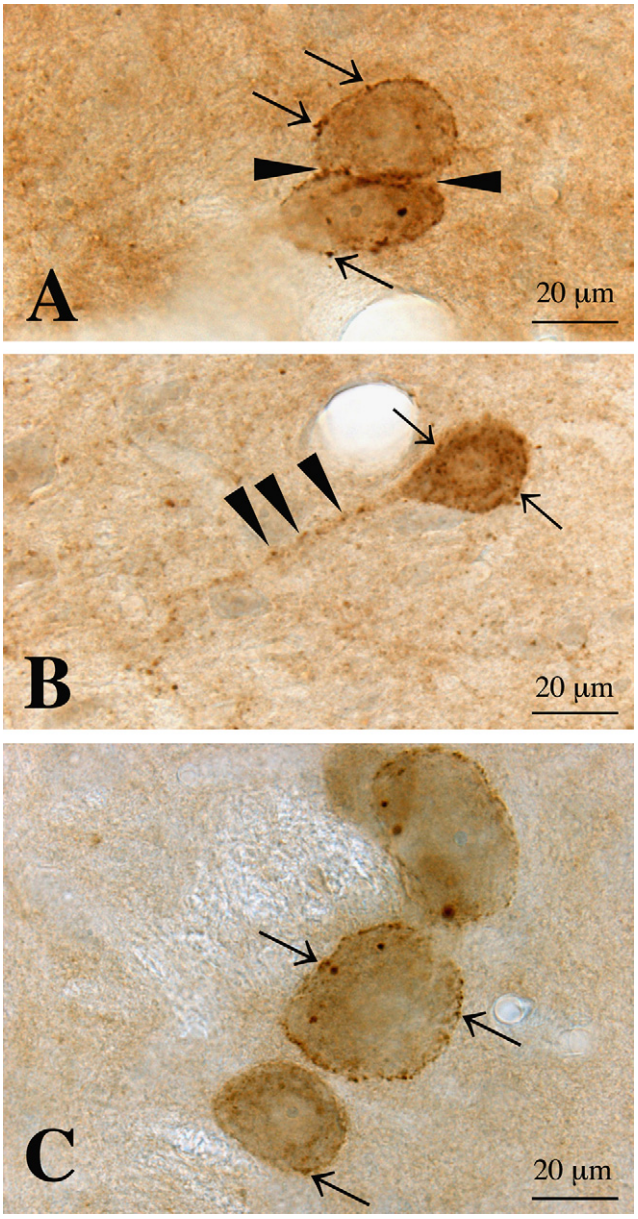

Fig. 1. High (A–C) magnification photographs showing drebrin-immunoreactive cell bodies and puncta. The latter are often associated with the cytoplasmic membrane (arrows in A–C) at the level of superior colliculus (A and B) and inferior colliculus (C) in transverse sections. Arrowheads in A indicate somato-somatic adhesion. Note that a dendrite-like process (arrowheads in B) extending in a ventro-lateral direction is recognized in a rare case. Scale bars in A–C = 20 μm.