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Research Report

Some γ -motoneurons contain γ -aminobutyric acid in the rat cervical spinal cord

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ARTICLE INFO

Article history:

Accepted 10 January 2008

Available online 2 February 2008

Keywords:

Colocalization

Immunohistochemistry

In situ hybridization

Ventral horn

Schwann cell

ABSTRACT

γ -aminobutyric acid (GABA) is utilized in the peripheral as well as central nervous system. In this study, fibers immunoreactive for 67 kDa isoform of glutamic acid decarboxylase (GAD67), an enzyme which synthesizes GABA, were found to terminate in the intercapsular region of muscle spindles of the upper limb. GABA-containing fibers were also found in the ventral roots of C5 to T5 spinal segments, brachial plexus, and radial nerve. These fibers were thin and immunoreactive for choline-acetyl transferase (ChAT). After transection of the brachial plexus, GABA immunoreactivity disappeared completely in the ipsilateral triceps brachii muscle (TBM). After the injection of fluorogold into the TBM, some retrogradely labeled medium-sized neurons were positive for GAD67, but not VGAT mRNA. All these observations clearly indicate that GABA-containing γ -motoneurons in the lower cervical spinal cord send their fibers to muscle spindles in the upper extremities. Since we detected neither GABA_A nor GABA_B receptors in the TBM by RT-PCR, the function of the GABA-containing γ -motoneurons remains unclear.

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1. Introduction

Somatic motor neurons, which include α -, β -, and γ -motoneurons, use acetylcholine as a neurotransmitter. Once released, acetylcholine excites both extrafusal and intrafusal muscles via nicotinic receptors. Thus, acetylcholine acts as an excitatory neurotransmitter. Years after “Dale’s law”, which claims that one neuron uses one transmitter (Dale, 1935),

much is now known about the colocalization of neurotransmitters. Namely, many types of neurons use more than one neurotransmitter (e.g. Johnson et al., 1992; Piehl et al., 1993; Shupliakov et al., 1993; Ito et al., 2005, 2007; Ito and Nojyo, 2008). Nevertheless, only a few studies (Johnson et al., 1992; Piehl et al., 1993; Shupliakov et al., 1993) have examined the colocalization of neurotransmitters in somatic motoneurons. In contrast, there have been many studies about colocalization

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Abbreviations: ir, immunoreactive; ChAT, choline-acetyl transferase; VH, ventral horn; TBM, triceps brachii muscle; DRG, dorsal root ganglion; VR, ventral root; DR, dorsal root; DAPI, 4',6-diamidino-2-phenylindole dihydrochloride; FG, fluorogold; GAD, glutamic acid decarboxylase; GAD67, 67 kDa isoform of GAD; GABA, γ -aminobutyric acid; PB, phosphate buffer; PBS, 0.05M phosphate-buffered saline; PBS-X, PBS containing 0.3%; Triton X-100, ; PBS-XD, PBS containing 0.3%; Triton X-100, 1%; normal donkey serum, ; RT, room temperature; NLS, N-lauroylsarcosine; DAB, diaminobenzidine; FITC, fluorescein isothiocyanate; VGAT, vesicular GABA transporter; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

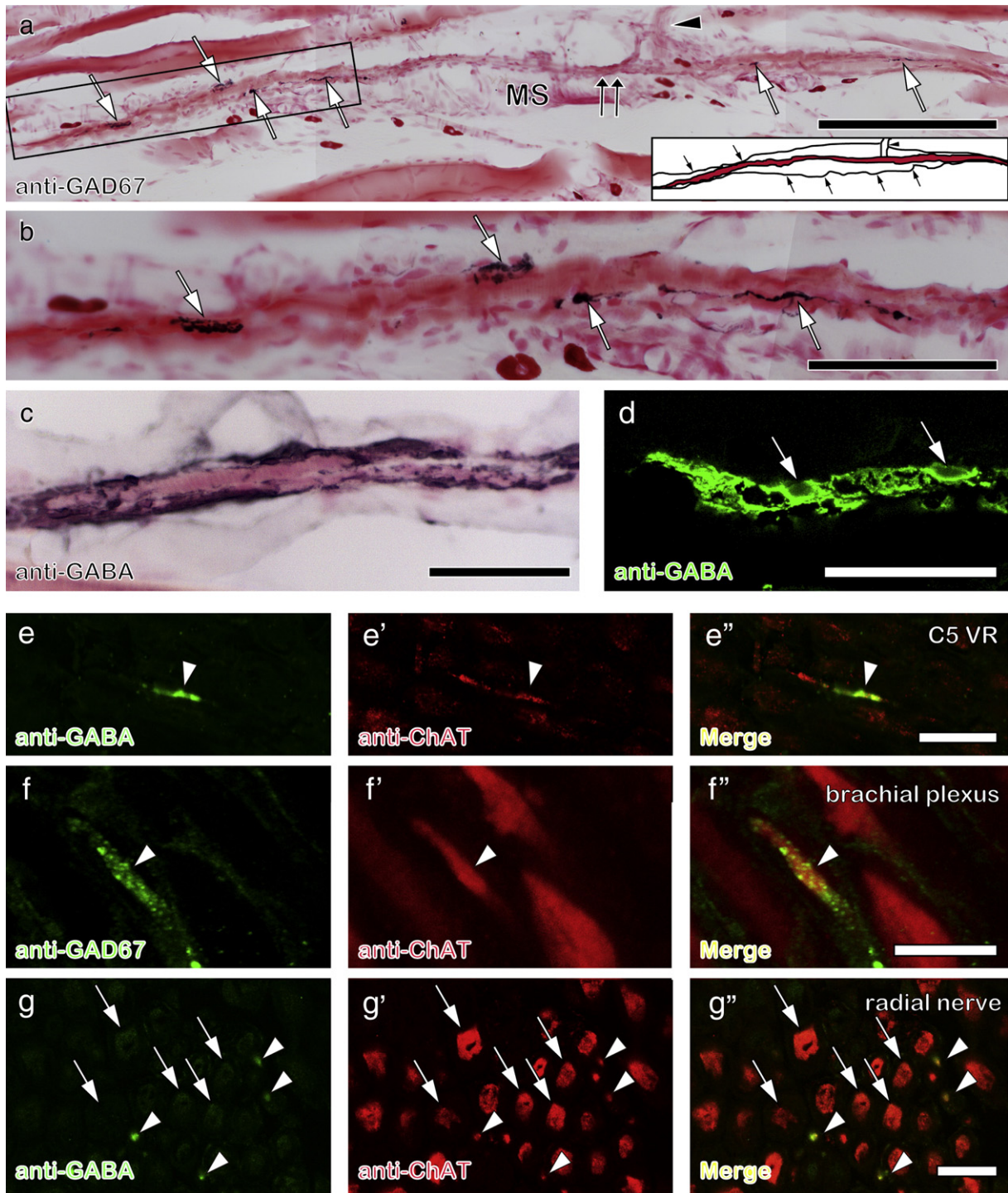


Fig. 1 – GABA-containing fibers in the upper extremities. In a muscle spindle (MS) of the triceps brachii muscle (TBM) counterstained with Neutral Red (a), GAD67-ir fibers (white arrows in a) were found in the intercapsular region. Note that a GAD67-negative nerve bundle (arrowhead in a) entered the equatorial region (double arrow in a). At a higher magnification of the boxed region in a (b), we can clearly observe that GAD67-ir fibers were apposed upon the intrafusal muscle fibers (white arrows in b). In sections immunostained for GABA, structures surrounding intrafusal muscle fibers were labeled (c, d). When the optical sections were projected into one Figure (12 optical sections collected from 2.63 μm z-axis range were processed for maximum projection; d), we can observe that these structures were composed of cell bodies (arrows in d) and their processes. Furthermore, GABA-containing fibers (arrowheads in e–g) were found in the ventral roots (VRs; e), brachial plexus (f), and radial nerve (g). All of these fibers were immunoreactive for choline-acetyl transferase (ChAT; red). ChAT-ir fibers which showed immunoreactivity for GABA or GAD67 (arrowheads in e–g) were much thinner than those immunonegative for GABA or GAD67 (arrows in f and g). Note: Inset in a shows a schematic diagram of a, and black arrows indicate sheath of the MS. Bar: 250 μm (a), 100 μm (b), 50 μm (c, d), 20 μm (e, g), and 10 μm (f).

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