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Neurochemical diversity of neurogliaform cells in the human primary motor cortex

S.G. Kalinichenko [a,b], I.I. Pushchin [c,*], I.V. Dyuizen [b,d]

[a] *Laboratory of Cell Physiology, Institute of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, 17 Palchevskogo street, Vladivostok 690041, Russia*
[b] *Department of Histology, Vladivostok State Medical University, 2 Ostryakova pr., Vladivostok 690650, Russia*
[c] *Laboratory of Physiology, Institute of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, 17 Palchevskogo street, Vladivostok 690041, Russia*
[d] *Laboratory of Pharmacology, Institute of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, 17 Palchevskogo street, Vladivostok 690041, Russia*

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Abstract

Neurogliaform cells of area 4 of the human motor cortex were found to express choline acetyl transferase (ChAT), gamma-aminobutyric acid, and calbindin. GABA- and calbindin-positive NGCs were mainly localized in layers II and VI and were relatively rare in layer I of the cortex. ChAT-positive NGCs were observed in the upper and middle thirds of layer II, occurring occasionally in layer I and the upper portion of layer III. Their numbers were low compared to those of GABA- and calbindin-positive NGCs in layers II/III. The dendrites of ChAT-positive NGCs were short and few in number. Axonal arborizations of neighboring ChAT-positive cells interpenetrated considerably so that each ChAT-positive cell body was normally surrounded by axonal trees of the parent and a few other ChAT-positive NGCs. NGC axon collaterals surrounded small neuropil areas containing perikarya presumptive pyramidal neurons. The findings are discussed in the context of information processing in cortical modules and interaction of excitatory and inhibitory interneurons.

Keywords: Cortical inhibitory interneurons; Interneuronal connections; Neuromediators; Choline acetyl transferase; GABA; Calbindin; Neocortex

1. Introduction

The complexity of cortical networks is due to the presence of more than 10 interneuron types comprising 20–25% of the total number of cells in the cortex (Beaulieu et al., 1992; Markram et al., 2004). The bulk of cortical neurons are GABAergic local circuit cells contributing to cortical inhibition on a different scale and via different mechanisms (Somogyi et al., 1998; Douglas and Martin, 2004). Some classes of these neurons are neurochemically diverse expressing different receptor molecules and neuropeptides and other neuromodulators. They include interneurons containing Ca-binding proteins: parvalbumin-positive basket and chandelier cells; calbindin-positive double bouquet, neurogliaform, and Martinotti cells; and calretinin-positive bipolar and Cajal-Retzius cells (Condé et al., 1994; Gabbott and Bacon, 1996; DeFelipe, 1997; Meskenaite, 1997). Despite interneurons fall into discrete subpopulations each expressing predominantly or exclusively one of these proteins, some GABAergic interneurons are reported to simultaneously express different Ca-binding proteins (del Río and DeFelipe, 1997).

A relatively small fraction of intrinsic cortical neurons contain choline acetyltransferase and receive inputs from cholinergic corticopetal fibers originating from neurons of the basal forebrain nuclei (Eckenstein and Baughman, 1983; Mesulam et al., 1983; Gritti et al., 1993). Cholinergic afferents are found in all layers of the neocortex, where they converge on pyramidal and non-pyramidal neurons (Foote and Morrison, 1987; Lewis, 1991; Mrzljak et al., 1995). Acetylcholine acts as both a conventional transmitter and as a paracrine agent, with the exact effects depending on the ratio between pre- and postsynaptic muscarinic and/or nicotinic receptors (Van der Zee

* Corresponding author. Tel.: +7 4232 311150; fax: +7 4232 310900.
E-mail address: pushchin@imb.dvo.ru (I.I. Pushchin).

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and Luiten, 1999). It may modulate the activity of pyramidal cells both directly and via activation of GABAergic interneurons (McCormick and Prince, 1986; Xiang et al., 1998). Cholinergic depolarization mainly involves calbindin-, cholecystokinin-, VIP-, and somatostatin-expressing interneurons are depolarized by acetylcholine administration, while parvalbumin-reactive inhibitory neurons remain unresponsive to acetylcholine (Kawaguchi, 1997).

Among known classes of interneurons, neurogliaform cells (NGCs) are least studied in terms of their neuromediator and transmitter/receptor systems. NGCs are the smallest cortical interneurons, morphologically similar to glial cells. They were first described by Cajal in the human neocortex and striatum (Ramon y Cajal, 1899, 1921). NGCs are the only interneurons that occur in all neocortical layers (Jones, 1984). They have smooth, slightly beaded dendrites. Primary dendrites emanate radially from the soma bending and branching dichotomously to form a near-spherical dense arborization 200–250 μm in diameter. A thin highly beaded axon produces numerous collaterals, which interlace with the parent cell's dendrites to form a 400-μm plexus resembling a loose thread clew. Axon terminals bear rare en passant varicosities and form solitary contacts on basal dendrites of the pyramidal neurons (Shkol'nik-Yarros, 1971; Jones, 1984; Valverde, 1985; Kisvárday et al., 1990). NGC axons also synapse onto non-pyramidal neurons. Thus, in layer IV of the human primary visual cortex, they come into contact with dendrites of spiny stellate neurons (Douglas and Martin, 2004). Here, NGCs are probably functionally coupled to clutch cells. This couple provides the major source of local inhibitory firing, controlling excitatory activity of spiny stellate cells (Okhotin and Kalinichenko, 2002).

In the mammalian neocortex, a single, calbindin-expressing NGC type was postulated (Lund and Lewis, 1993; Condé et al., 1994; Gabbott and Bacon, 1996). However, in the human brain, NGCs are predominantly GABA-positive (Kisvárday et al., 1990). These cells also express a number of membrane galactosamines, absent in other GABAergic cortical interneurons (Naegele and Katz, 1990).

The present study was aimed at revealing ChAT, GABA, and calbindin activity in neurogliaform cells of the human primary motor neocortex.

2. Materials and methods

The material came from six human subjects aged 25–37 without neurological pathologies. The brain tissue was taken at autopsy within 6–12 h postmortem. The cortex was placed onto glass and cut into 1 cm × 0.5 cm pieces.

Neurons were visualized by rapid Golgi staining as described in Fairén et al. (1977).

GABA and calbindin immunocytochemistry was performed as described in (Gabbott and Bacon, 1996) using rabbit polyclonal antibodies against GABA (Sigma) and calbindin (Swant). In brief, cortical pieces were fixed in cold 4% paraformaldehyde fixative for 3 h, post-fixed in 2% paraformaldehyde and 0.2% glutaraldehyde at 4 °C for 24 h, and washed in several changes of 30% buffered sucrose solution at 4 °C for 24 h.

Cryostat sections were prepared and incubated in 3% normal goat serum and 0.25% Triton X-100 (Serva) solution in 0.1 M phosphate buffer (pH 7.4) at 4 °C for 3–4 h, placed in biotinylated goat antiserum against rabbit IgG (Vector Laboratories, dilution 1:200) and 3% normal goat serum on 0.1 M phsphate buffer (pH 7.4) at room temperature for 1 h, washed in three changes of phosphate buffer, and incubated in avidin–biotin–horseradish peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories, dilution 1:100) at room temperature for 1 h. After that, sections were washed in phosphate buffer and incubated in 0.05% 3-3′diaminobenzidine tetrahydrochloride (Sigma) solution and 0.01% hydrogen peroxide in 0.1 M phasphate buffer (pH 7.4) for 20 min. They were then washed thoroughly in phosphate buffer, dehydrated, and mounted.

Control sections were incubated in the same media without primary antibodies.

ChAT reactivity was revealed using the method of Burt and Silver (1973) adapted for human brain (Okhotin et al., 1999). In brief, cortical pieces 4–5 mm in size were fixed in 1% paraformaldehyde and 0.32 M sucrose solution in 0.1 M cacodylate buffer (pH 5.0) at 4 °C for 2 h and rinsed in seven changes of sucrose solution in cacodylate buffer (pH 5.2) for 18 h.

Cryostat sections 12–15 μm thick were pre-incubated in 2 mM di-isopropyl fluorophosphate and 10% sucrose in 0.1 M cacodylate buffer (pH 6.0) at 4 °C for 1 h, incubated in 1 mM di-isopropyl fluorophosphate, 4 mM choline chloride, 1 mM lead nitrate, 0.3 mM acetyl-CoA, and 5% sucrose in 0.1M cacodylate buffer (pH 6.0) at 37 °C for 2.5 h. They were then washed in three changes of distilled water, treated with 5% ammonium sulphide solution, dehydrated, and mounted.

Reaction specificity was confirmed in a series of control experiments. In the first experiment, di-isopropyl fluorophosphate was excluded from the incubation media. In the second, the media either acetyl-CoA or choline chloride were excluded. In the third, 10 mM chloride acetylcholine perchlorate was added to di-isopropyl fluorophosphates-containing incubation medium and the time of section pre-incubation in the ice bath was increased to 1.5–2 h.

3. Results

In Golgi sections, NGCs occurred in all neocortical layers. Their bodies were rounded or slightly elongated, 10–15 μm in diameter. They gave rise to several smooth primary dendrites, which branched rarely and had rare en passant and terminal varicosities (Figs. 1 and 2). The morphology of the NGCs revealed was generally similar to that of NGCs described by other authors (Meyer, 1983; Lund and Yoshioka, 1991).

GABA-, ChAT-, and calbindin-positive NGCs were identified by their similarity in soma size, proximal dendrite ramification, axonal arborization, and distribution to confirmed NGCs in Golgi sections.

GABA- and calbindin-positive NGCs were found in all cortical layers (Fig. 3). They were mainly localized in layers II and VI and were relatively rare in layer I (Table 1). ChAT-positive NGCs were observed in the upper and middle thirds of layer II, occurring occasionally in layer I and the upper portion of layer III (Fig. 4). Their numbers were low compared to those of GABA- and calbindin-positive NGCs in layers II/III. Their dendrites were short and few in number. Axonal arborizations of neighboring ChAT-positive cells interpenetrated considerably so that each ChAT-positive cell body was normally surrounded by axonal trees of the parent and a few other ChAT-positive NGCs (Fig. 4B, C). NGC axon collaterals surrounded small neuropil areas containing perikarya presumptive pyramidal neurons (Fig. 4D). It is not clear whether these perikarya are targets of NGC axon terminals or whether they make contacts with cortical afferents or other cholinergic neuron types.

In all control experiments (see Section 2 for details), no specific labeling was observed, confirming the specificity of the