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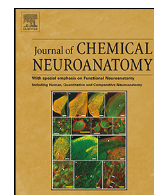

Tyrosine hydroxylase-producing neurons in the human cerebral cortex do not colocalize with calcium-binding proteins or the serotonin 3A receptor

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ABSTRACT

Interneurons of the cerebral cortex play a significant role in cortical information processing and are of clinical interest due to their involvement in neurological disorders. In the human neocortex, three subsets of interneurons can be identified based on the production of the calcium-binding proteins parvalbumin, calretinin or calbindin. A subset of interneurons in the mouse cortex expresses the serotonin 3A receptor ($5\text{-}HT_{3A}R$). Previous work in humans has also demonstrated the presence of a subgroup of cortical neurons that produces the catecholaminergic enzyme tyrosine hydroxylase (TH). Many TH-producing cells in the rat cortex coexpress calretinin and are adjacent to blood vessels. However, little is known about the phenotype of these TH interneurons in humans. Here we immunohistochemically examined the coexpression of TH with parvalbumin, calretinin, calbindin or $5\text{-}HT_{3A}R$ in human Brodmann's areas 10 and 24, cortical regions with high densities of TH-containing neurons. Colocalization of TH with these calcium-binding proteins and with $5\text{-}HT_{3A}R$ was not detected in either area. Cortical TH cells were rarely apposed to blood vessels, denoted by immunolabeling for the gliovascular marker aquaporin-4. Our results suggest that the TH-immunoreactive cells in the human cortex do not overlap with any known neurochemically-defined subsets of interneurons and provide further evidence of differences in the phenotype of these cells across species.

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1. Introduction

Interneurons in the cerebral cortex are predominantly non-pyramidal, γ-aminobutyric acid (GABA)-producing cells that provide inhibitory input to the more numerous projection neurons, thereby regulating the cortical microcircuitry (DeFelipe, 2002; Markram et al., 2004). Cortical interneurons are clinically relevant; their dysfunction has been implicated in a variety of neurological disorders, including schizophrenia and epilepsy (Benes and Berretta, 2001; Chu and Anderson, 2015; Daskalakis et al., 2007; Di Cristo, 2007; Levitt et al., 2004; Lewis et al., 2012; Marin, 2012). Diverse subgroups of interneurons have been identified on the basis of their morphology, electrophysiological characteristics, embryonic origin, and molecular and neurochemical attributes (Ascoli et al., 2008; Chu and Anderson, 2015; Vitalis and Rossier, 2011; Wonders and Anderson, 2006). In the rodent neocortex, three non-overlapping subsets of interneurons have been defined based on the production of the calcium-binding proteins parvalbumin (PV) or calretinin (CR) or the peptide somatostatin (SST) (Gonchar and Burkhalter, 1997; Kubota et al., 1994; Xu et al., 2004). In the mouse, a subset of cortical interneurons can be identified by the expression of the ionotropic serotonergic receptor subtype, 5-hydroxytryptamine 3A ($5\text{-}HT_{3A}R$), and this subgroup overlaps with the previously-identified CR subset (Lee et al., 2010; Rudy et al., 2011; Vucurovic et al., 2010). Interneurons in the primate neocortex, likewise, have been subdivided into three neurochemically-distinct groups that contain PV, CR, or another calcium-binding protein calbindin (CB) (Carder et al., 1996; Conde et al., 1994; DeFelipe, 1997; Gabbott et al., 1997; Glezer et al., 1993; Sherwood et al., 2007, 2010; Zaitsev et al., 2009). The presence of $5\text{-}HT_{3A}R$ in human interneurons has not been reported.

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A subpopulation of neurons in the neocortex of rodents (Asmus et al., 2008, 2011; Berger et al., 1985; Kosaka et al., 1987a,b; Satoh and Suzuki, 1990; Wachter et al., 2014), non-human primates (Raghanti et al., 2009; Weihe et al., 2006), and humans (Benavides-Piccione and DeFelipe, 2003, 2007; Fukuda et al., 1999; Gaspar et al., 1987; Hornung et al., 1989; Ikemoto et al., 1999; Kuljis et al., 1989; Marui et al., 2003; Raghanti et al., 2009; Trottier et al., 1989), is immunoreactive (IR) for tyrosine hydroxylase (TH), the first enzyme in the catecholamine biosynthesis pathway. Cortical TH-IR cells are considered to be inhibitory interneurons because they are small, non-pyramidal cells, many of which coexpress GABA or its biosynthetic enzyme, glutamic acid decarboxylase (GAD). These cells are outside the classically-defined catecholaminergic cell groups that are present in subcortical brain regions (Hokfelt et al., 1984). Moreover, in all species examined, these TH-IR cells do not contain any subsequent catecholaminergic enzymes (Berger et al., 1985; Gaspar et al., 1987; Ikemoto et al., 1999; Satoh and Suzuki, 1990; Weihe et al., 2006), leaving their end-product in question. In rats TH-IR cells are present in all cortical laminae but are most abundant in layers II/III (Asmus et al., 2008; Berger et al., 1985; Kosaka et al., 1987a, 1987b). Coexpression of CR but not PV or SST was observed in many cortical TH-IR cells in normal rats (Asmus et al., 2008) and in rats treated intraventricularly with the catecholaminergic neurotoxin 6-hydryoxydopamine (6-OHDA) (Wachter et al., 2014). Furthermore, in rats many of the TH-IR somata are adjacent to cortical blood vessels that are outlined by aquaporin-4 (AQP4) (Asmus et al., 2011), which is a channel protein found in the astrocytic end-feet surrounding central nervous system (CNS) microvessels (Tait et al., 2008). In humans, TH-IR cells are found predominantly in layers V and VI, although more superficially-located TH-IR cells are observed in some areas (Benavides-Piccione and DeFelipe, 2003, 2007; Fukuda et al., 1999; Gaspar et al., 1987; Hornung et al., 1989; Ikemoto et al., 1999; Kuljis et al., 1989; Marui et al., 2003; Raghanti et al., 2009; Trottier et al., 1989). Neurons that produce TH are widely distributed throughout the human neocortex, with two of the highest densities of these cells being reported in the associative dorsolateral frontal cortex (Brodmann's area (BA) 10) and in the limbic cingulate cortex (BA 24) (Benavides-Piccione and DeFelipe, 2007). In the human temporal cortex, approximately one-fourth of the TH-IR neurons coproduce neuronal nitric oxide synthase (nNOS), the biosynthetic enzyme for nitric oxide (Benavides-Piccione and DeFelipe, 2003). Colocalization of TH with other neurochemical markers and proximity of TH-IR cells to the cortical vasculature have not been reported in the human cortex.

The paucity of information regarding the neurochemical phenotype and possible function of cortical TH-IR neurons, especially in humans, is surprising given that a loss of these cells is seen in Parkinson's disease (Fukuda et al., 1999) and dementia (Marui et al., 2003). Here, we tested the hypothesis that human cortical TH-IR somata in BA 10 and 24 overlap with one or more of the neurochemically-defined subgroups of interneurons that produce PV, CR, CB, or 5-HT_{3A}R. The close association of TH-IR cell bodies and cortical microvessels was also examined in these areas using the gliovascular marker AQP4.

2. Materials and methods

2.1. Specimens, fixation and processing

Human cortical samples (BA 10 and 24) derived from the left hemisphere were provided by the Northwestern University Alzheimer's Disease Center Brain Bank. The human subjects were non-geriatric adults, exhibited no evidence of cognitive changes before death, and all received a score of zero for the CERAD senile plaque grade (Mirra et al., 1991) and the Braak and Braak (1991) neurofibrillary tangle stage. Cortical samples were analyzed from a subset of seven brains, four female (age range 40–53) and three male (age range 54–56). The postmortem interval (PMI) prior to immersion-fixation did not exceed 17 h (see Table 1 for complete demographic and PMI information). Because this research involved the use of postmortem specimens, the project was exempt from the need for approval of Centre College's Institutional Review Board regarding human subjects.

Brain samples were immersion-fixed in 10% buffered formalin for approximately one week, then transferred to 0.1 M phosphate buffered saline (PBS, pH 7.4) containing 0.1% sodium azide at 4 °C. Prior to sectioning, specimens were cryoprotected in a series of sucrose solutions (10, 20, and 30%) until saturated. Brains were frozen in dry ice and sectioned at 40 μm using a freezing sliding microtome (Leica SM2000R, Buffalo Grove, IL). Sections were placed into individual microcentrifuge tubes containing a freezer storage solution (30% of each distilled water, ethylene glycol, and glycerol and 10% 0.244 M phosphate buffered saline) and numbered sequentially. Sections were stored at −20 °C until further processing.

2.2. Immunohistochemistry

The protocol followed here was a modification of a previously-reported protocol (Raghanti et al., 2009). To examine the colocalization of TH with calcium-binding proteins, rabbit anti-TH (1:200, Pel-Freez Biologicals, Rogers, AR) was used in combination with one of the following antibodies: mouse anti-PV (1:500, Millipore, Billerica, MA), mouse anti-CR (1:1000, Millipore), or mouse anti-CB (1:8000, Swant, Bellinzona, Switzerland). To examine the colocalization of TH with 5-HT_{3A}R, mouse anti-TH (1:200, ImmunoStar, Hudson, WI) was used in combination with rabbit anti-5-HT_{3A}R (1:50, Sigma, St. Louis, MO). To determine the apposition of TH-IR cells and microvessels, mouse anti-TH (1:200, ImmunoStar) was used in combination with a rabbit antibody against the gliovascular marker AQP4 (1:100, Millipore).

The following secondary antibodies (Jackson ImmunoResearch, West Grove, PA) were used in combination: Alexa Fluor 488- or DyLight 488-conjugated donkey anti-rabbit (1:100) and Rhodamine Red-X-conjugated donkey anti-mouse (1:50). Control experiments confirmed that secondary antibodies bound only to the appropriate primary antibodies.

All incubations and wash steps were conducted with free-floating sections in a 24-well plate on an orbital shaker. The brain tissue sections were washed with PBS 10 times for 5 min each (10×5). All subsequent incubations were followed by 6×5 washes in PBS. For antigen retrieval, the sections were incubated in ImmunoSaver Antigen Retriever (1:200, Electron Microscopy Sciences, Hatfield, PA) in distilled water and heated for 30 min in a water bath at either 85° C (for TH/CB labeling) or 100° C (for TH/PV, TH/CR, TH/5-HT_{3A}R, and TH/AQP4 labeling). The sections were allowed to cool at room temperature for 10–20 min and then were incubated for one hour at room temperature in dilution buffer (PBS

Table 1
Demographics of cases used in this study.

Case	Age (years)	Gender	PMI (hours)
1	54	M	12
2	56	M	5
3	56	M	5
4	40	F	17
5	43	F	10
6	43	F	6
7	54	F	13

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