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Immunoreactivity pattern of calretinin in the developing human cerebellar cortex

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ABSTRACT

The immunohistochemical expression of the calcium-binding protein calretinin during human cerebellar development has been investigated in this study. Human cerebellum samples, obtained from 7 fetuses and newborns ranging from 11 to 38 weeks of gestation, were 10% formalin-fixed, routinely processed and paraffin-embedded. 3 μm-tick sections were immunostained with an anti-calretinin antibody. Our study evidenced a different immunoreactivity for calretinin in Purkinje cells and in several cerebellar interneurons at different intrauterine developmental stages. Whereas at 11 weeks of gestation calretinin immunoreactivity was not detected in the developing cerebellum, from the 18th to the 24th week, calretinin expression was found in Purkinje cells migrating from the ventricular neuroepithelium and in migrating cerebellar interneurons. From the 30th to the 38th week, calretinin was expressed by most of Purkinje cells and by migrating cerebellar interneurons. Furthermore, granule cells in the internal granular layer were also immunoreactive for calretinin. Our data show that calretinin, other than for developing Purkinje cells, is a useful marker also for migrating cerebellar interneurons and for some neuronal elements related to the granular layer. Moreover, given the critical role of calcium in a great variety of neuronal processes in the central nervous system, our findings suggest that calretinin may play a pivotal role in the regulation of neuronal excitability during intrauterine cerebellar development.

1. Introduction

The development of the cerebellum occurs throughout the intrauterine life and during early postnatal life (Laure-Kamionowska and Maślińska, 2009). The cerebellum is tightly associated with motor function, concerned to fine movement execution, co-ordination of voluntary movement, control of muscle tone, posture and gait (Swinny et al., 2005; Proville et al., 2014). Cerebellum also participates to cognitive, emotional and behavioral development (Dolan, 1998; Baumann and Mattingley, 2012; Martinez, 2014; Noroozian, 2014; Grossauer et al., 2015). Structurally, the cerebellum consists of: a) the cerebellar cortex, b) the internal white matter and c) cerebellar nuclei. Superficially, the mature cerebellar cortex comprises a simple and uniform three-layered structure, containing different types of neurons. From outside to inside, these layers are: a) the molecular layer, b) the Purkinje cell layer and c) the granular layer. The developing human cerebellar cortex has an additional superficial layer, the "external granular layer", consisting of granule cells progenitors that migrate inward to form the internal granular layer and disappear up to 1 year postnatally (Komuro et al., 2001; Volpe, 2009).

Neural connections exist between different areas of the brain (prefrontal cortex, dorso-lateral cortex, parietal lobe, cingulate cortex, the upper part of the temporal lobe) and cerebellum, allowing a careful implementation of cerebellar functions (Middleton and Strick, 1994; Apps and Garwicz, 2005). In order to exert its roles, cerebellum utilizes a neural network composed of specific neuroelements (Kwong et al., 2000).

The purpose of this study was to define, by immunohistochemistry, cerebellar neuronal populations containing calretinin during the prenatal development (11–38 week of gestation) of human brain. Calretinin (CR) is a calcium-binding protein (CaBPs) involved in the maintenance of intracellular calcium homeostasis. Similarly as other CaBP parvalbumin and calbindin, CR also belongs to the EF-family of CaBPs, characterized by six domains with different affinity to bind calcium ions (Rogers, 1987). Immunoreactivity to CR has been reported in different areas of the central nervous system of various animal species (Resibois and Rogers, 1992). In humans, few studies regarding the immunoreactivity for CR in the cerebellum exist, particularly during development. The study by Yew

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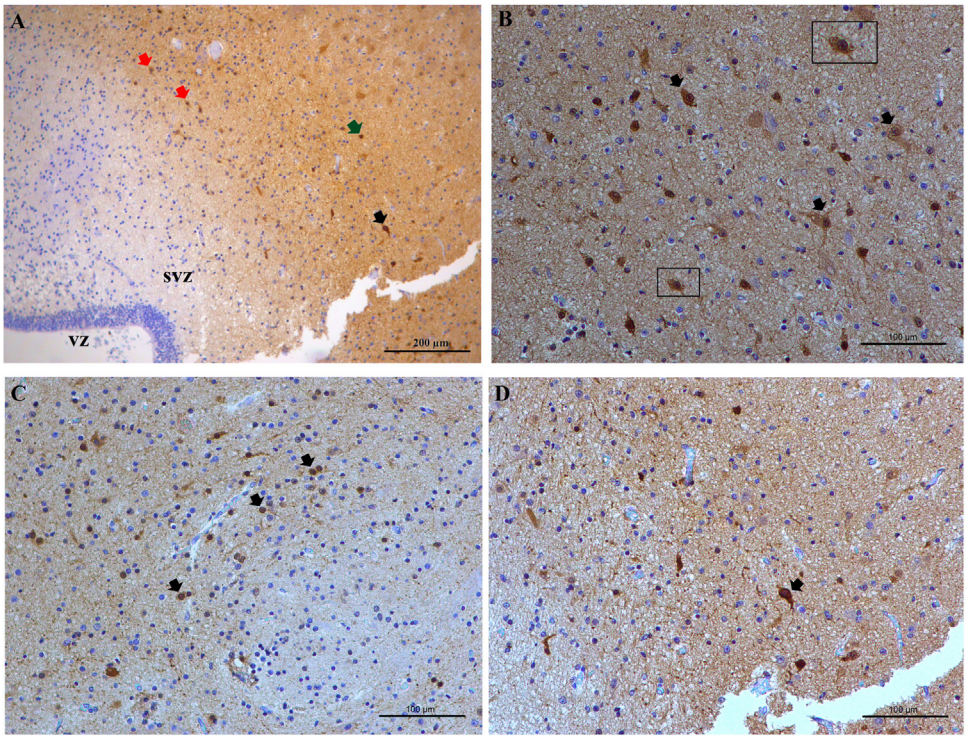

Fig. 1. 18 weeks of gestation. A: CR expression in migrating Purkinje cells (red arrows), Golgi cell (green arrow) and unipolar brush cell (black arrow) from the SVZ of the neuroepithelium. B: CR expression in migrating Purkinje cells (black arrows). Insert: 50 µm. C: CR expression in migrating round cells concerning to cerebellar Golgi cells (black arrows). D: CR expression in migrating unipolar brush cell, characterized by a small soma and a short dendritic process similar to a brush (black arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al. (1997) demonstrated that, in the developing human cerebellum, CR appeared approximately at the 21st week, more later than calbindin and parvalbumin, its expression increasing with developmental age. Since the 21st week, CR-immunostaining cells was found in Purkinje cells, basket cells and in neurons of deep nuclei (Yew et al., 1997). Few data regarding the distribution of CR in the human cerebellum during postnatal development and adulthood have been reported (Víg et al., 2005). The number of CR-reactive unipolar brush cells (UBCs) increases after birth because their proliferation is prolonged during the postnatal period, till the 11th month of age (Abrahám et al., 2001). Therefore, the distribution of CR-immunoreactivity within the granule cell layer of the adult human cerebellar cortex has been reported (Braak and Braak, 1993).

On these bases, our study was designed to describe CR-immunopositive cerebellar cells and their localization, during development.

2. Materials and methods

Cerebellum samples were obtained from 4 human fetuses ranging from 11 to 24 weeks of gestation and 3 neonates, from 30 up to 38 weeks of gestation, from the Obstetric Division of the University of Cagliari. All the fetuses and newborns included in this study had no congenital brain malformation. Regarding the cause of death, the 11 week-old fetus underwent voluntary termination of pregnancy (VTOP); the 20 week-old fetus underwent therapeutic abortion following the diagnosis of diaphragmatic hernia; placental detachment was the cause of death in the 24 week-old fetus, in newborns of 30, 34 and 38 weeks of gestation the cause of death was sepsis. All procedures were approved by the Ethic Human Studies Committee of University Medical Centre of Cagliari (according to the instructions of the Declaration of Helsinki). Samples were fixed in 10% buffered formalin, routinely processed, and paraffin-embedded. For each cerebellar sample, we used one paraffin block and 2 sections per case were examined. Serial 3 µm-tick sections were obtained from each paraffin block; after dewaxing and rehydrating, one section was stained with hematoxylin-eosin, while the others were pre-treated for immunohistochemistry, with 10 min heat-induced epitope retrieval in buffer pH 9.00 (*EnVision™ FLEX Target Retrieval Solution High pH*; *Dako Denmark A/S, Glostrup, Denmark; Code K8004)*. Slides were then incubated for 20 min at room temperature with an anti-calretinin antibody (*Dako*; *Code* M- *7245*; monoclonal mouse antibody; clone DAK-Calret1 at 1:1000 dilution). Staining procedures were performed by Envision™ FLEX+ (*Dako; Code K8002*) Detection System and *AutostainerLink 48* instrument following dealer's instructions. As positive control, a section of human malignant mesothelioma was utilized. Data were obtained by evaluation of positivity (+) and negativity (−) for CR immunoreactivity in each cerebellar sample.

3. Results

Immunoreactivity for CR was detected in 6 out of 7 of the human cerebellar samples considered in this study. Among the several gestational ages analyzed, differences were found regarding CR-immunoreactive cells in the cerebellar cortex, suggesting that CR has not a homogenous expression during human cerebellar development. Moreover, a different immunohistochemical localization of CR was found in a specific population during development of human cerebellum. Whereas at 11 weeks of gestation immunoreactivity for CR was not detected, in the cerebellar specimens from the 18th to the 38th week CR immunoreactivity was found in different neuronal populations in the developing cerebellum. Furthermore, in the internal granular layer, immunoreactivity for CR was detected in the cytoplasm of granule cell precursors, from the 30th to the 38th week. Immunoreactivity for CR in the different cerebellar cell types at the different gestational ages was summarized in Table 1.

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