Int. J. Devl Neuroscience 30 (2012) 333–341

Contents lists available at SciVerse ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Expression of coat proteins changes during postnatal development in selected areas of the rat brain

Janina Borgonovo[a,1], Pablo Capella[a,1], Alicia Seltzer[c,1], Miguel A. Sosa[a,b,*,1]

[a] Laboratorio de Biología y Fisiología Celular "Dr. Francisco Bertini", Instituto de Histología y Embriología, FCM, Universidad Nacional de Cuyo, Mendoza, Argentina
[b] Instituto de Ciencias Básicas–Universidad Nacional de Cuyo, Mendoza, Argentina
[c] Laboratorio de Desarrollo Perinatal. Instituto de Histología y Embriología, FCM, Universidad Nacional de Cuyo, Mendoza, Argentina

ARTICLE INFO

Article history:
Received 7 September 2011
Received in revised form 3 January 2012
Accepted 17 January 2012

Keywords:
Clathrin coated vesicles
Adaptins
Endocytosis
Intracellular transport
Brain development

ABSTRACT

It is well known that clathrin-mediated endocytosis is crucial for the normal functioning and integrity of neurons in the central nervous system. In this study we attempted to correlate the expression of coat proteins with development in different areas of rat brain. By Western blot, we studied the expression of AP-2, GGA1 and GGA2 in striatum, cerebellum, brain stem, cerebral cortex and hippocampus of newborn rats and during post-natal development; 5, 15, 30, 60, 90 or 150 days after birth. We observed that the expression of the α2 subunit of AP-2 increased substantially between the 15th and 30th day after birth in all areas studied, excepting the cerebellum and cortex. On the other hand, the expression of the α1 subunit does not change significantly during the development in any of the areas under study. We also noted that the expression of the μ2 subunit did not follow the pattern of α2 during development. In general terms, the expression of GGA1 and GGA2 followed a similar pattern to that of AP-2, although these proteins increased significantly in the cerebral cortex from the 15th day after birth. Moreover, presenilin-1, a protein associated with aging and neurodegeneration, shows an expression pattern similar to coat proteins in the striatum and cortex. These results suggest that proteins that conform the intracellular transport machinery in the brain cells seems to accompany development, according to the maturation of the different brain areas.

1. Introduction

Vesicle-mediated transport is one of the main ways to carry material from one area to another within the cell, or in the exchange with the extracellular environment or with other cells (Conner and Schmidt, 2003). In the central nervous system, the clathrin-mediated transport appears crucial for the proper functioning and maintenance of the integrity of neurons (Hirst and Robinson, 1998). Thus, the study of proteins that form the coat of the vesicles could give us some clues about the conditions of the cells on the different areas of the brain. These clathrin-coated vesicles (CCVs) are involved in endocytosis and transport from the trans-Golgi network (TGN) to endosomes (reviewed by Kirchhausen, 1999). Although clathrin is a major component of the coat of the vesicles, from the functional standpoint adaptins (APs) play a key role in the formation of the coat, as they confer selectivity. Four families of APs (AP 1–4) have been described so far, of which AP-2 is specific to the CCV-mediated endocytosis and plays an important role in membrane homeostasis of nerve terminals (Granseth et al., 2007; Granseth and Lagnado, 2008). AP-2 is a heterotetramer composed of two major subunits (α, β2), a medium subunit (μ2) and a small subunit (δ2). Interestingly, in nervous tissue two isotypes of α subunit have been described, α1 and α2, but the co-existence of these isotypes is not yet well understood. Other proteins functionally related to CCVs have been identified, such as the family of the Golgi-localised, gamma-ear containing, ARF-binding proteins (GGA1–3) (Robinson and Bonifacino, 2001; Hirst et al., 2007; Zhang et al., 2007). These proteins, along with a myriad of accessory proteins (Owen et al., 1999; Collins et al., 2002) form a complex machinery, leading to the formation of the CCVs. In addition, some proteins and lipids in membranes are the partners for the recruitment of coat proteins.

It has been shown that the dynamics of formation of CCVs changes during the growth of neurons in primary cultures, to the point that the cycle of assembly–disassembly of the coat is faster in young neurons (Blanpied et al., 2002). Furthermore, it seems that changes in the dynamics of uptake of molecules by endocytosis plays a considerable role in neuronal aging (Benzi and Moretti, 1995; Samson and Nelson, 2000), as well as in age-related diseases such as Parkinson's and Alzheimer's disease (Herbert et al., 1994;

* Corresponding author at: Instituto de Histología y Embriología, FCM, Universidad Nacional de Cuyo, Mendoza-Argentina. Tel.: +54 261 413500x2677; fax: +54 261 4494117.
E-mail address: msosa@fcm.uncu.edu.ar (M.A. Sosa).
[1] Equal contribution.

0736-5748/$36.00 © 2012 ISDN. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijdevneu.2012.01.005

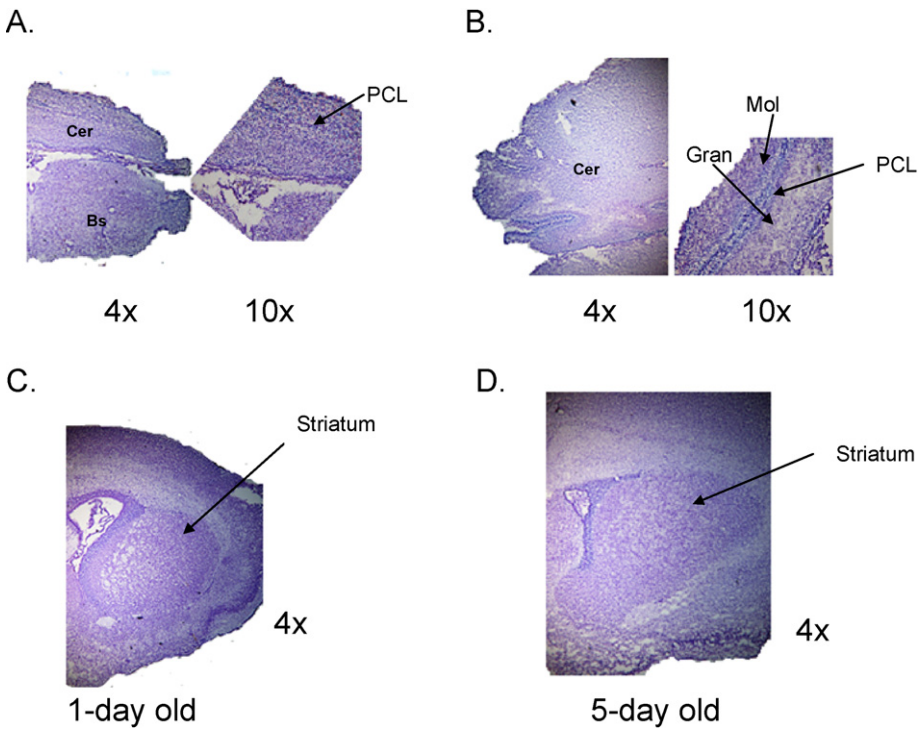

Fig. 1. Identification of brain areas from 1 and 5-day-old rat pups. Sections of 20 μm were cut with a cryostat and stained with cresyl violet. (A) The newborn animal shows an incipient cerebellum where Purkinje cells can be identified. (B) The 5-day-old cerebellum displays lobes and molecular and granular layers. (C) and (D) Striatum is a well defined brain area in both 1 and 5-day-old animals; Cer: cerebellum; PCL: Purkinje cell layer; Mol: molecular layer of the cerebellum; Gran: granular layer; Bs: brainstem.

Jellinger, 1999; Gaidarov and Keen, 1999; Foley and Riederer, 2000; Double et al., 2000; Halliwell, 2001).

In recent years, studies on endocytosis in nerve terminals became a priority, as it could have implications for neuromodulation and neuronal growth (Nagappan and Lu, 2005; Yamashita et al., 2010; Hines et al., 2010; Assaife-Lopes et al., 2010). The importance of the endocytic machinery in the maintenance of neuronal integrity is also supported by evidence showing changes in the expression of AP-2 and loss of its affinity for membranes in certain brain areas when subjected to the effect of excitotoxic drugs (Borgonovo et al., 2009).

This study attempted to determine whether the intracellular transport machinery accompanies neuronal development in different areas during postnatal development. To address this, by applying the immunoblot and immunohistochemistry methodologies, we studied the expression of proteins involved in the intracellular transport, in the cerebral cortex, *Corpus striatum*, hippocampus, cerebellum and brain stem from newborn rats and at different ages after birth.

2. Materials and methods

2.1. Subjects

Wistar rats used in this study were newborn (less than 1 day), 5, 15, 30, 60, 90 and 150 days after birth. The different brain areas from newborn (24 pups) and 5–15-day-old animals were pooled into three independent groups, ($n = 3$). Older animals (age 30–150 days) were processed individually (three animals for each age). The newborn were sacrificed 3 h after birth, and older pups were kept with their mothers and siblings until sacrifice. The rats aged 15–150 days were kept under controlled temperature, humidity and light–dark cycles with food and water available *ad libitum*. All experiments were carried out in accordance with the rules set in the National Institute of Health guide for the care and use of laboratory animals and the local animal-ethic instructions set by the CICUAL (Comité Institucional para el cuidado y uso de Animales de Laboratorio) de Argentina.

2.2. Materials and reagents

The monoclonal IgG against α-adaptin (AP-2) (Sigma Chemicals, St. Louis, MO, USA, A 4325) was prepared in mouse against AP-2 adaptor polypeptides from bovine brain, and it reacts with the 105 and 110 kDa α-subunits of adaptor complex AP-2 in bovine and rats. The mouse monoclonal antibody against the N-terminus (R11-29; amino acids 11–29) of μ2 subunit was kindly provided by Dr. Bonifacino (Bethesda, USA). The goat polyclonal IgG against GGA-1 was purchased from Santa Cruz (sc-23261) raised against an epitope mapping at the N-terminus of human GGA1, and it recognizes a unique band arround 67 kDa. The goat polyclonal IgG against GGA2 (Santa Cruz, sc-19326) was raised against an epitope

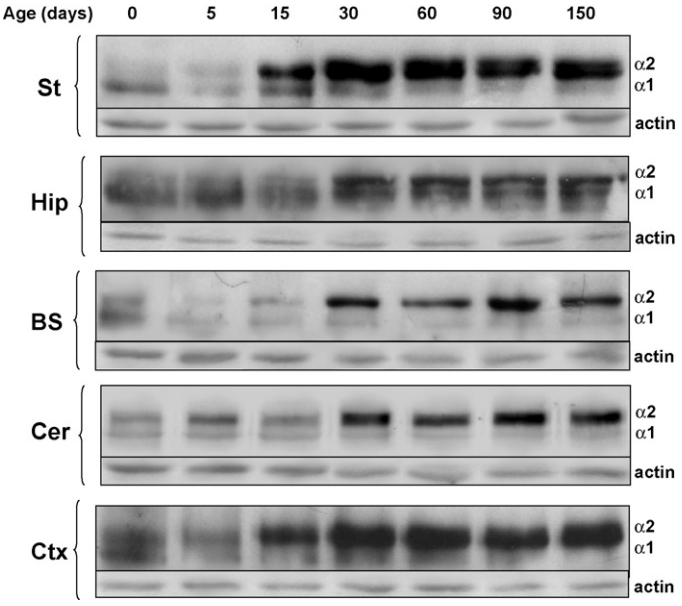

Fig. 2. Immunoblot analysis of AP-2 (α subunits) in different areas of rat brain. Proteins (45 μg) from homogenates of each brain area, at the indicated ages, were subjected to electrophoresis, transferred to nitrocellulose membranes and tested for α1 and α2 subunits of AP-2 with the specific monoclonal antibody that recognize both isoforms. The image shows a representative immunoblot from three independent experiments at each age, as detailed in Section 2. The α1 and α2 isoforms of AP-2 are indicated in the figure. St: striatum; Hip: hippocampus; BS: brainstem; Cer: cerebellum; Ctx: cerebral cortex.