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Developmental expression of SNAP-25 protein in the rat striatum and cerebral cortex

Justyna Sidor-Kaczmarek, Cezary Labuda, Barbara Litwinowicz, Jan Henryk Spodnik, Przemysław Kowiański, Jerzy Dziewiątkowski, Janusz Moryś

Department of Anatomy and Neurobiology, Medical University, Gdańsk, Poland

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The developmental changes of 25-kDa synaptosomal-associated protein (SNAP-25) expression in the rat striatum and cerebral cortex were examined using Western-blotting and densitometric scanning of immunoblots. Analysis of the striatum extracts from postnatal day 0 (P0) to postnatal day 120 (P120) demonstrated that SNAP-25 is poorly expressed until P14. From this point the expression level gradually increases to reach a maximum on P60 and then decreases. The pattern of SNAP-25 expression in the rat cerebral cortex is different. Two peaks are observed, the first on P10 and the second on P60, after which the expression level decreases. These results appear to confirm the role of SNAP-25 protein in axon outgrowth and synaptogenesis in the nervous system.

Key words: SNAP-25, protein expression, Western blotting, development, cerebral cortex, striatum, rat

INTRODUCTION

SNAP-25 is a member of the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) family of proteins, which are crucial for exocytosis and neurotransmitter release. It is a hydrophilic membrane protein of 206 amino acids localised on the cytoplasmic face of the plasma membrane and on secretory vesicles. Alternative splicing of exon 5 gives two isoforms (SNAP-25a and b) that differ in a 9-amino-acid sequence [7]. SNAP-25 forms complexes with syntaxin and synaptobrevin, two proteins essential for Ca^{2+}— regulated exocytosis. The role of the SNARE complex in vesicle docking and fusion is well characterised [4].

SNAP-25 has been identified in developing neurons, where it plays a role in neurite outgrowth and synaptogenesis, both in the central and peripheral nervous systems [6]. SNAP-25 and other SNARE proteins are also present in mature neurons of all parts of the brain [3].

It has been shown that SNAP-25 is also expressed in neuroendocrine glands (the hypophysis, the pineal gland and the adrenal glands [1]) as well as in the retina [11].

Although SNAP-25 expression during development has previously been determined in whole brain [9] or hippocampal extracts [2, 10], the previous studies have indicated that the expression pattern of SNAP-25 is tissue and age specific and can be different in various brain regions as a result of local conditions. To date the only factors that have been found that can regulate SNAP-25 expression are certain hormones, intracellular messengers and depolarisation [5].

The present study was established to examine the developmental changes of SNAP-25 protein expression in two rat brain structures, the striatum and the cerebral cortex, with the use of the chemiluminescent immunoblotting technique.

Address for correspondence: Justyna Sidor-Kaczmarek, MSc, Department of Anatomy and Neurobiology, Medical University of Gdańsk, ul. Dębinki 1, 80–211 Gdańsk, Poland, tel: +48 58 349 14 01, fax: +48 58 349 14 21, e-mail: jusid@biology.pl

MATERIAL AND METHODS

A total of 32 Wistar rats were divided into groups according to survival period (P0, P2, P7, P10, P14, P21, P28, P60 and P120; P — postnatal day). Care and treatment of the animals was in accordance with the guidelines for laboratory animals established by the National Institute of Health as well as by the local ethical committee. All animals were deeply anaesthetised with lethal doses of Nembutal and then decapitated. The brains were removed and the samples of the striatum and neocortex of the parietal region were rapidly microdissected under the control of a surgical microscope.

The tissues were manually homogenised in buffer including protease inhibitors. The total protein concentration was evaluated using colorimetric Lowry assay.

Samples containing equivalent amounts of proteins were electrophoretically separated and transferred onto nitrocellulose membrane by semi-dry electroblotting. The reliability of sample loading and protein transfer was evaluated by reversible staining of the membrane with Ponceau S solution before immunoblotting. Non specific binding sites on the membrane were blocked in non-fat dry milk. The membrane was incubated overnight with primary monoclonal antibody (mouse anti-SNAP-25, Chemicon International, USA) diluted 1:1000. After this the blots were incubated with rabbit anti-mouse IgG, horseradish peroxidase conjugate (Sigma, USA) diluted 1:40000. SuperSignal West Pico chemiluminescent substrate for peroxidase (Pierce, USA) was used for the visualisation of bands on autoradiographic film. After visualisation of SNAP-25 the antibodies were stripped from the membranes using Restore Stripping Buffer (Pierce, USA).

The whole procedure was then repeated for the reference protein β-actin but with a different set of antibodies. Antibodies that identified and visualised β-actin were mouse monoclonal anti-β-actin (Sigma, USA) diluted 1:5000 and biotinylated goat anti-mouse IgG (Jackson Immuno Research Laboratories, USA) diluted 1:10000.

RESULTS

In the striatum we observed low band colour intensity in the early postnatal period (from P0 to P14 groups). The intensity of protein staining increased from the 14th day of postnatal life, reaching a maximum in the P60 group, and then decreased slightly in the group of adult animals (P120). However, the band intensity in the P120 group was higher than in the early P0 to P14 groups (Fig. 1).

An analysis of band colour intensity carried out in the cerebral cortex revealed two peaks of relative SNAP-25 concentration. At birth the protein concentration level was low and increased until the 10th day of the postnatal life. Then in the P14, P21 and P28 groups the protein concentration level decreased. In the P60 group a second increase in band colour intensity was observed, after which, in the P120 group, the protein concentration decreased again (Fig. 2).

To summarise, in the course of this work we found considerable differences in SNAP-25 expression pattern between the rat striatum and the cerebral cortex during the postnatal period. In general, the SNAP-25 level was much higher in the cerebral cortex than in the striatum. Although protein concentration in both brain structures was initially similar and very low (the P0 and P2 groups) the main differences appeared later. These concerned groups P7–P21. In the cerebral cortex the SNAP-25 level increased rap-

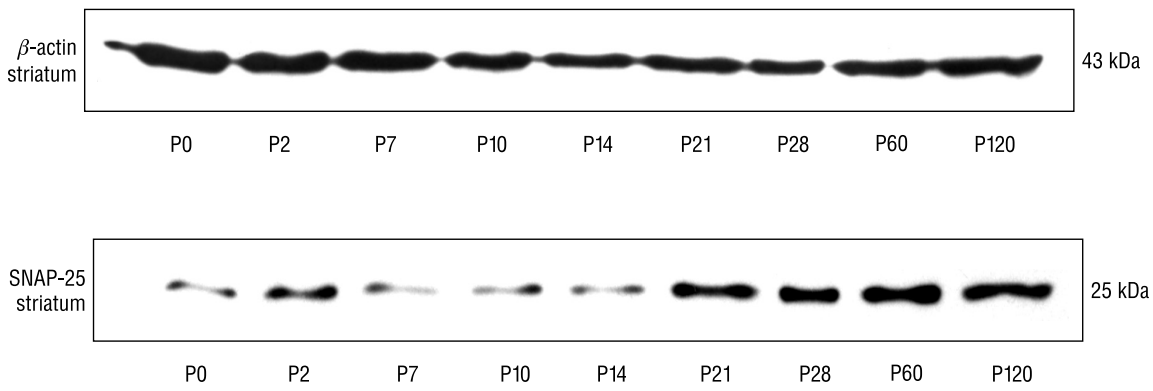

Figure 1. Age-related comparative analysis of SNAP-25 expression in the striatum. Upper panel: Western blots of β-actin carried out in 9 groups (P0–P120 days of postnatal life). Bottom panels: Western blots of SNAP-25 at different ages. The same membrane was used in β-actin and SNAP-25 detection.

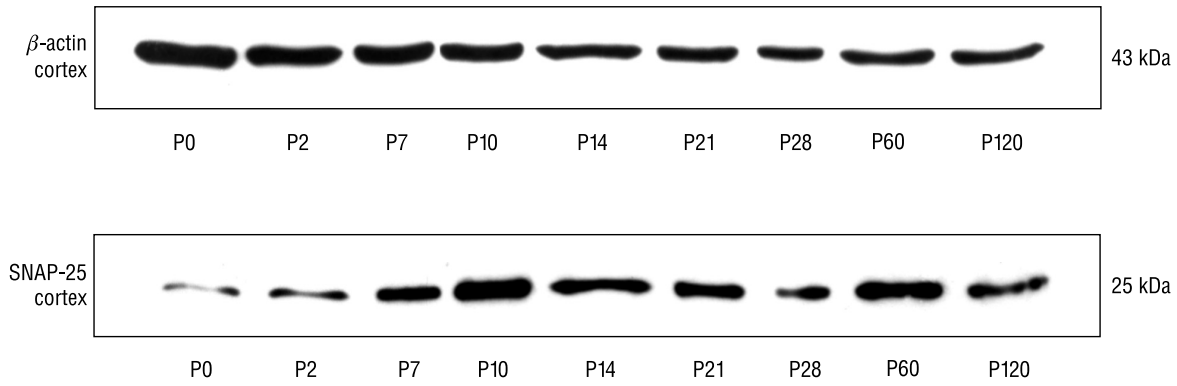

Figure 2. Age-related comparative analysis of SNAP-25 expression in the cerebral cortex. Upper panel: Western blots of β-actin carried out in 9 groups (P0–P120 days of postnatal life). Bottom panel: Western blots of SNAP-25 at different ages. The same membrane was used in β-actin and SNAP-25 detection.

idly from P7 to reach a maximum in P10, while in the striatum it remained low until the 14th postnatal day. The highest SNAP-25 levels in the cerebral cortex and striatum were reached on the 60th day of postnatal life.

The antibody used in our investigation did not detect a 27-kDa protein band.

DISCUSSION

Using Northern-blot and immunoblotting procedures, Oyler et al. [9] have demonstrated that both SNAP-25 protein and its mRNA could be detected in whole rat brains at an early embryonic (E15) stage of development. The abundance of SNAP-25 and its mRNA increased during brain development, with the largest increase occurring between birth and the 5th postnatal day. A 25-kDa peptide was the major isoform which increased from E15 to adulthood.

The antibody used in our investigation did not detect a 27-kDa protein band. According to Oyler and colleagues this isoform was present only in the early stages of development [9]. This might be a reason for the low SNAP-25 protein levels that we observed both in the striatum and cerebral cortex at the P0 stage.

Bark et al. [1] found a different pattern of SNAP-25 protein expression in the developing mouse brain. The mRNA level of two SNAP-25 isoforms (a and b) was measured in the whole brain extracts by means of RNase Protection Assay. The results showed that at early stages of development the total level of SNAP-25 mRNA was low and increased from the 1st to the 8th postnatal week. This pattern is similar to our findings regarding SNAP-25 level in the rat striatum

The changes in SNAP-25 protein concentration in the striatum revealed in our work also correspond to the results of Shimohama et al. [10] on SNAP-25 protein levels in the rat hippocampus detected by immunoblotting. SNAP-25 was apparently not expressed until stage P14 and its level was highest at P28. It then gradually decreased with maturation. Biranowska et al. [2] revealed in a immunohistochemical study a similar expression pattern of SNAP-25 in the hippocampus but the onset of its expression was shifted to P4.

It may be supposed that at the time of birth the striatum is very immature. As SNAP-25 is considered to be the marker of synaptogenesis, we can suspect that synaptogenesis in the striatum begins on P14 and lasts till P28 or P60. After this relative protein concentration decreases slightly and remains at the lower level in adult animals.

The SNAP-25 expression pattern in the cerebral cortex is more heterogenous. Two peaks were observed in our analysis, on P10 and P60, with a decrease in protein concentration between these periods. A similar observation was presented by Moryś et al. [8] in their immunohistochemical study on synaptophysin expression changes in the rat hippocampus. Synaptophysin is also a synaptic membrane protein which plays a role in synaptogenesis. Moryś et al. [8] observed two increases in synaptophysin level, the first at P7, followed by a rapid decrease at P14, with the second at P90. The first increase in SNAP-25 expression in the cerebral cortex may be explained by the overproduction of synapses during early postnatal development, while the second increase may be a result of the experience gathering of the adult animal.

CONCLUSIONS

In conclusion, the differences in changes of SNAP-25 expression levels in the rat striatum and cerebral cortex may be the result of distinct synapse maturation periods in these two brain structures, as SNAP-25 is known to be strongly connected with neurite outgrowth and transformation of the growth cone into the mature synapses.

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