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Study of Amyloid Precursor Protein Developmental Changes in Homogenate, Membrane and Soluble Fractions Derived from Rat Brain, Skeletal Muscle, Kidney and Liver

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In previous studies we have shown marked changes in the amyloid precursor protein (APP) expression during the period of synaptic contact formation, indicating an important role of APP in the synaptogenetic process. In the present study we investigated the changes in APP expression during ontogenesis at protein level in homogenate, membrane and soluble fractions from rat brain in order to obtain further data on the changes of APP processing/secretion. We also followed up the changes of the content of APP in skeletal muscle, kidney and liver.

The results show an increased expression of APP during synaptogenesis in brain. In the other organs a clear tendency of change in the content of APP is observed only in skeletal muscle. The lack of changes in postnatal development in kidney and liver confirms the hypothesis that the secretion of APP is a brain/nervous tissue-specific process.

Key words: amyloid precursor protein, ontogenetic changes, expression, peripheral organs

Introduction

Alzheimer's disease (AD) is the most common degenerative disease of the human brain and causes about 50% of dementias. It is generally accepted that APP is the centerpiece of the etiology of AD. APP is an integral membrane glycoprotein and contains the amino acid sequence of the amyloid beta peptide (Aβ). In the healthy brain APP is processed by an enzyme called alpha secretase, which cuts within the sequence of Aβ. As a result a large extracellular portion of APP is secreted into the intercellular space. There is also an alternative route of degradation via beta and gamma secretases that act at the ends of Aβ. In the diseased brain the balance between the secretases is disturbed and increased amounts of intact Aβ are formed, which is available for deposition in the senile plaques – the basic morphological hallmark of AD. Because senile plaques are

only observed in the brain, the interest in the metabolizing of APP and the role of its metabolites in the brain is enormous.

Despite the intensive research there is still no clarity about the physiological role of APP and its derivatives/metabolites. APP is believed to play the role of receptor as well as to participate in cell adhesion processes in embryonic tissue. Intact APP and its secreted forms can participate in cellular growth, cell-cell interactions, neurite outgrowth, synapse formation and maintenance, neuroprotection, homeostasis, blood coagulation and interact with receptors (e.g. p75 neurotrophin receptor [3]). APP can act in gene regulation through the cleaved C-terminal domain which translocates to the nucleus and activates gene transcription. On the other hand, Aβ has a neurotoxic effect [9] and leads to the loss of synapses [for reviews see 2, 4, 12, 13].

APP is expressed to a considerable extent in peripheral tissues and organs, however, the deposition of Aβ in senile plaques is specific to the brain alone. Very little is known about the role of APP and its derivatives in peripheral organs.

Interestingly, Maarouf et al. [11] observed that in AD patients there is an abnormal degradation of APP in the liver and hypothesize that this can contribute to the development of the disease.

In this study we followed up the changes of APP at the protein level in the homogenates, the membrane and soluble fractions prepared from brain, skeletal muscle, liver and kidney of rats during ontogenesis, starting at embryonic day (ED) 16 through to postnatal day (PD) 90. A comparative analysis of the results would help to clarify the role of APP metabolism in the brain and other organs.

Materials and Methods

The monoclonal Anti-APP A4 Antibody, clone 22C11 was purchased from Sigma Aldrich (Merck), Germany. All other reagents were commercial products of highest purity.

Pregnant Wistar rats were sacrificed on ED16. The fetuses were rapidly removed and brain, skeletal muscle and liver were dissected. For postnatal studies the brain, skeletal muscle, kidney and liver of Wistar rats at ages 1, 4, 7, 14, 20, 30, 60 and 90PD were rapidly removed.

The organs were carefully homogenized and the homogenates (H) were centrifuged at 100 000 × g for 1h at 4°C to yield the pelleted membrane fraction (P) and the soluble fraction (S).

Protein content was estimated by the method of Folin and Lowry as described earlier [6].

The proteins of the three fractions were separated by sodiumdodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) on 0.75 mm thick 12% slab gels. The protein load was 15 µg of protein per well. Western blotting and visualization of immunoreactive bands was performed as described [8]. The quantification of grey values of the immunoreactive bands was performed by densitometric scanning using a computer assisted imaging device and the 1D Image AnalysisSoftware from KODAK – EDAS 290. We have previously shown that the grey values produced by the visualization of immunoreactive bands are linearly dependent on the amount of protein loaded on the gel [7]. Electrophoretic separation of H, P and S fractions of the studied organs derived from different ages were blotted together to allow direct quantitative comparison of the age-related changes of APP levels.

The data represented on the figures are the means of two experiments, each performed in duplicate. The standard deviations did not exceed 15%.

Results and Discussion

The monoclonal antibody 22C11 employed in this study binds to an epitope in the N-terminal portion of the APP molecule, i.e. it recognizes both the intact APP molecules as well as the metabolites, obtained as a result of the activity of the proteases acting at the C-terminal. These comprise the secreted forms of APP which are found in the soluble fraction.

The changes in the levels of APP, assayed in the homogenates, membrane and soluble fractions from rat brain during the studied ontogenetic period are shown on **Fig. 1.** A clear-cut maximum in all fractions is evident during the period of vigorous synaptogenesis between PD1 and PD14, suggesting an essential role of APP in the process of targeting and establishing the synaptic contacts. This is accentuated by an increased secretion of APP metabolites during and after the onset of synaptic activity.

A comparative analysis of the distribution of protein and APP between the membrane and soluble fractions is presented on **Fig. 2.** Following the period of active synaptogenesis the amount of protein in the membrane fraction increases from some 53% to 72%, while the concentration of APP remains grossly unchanged, reflecting a decrease of APP content as a fraction of membrane protein. On the other hand the amount of protein in the soluble fraction decreases from some 47% to 38% and the amount of APP remains almost unchanged, suggesting that after synaptogenesis there is an increase of APP as percentage of the soluble fraction protein content. This is a further indication that the secretion of APP is under neurotransmitter control, since synapses have now been established and fully functional, confirming the view that it's processing is under neurotransmitter control [10].

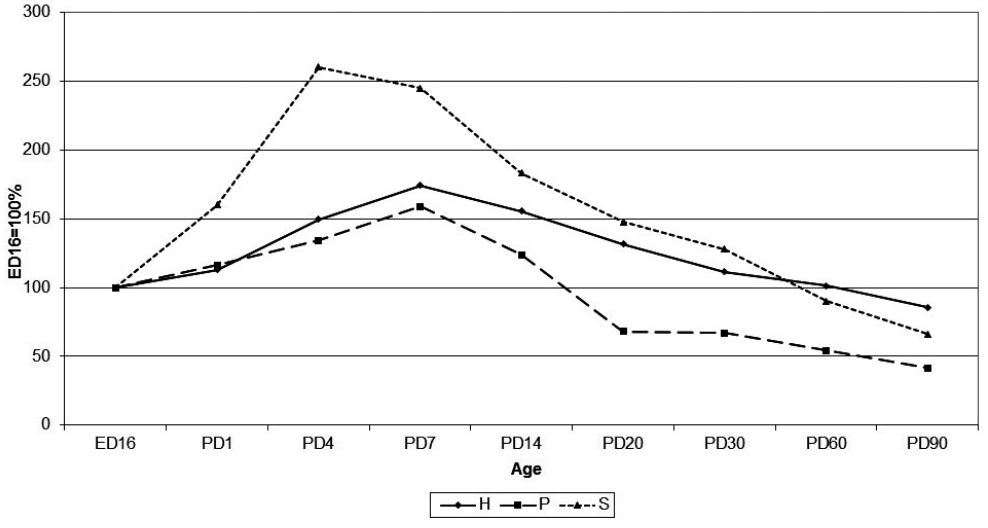

Fig.1. Age-related changes in APP level in rat brain H, P and S fractions. The data are calculated as grey values/μg protein and the value at ED16 is taken as 100%. The data are the means of two experiments, each performed in duplicate. The standard deviations did not exceed 15%.

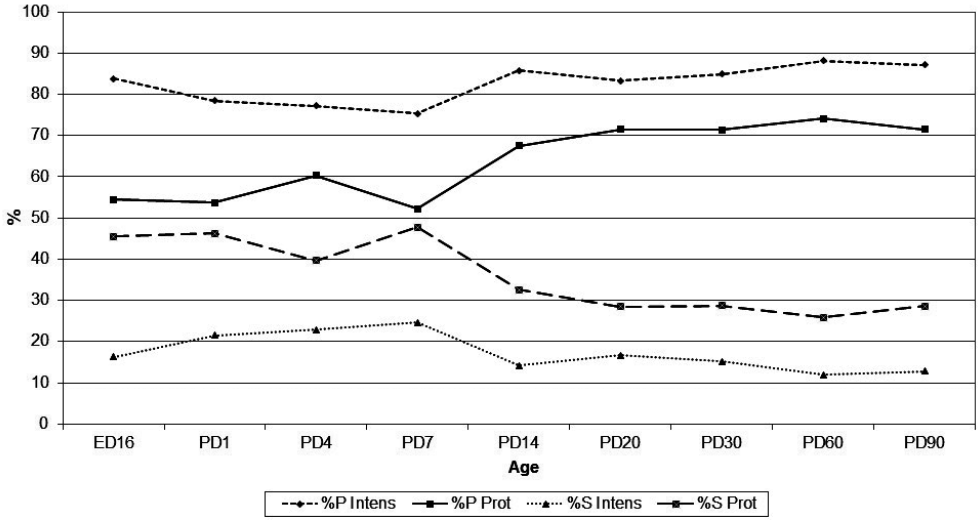

Fig. 2. Distribution (%) of protein and APP (grey values) between P and S fractions from rat brain during ontogenesis. The data are the means of two experiments, each performed in duplicate. The standard deviations did not exceed 15%.

A well-defined change of APP expression during the studied period was observed in skeletal muscle. After an initial high level of APP in embryonic muscle there is a decrease towards PD1 (**Fig. 3**). This change is most probably due to the participation of APP in the process of formation and consolidation of the neuromuscular junctions [15]. This suggestion is supported by histochemical studies, revealing the abundance of APP in the cytoplasm of myotubes at 16ED and an ensuing progressive concentration at the neuromuscular junction at birth [1, 14]. The continuous decline after PD1 to PD14 (**Fig. 3**) most probably reflects the process of elimination of synapses. At birth nearly all muscle fibers are polyneuronaly innervated. During the next two weeks the multiple innervation disappears until each muscle fiber is innervated by only one axon [5].

It has also been suggested that atypical processing of APP plays a role in the etiology of amyotrophic lateral sclerosis, a neurodegenerative muscle disease [16].

Examining the course of APP change in homogenates from kidney and liver we did not find any pronounced changes during ontogenesis (**Fig. 4**). The changes of APP in the membrane and soluble fractions follow the same course as these in homogenate. From the results shown in this figure it is also evident that APP is expressed to a much higher extent in the brain as compared to the other organs studied.

Conclusions

On the basis of our findings we can conclude that: a) The expression and procession/secretion of APP in brain during ontogenesis has unique features; b) The secretion of APP is a brain/nervous tissue-specific process; c) APP is expressed predominantly in the brain where its concentration exceeds several fold this in the other organs.

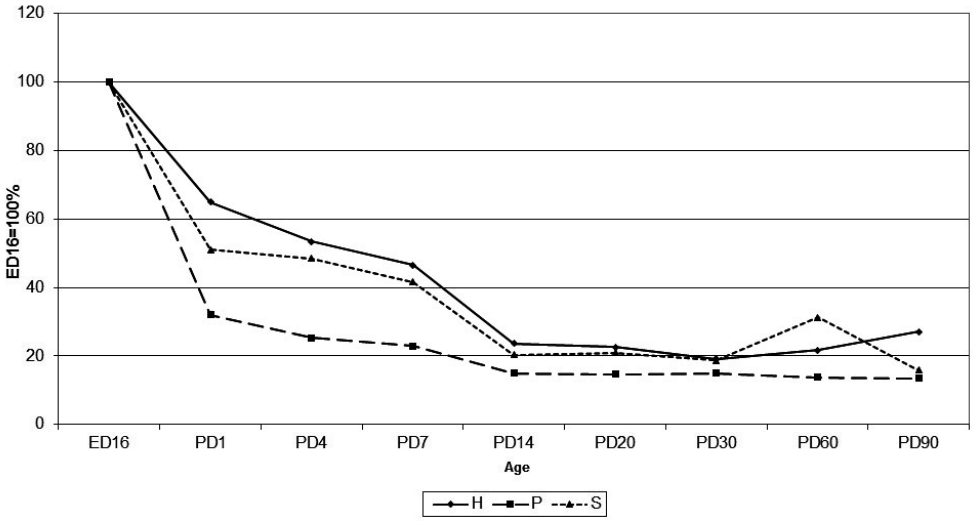

Fig. 3. Developmental changes of APP in H, P and S fractions from skeletal muscle. The data are calculated as grey values/µg protein and the value at ED16 is taken as 100%. The data are the means of two experiments, each performed in duplicate. The standard deviations did not exceed 15%.

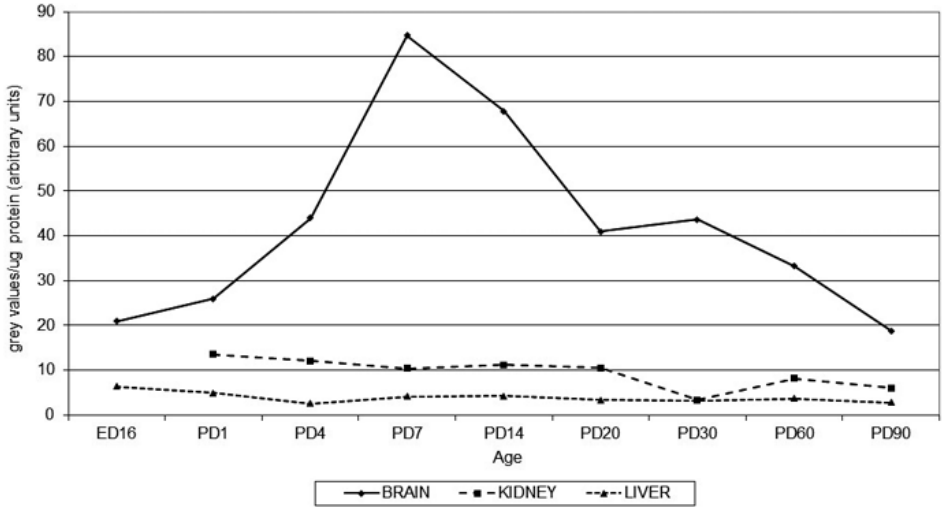

Fig. 4. Developmental changes of APP in H fractions from rat brain, kidney and liver. The data are expressed as arbitrary units, calculated on a grey values/µg protein basis.The data are the means of two experiments, each performed in duplicate. The standard deviations did not exceed 15%.

References

1. **Akaaboune, M., B. Alliquant, H. Farza, K. Roy, R. Magoul, M. Fiszman, B. W. Festoff, D. Hantai.** Developmental regulation of amyloid precursor protein at the neuromuscular junction in mouse skeletal muscle. – *Mol. Cell. Neurosci.,* **15**(**4**), 2000, 355-367.
2. **Dawkins, E., D. H. Small.** Insights into the physiological function of the β-amyloid precursor protein: beyond Azlheimer's disease. – *J. Neurochem.,* **129**(**5**), 2014, 756-769.
3. **Hasebe, N., Y. Fujita, M. Ueno, K. Yoshimura, Y. Fujino, T. Yamashita.** Soluble b-amyloid precursor protein alpha binds to p75 neurotrophin receptor to promote neurite outgrowth. – *PlOS ONE,* **8**(**12**), 2013, e82321.
4. **Kant, R. v. d., L. S. B. Goldstein.** Cellular functions of the amyloid precursor protein from development to dementia. – *Dev. Cell,* **32**, 2015, 502-515.
5. **Kasthuri, N., J. W. Lichtman.** The role of neuronal identity in synaptic competition. – *Nature,* **424**, 2003, 426-430.
6. **Kirazov, L., L. Venkov, E. Kirazov.** A comparison of the Lowry and the Bradford protein assays as applied for protein estimation of membrane-containing fractions. – *Anal. Biochem.,* **208**, 1993, 44-48.
7. **Kirazov, L., T. Loeffler, R. Schliebs, V. Bigl.** Glutamate-stimulated secretion of amyloid precursor protein from cortical rat brain slices. – *Neurochem. Int.,* **30**, 1997, 557-563.
8. **Kirazov, E., L. Kirazov, V. Bigl, R. Schliebs.** Ontogenetic changes in protein level of amyloid precursor protein (APP) in growth cones and synaptosomes from rat brain and prenatal expression pattern of APP mRNA isoforms in developing rat embryo. – *Int. J. Dev. Neurosci.,* **19**, 2001, 287-296.
9. **Kirazov, E., L. Kirazov, L. Venkov, E. Vassileva, S. Stuewe, D. G. Weiss.** Studies on the effects of the amyloidogenicA-ß-peptide on the electrical activity of neuronal networks cultured on microelectrode arrays. – *Acta morphol. anthropol.,* **7**, 2002, 9-16.
10. **Kirazov, L. P., E. P. Kirazov, C. L. Naydenov, V. I. Mitev.** Model systems and approaches to the study of the metabolism of Alzheimer's amyloid precursor protein. – *Acta morphol. anthropol.,* **21**, 2015, 55-61.
11. **Maarouf, C. L., J. E. Walker, L. I. Sue, B. N. Dugger, T. G. Beach, G. E. Serrano.** Impaired hepatic amyloid-beta degradation in Alzheimer's disease. – *PLOS ONE,* **13**(**9**), 2018, e0203659.
12. **Mueller, U. C., T. Deller, M. Korte.** Not just amyloid: physiological functions of the amyloid precursor protein family. – *Nat. Rev. Neurosci.,* **18**, 2017, 281-298.
13. **Nalivaeva, N. N., A. J. Turner.** The amyloid precursor protein: a biochemical enigma in brain development, function and disease. – *FEBS Lett.,* **587**, 2013, 2046-2054.
14. **Puig, K. L., C. K. Combs.** Expression and function of APP and its metabolites outside the central nervous system. – *Exp. Gerontol.,* **48**(**7**), 2013, 608-611.
15. **Wang, P., G. Yang, D. R. Mosier, P. Chang, T. Zaidi, Y. D. Gong, N. M. Zhao, B. Dominguez, K. F. Lee, W. B. Gan, H. Zheng.** Defective neuromuscular synapses in mice lacking amyloid precursor protein (APP) and APP-like protein 2. – *J. Neurosci.,* **25**, 2005, 1219-1225.
16. **Yang, H.** Enhanced β-secretase processing of amyloid precursor protein in the skeletal muscle of ALS animal models. – *bioRxiv*, **352401**; 2018, doi: https://doi.org/10.1101/352401.