Vol. 184, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 15, 1992 Pages 292-299

Dynamics of Gene Expression for Microtubule-Associated Protein MAP1B, Embryonic α-Tubulin and Late Neural ß-Tubulin mRNAs in the Hippocampus of Aged Rats

Aurel Popa Wagner*[1], Gunhild Reck[§], and Dieter Platt*

*Institute of Gerontology, University of Erlangen-Nürnberg, Nürnberg, and [§]Institute of Organic Chemistry, Department of Biochemistry, University of Karlsruhe, Germany

Received February 27, 1992

SUMMARY: In the present study we characterized the developmental changes in the prevalence of mRNA coding for microtubule-associated protein, MAP1B, embryonic α-tubulin and late neural ß-tubulin in rat hippocampus and forebrain from 1 to 720 days of age using RNA gel-blot analysis. We find that (i) the microtubule-associated protein, MAP1B, signal was relatively abundant at early postnatal stages when compared with mature animals. The hybridization signal in the 24-month-old rats was was ~1.7 times that observed in 6-month-old rats. (ii) Embryonic α-tubulin and late neural ß-tubulin were differentially regulated during rat brain development. This regulation is characterized by a dramatic decrease in the amount of α-tubulin after day-1 and a coincident increase in the production of late neural ß-tubulin. Both messages became stabilized at moderate levels during the subsequent developmental stages. However, the averaged signal for ß-tubulin was then ~1.8-fold increased in 24- vs. 6-month-old rats. These results are consistent with hypothesis of an age-associated increase in reactive synaptogenesis where the healthy neurons sprout new connections to compensate for neuronal loss occurring in neighboring neurons.

Stabilization of the neuroarchitecture provides a substrate for vectorial metabolism that is insensitive to macromolecular turnover events. On the other hand if a living system is going to maintain its structure and function then the newly synthesized molecules should replace the faulty ones at the correct time and in the correct places so that the previously established cellular topology will be preserved. In addition, pre-existing spatial determinants (topological organizer, TO) which will direct the

[1]To whom correspondence should be addressed. Fax: 49-911-398-2999.

Abbreviations: FN, fibronectin; FN-V, fibronectin mRNA containing the V95 segment; GFAP, glial fibrillary acidic protein; MAP, microtubule-associated protein; ECM, extracellular matrix; GAP, growth-associated protein; CNS, central nervous system; TO, topological organizer; nt, nucleotide.

0006-291X/92 $1.50

asymmetrical assembly of the newly synthesized molecules should be available [1]. Examples of TO include: fibronectin (FN), neural cell adhesion molecules (NCAM, N-cadherin), collagen, entactin, tenascin, laminin, proteoglycans. It is conceivably that gene regulation for these proteins will play a significant part in modulating the aging process. Therefore we decided to characterize the time course of changes in the prevalence of rat mRNA species coding for molecules which help to maintain the cellular structure (and hence function) over the entire rodent life-span (1-720 days). In previous studies we found both age-specific increases in the prevalence of FN mRNA isoform containing the V segment (FN-V) and very low levels of expression of mRNAs coding for FN isoforms containing the A and B alternatively spliced segments and NCAM and N-cadherin [2,3]. In the present study, using RNA gel-blot analysis, we characterized the time course, from 1 to 720 days of age, of changes in the prevalence of mRNAs coding for microtubule-associated protein, MAP1B (alternatively known as MAP5), embryonic α-tubulin and late neural ß-tubulin in rat hippocampus and forebrain.

MATERIALS AND METHODS

Pathogen-free male Fischer-344 rats were divided in 3 age groups: 6 month (n=8); 15 month (n=8), and 24 month (n=12). The weights varied between 366 and 495 g. After sacrifice by decapitation, a necropsy was performed to eliminate those with pituitary tumors, advanced kidney lesions, and other gross pathological lesions. The brains were removed, and the hippocampus microdissected, immediately placed on dry ice and kept at -80°C until homogenized. Postnatal day-1 and day-21 rat forebrain excluded the cerebellum and brain stem. The forebrain as dissected contains the hippocampus, whose development is incomplete at birth; neuronogenesis continues, depending on the rat genotype, for several or more postnatal months.

RNA extraction and electrophoresis: Total RNA was extracted and 5 μ g electrophoresed on denaturing agarose-formaldehyde gels using established procedures [4,5]. After electrophoresis the RNA was transferred to positively charged Nylon-1 membranes (BRL, Gaithersburg) and linked to the membrane by UV cross-linking (Stratagene, La Jolla, CA). The uniformity and quality of RNA transfer was evaluated by staining of the 18S and 28S rRNA with methylene blue.

Hybridization and quantitative analysis: Hybridization of all blots was carried out with 1-3x10^6 cpm/ml in 50% formamide, 1.5xSSPE, 1% SDS, 0.5% dry milk, 100 g/ml yeast total RNA, and 300 g/ml salmon sperm DNA, at 50 °C for 15 h. After hybridization the filters were washed at a final stringency of 0.2x SSC, 0.2% SDS at 75°C. Filters were exposed to Kodak XAR-5 pre-flashed x-ray film with intensifying

screens at -70°C. Integrated optical densities were collected via computerized laser densitometry (Enhanced Laser Densitometry, Uppsala, Sweden).

Preparation of probes:MAP1B Rat cDNA (clone 36a in pUC vector) was kindly provided by Dr. C.C. Garner (Zentrum Molekulare Neurobiologie, Hamburg) and subcloned into a pBluescript [pBSSKI+] vector. ß-Tubulin: A clone corresponding to the 3'-untranslated region of late neural ß-tubulin (clone RBT.1 in pSP64 vector) was a gift from Dr. S.R. Farmer (Boston University School of Medicine, MA). α-Tubulin: An embryonic cDNA clone (ECL-24, in pBSSK+) corresponding to the 3'-non-coding region (nt 1319-1460) of rat Tα1 mRNA was provided by Dr. C.E. Finch (Ethel Percy Andrus Gerontology Center, USC, CA). Fibronectin: Rat cDNA (in pGEM-2 vector) corresponding to the V95-spliced region of FN mRNAs was kindly provided by Dr. R.O. Hynes (MIT, Cambridge, MA). GFAP: Mouse cDNA (clone G1 in pUC vector) was kindly provided by Dr. N. Cowan (NYU Medical Center, New York) and subcloned into a pBluescript [pBSKSII-] vector.These plasmids allowed the synthesis of both sense and antisense probes using [^{35}S]UTP. Finally the RNA probes were purified by gel filtration (push columns, Stratagene).

RESULTS

The levels of mRNA for MAP1B, α-tubulin and ß-tubulin molecules were examined in the forebrain from 1d and 21d rats; the hippocampus of 180d, 450d and 720d old rats. Since previous findings have identified age-related increases in the prevalence of FN-V and GFAP transcripts [ref. 2, and Wagner, A.P., G. Reck and D. Platt, submitted] we have used FN-V and GFAP probes as positive controls. One band of about 11 kb was detected in rat hippocampus and forebrain using the rat MAP1B probe. The hybridization signal was relatively abundant at early postnatal stages but few transcripts were detected in adult rats (Fig.1A). However, the hybridization signal for the MAP1B mRNA then increased 1.7-fold, on the average, in 720d vs. 180d old rats (Fig. 2)($p < 0.05$, Friedman & Quade test). An almost identical pattern of expression has been previously observed for FN-V mRNA [2]. α-Tubulin mRNA was used to normalize mRNA transfer levels. One α-tubulin mRNA band hybridizing at 1.8-kb and one ß-tubulin band of 2.5-kb identical to those described for the mouse and rat brain respectively [6,7] were detected (Fig.1A). At an early developmental stage there is a mirrored expression of the two messages, with α-tubulin being heavily expressed in 1-day-old rats while ß-tubulin was expressed at rather moderate levels. The expression of ß-tubulin then increased by 21d while that of α-tubulin largely decreased in 21-day-old rats. Both signals remained then at moderate levels during the subsequent developmental stages (Fig.1A and Fig.2). However, the ß-tubulin message

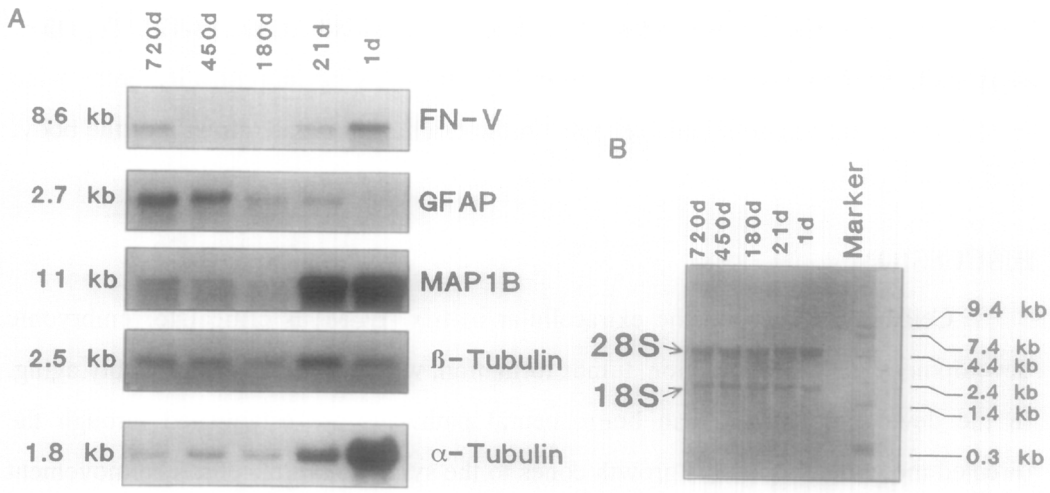

Figure 1. (**A**) Northern blot hybridization of FN-V, GFAP, MAP1B, α-tubulin and ß-tubulin mRNAs expressed during development and aging. Each lane contains 5μg total forebrain (1 and 21 day old rats) or hippocampal RNA from 180, 450 and 720 day old rats. Sizes were determined by comparison with an RNA ladder and the uniformity and quality of RNA transfer were evaluated by staining with methylene blue (**B**)

then increased ~1.8-fold, on the average, in the 24- vs. 6-month-old rats. We note the considerable variability among the 24-month-old animals that were studied: whilst some animals showed *simultaneous* increases in the MAP1B, FN-V, ß-tubulin and GFAP messages of up to 8-fold as compared to 180d old rats, still others, although showing an increase, it was to a lesser extent and the remaining animals showed no change at all. An elevated inter-animal variability upon reaching senescence seems to be the rule rather the exception and this has been reported for the steroid 17α-

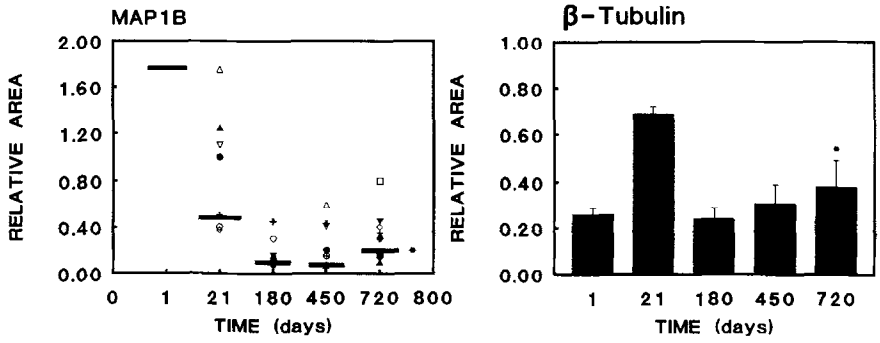

Figure 2. Developmental changes in the MAP1B and ß-tubulin mRNAs.
The optical density of a band was determined by computerized videodensitometry with background substraction. The horizontal bars represent the means and the symbols represent values from individual animals. *P<0.05 vs 6-month-old rats by Friedman & Quade test. Note that for MAP1B from the forebrain of 1-day-old rats only the mean has been represented.

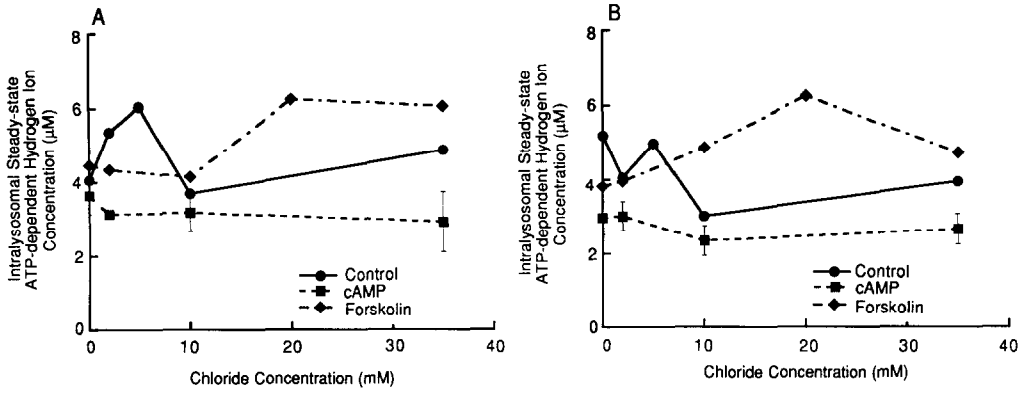

Figure 3. Steady-state ATP-dependent intralysosomal hydrogen ion concentration in lysosomes isolated from PLJ-CFPAC cells (A) or CFTR-CFPAC cells (B) under control conditions (circles) or after pre-treatment of cells with 0.5mM dibutyryl cAMP (squares) or 10μM forskolin (diamonds). Symbols represent mean (± SEM for n ≥ 3) of duplicate assays on 1-3 different preparations of lysosomes.

cAMP or 10 μM forskolin. Similarly, steady-state ATP-dependent intralysosomal hydrogen ion concentrations ranged from 2.74 to 6.25 x 10^{-6}M and were not significantly affected by medium chloride, presence of normal CFTR or pretreatment with 0.5 mM dibutyryl cAMP or 10 μM forskolin (Figure 3).

Since most endocytic vesicles appear to exhibit chloride, and perhaps potassium, conductances which stimulate vesicle acidification by 200-300% (9,12,13) by minimizing the vesicle interior-positive membrane potential generated by the electrogenic H^+- ATPase, further studies were performed to look at the effects of chloride and potassium on acidification of lysosomes from PLJ-CFPAC cells. When lysosomes (Fig. 4) were suspended initially in isotonic medium containing large,

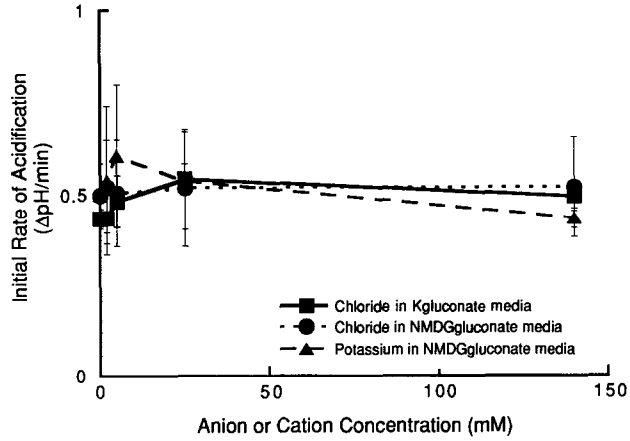

Figure 4. Effects of chloride or potassium on initital rates of ATP-dependent acidification of lysosomes prepared from PLJ-CFPAC cells. Lysosomes were prepared in Kgluconate (squares) or NMDGgluconate (circle, triangles) media and were assayed in media in which chloride replaced gluconate (squares, circles) or potassium replaced NMDG (triangles). Symbols represent the mean ± SEM of results from three separate preparations of lysosomes.

degenerating axon terminals by activated glia and axon sprouting from undamaged neurons to form new connections to replace those lost. The aged rodent brain retains the capacity, albeit to a lesser extent, for reactive synaptogenesis [22-24]. We hypothesize that MAP1B and ß-tubulin might be synthesized by neuronal processes that innervate vacant postsynaptic sites left by dying neurons while FN-V, GFAP and S100ß messages are synthesized by reactive astrocytes assisting the sprouting of these processes. This conclusion is supported by the recent findings that (i) MAP1B is persistent in those rare situations where neuronal growth occur in the adult CNS: in photosensitive cells of the retina [25] and in the olfactory system [26,27]. MAP1B expression is also induced in response to nerve growth factor in PC12 cells [28]; (ii) late neural ß-tubulin mRNA has been shown to be selectively induced during neuronal development and axonal regeneration [29]; (ii) growth-associated protein mRNA (GAP-43), which is considered a marker of *neuronal* plasticity, is still well expressed in aging hippocampus [2]; (iii) immunocytochemistry in conjunction with electron microscopy studies, indicated that FN was the first among other ECM components, to accumulate during the repair of a fronto-parietal cryogenic injury in the adult rat cerebral cortex [31]; (iv) the level of GFAP mRNA and protein has been found to increase in aging mouse and rat brain [32,33]; (v) although the exact *in vivo* function of S100ß is not known, increasing evidence suggest that S100ß may influence development of the CNS by stimulating the differentiation of neurons and the proliferation of glial cells. In addition, S100ß immunoreactivity levels are elevated in reactive glial cells of Down syndrome and Alzheimer disease [34], suggesting that S100ß may be involved in pathophysiological disorders. The finding that reactive astrocyte secrete neurotrophic factors at the site of injury [for a review, see ref. 35] is consistent with this hypothesis. However, since no increases in the NCAM, N-cadherin, GAP-43 and embryonic α-tubulin transcripts have been observed in the hippocampus of old rats, the molecular events associated with reactive synaptogenesis in the aging rat brain appear to differ to a certain extent both from those occurring during experimental-induced reactive synaptogenesis and embryonic development [29,30,36]. Since development of a biological structure is a highly spatio-temporally coordinated process, in which previously synthesized molecules serve as nucleation points for the newly synthesized molecules the accuracy of reconnection would seem impractical.

REFERENCES

1. Lazarides, E. (1987) *Cell* **51,** 345-356.
2. Wagner, A.P., G.Reck, and Beck, K.D. (1991) *NeuroReport* **2**, 691-694.
3. Wagner, A.P., Beck, K.D., and Reck, G. (1992) *Mech. Ageing Dev.*, in press.
4. Chomczynski, P. and Sachi, N. (1987) *Anal.Biochem.* **162,** 156-159.
5. Sambrook, J., Fritsch, E.F. and Maniatis, T. *Molecular cloning, a Laboratory Manual*, Vol.3, 2nd ed., Cold Spring Harbor, NY., 1989.
6. Bond, J.F., Robinson, G.S., and Farmer, S.R. (1984) *Molec. Cell. Biol.* **4**, 1313-1319.
7. Miller, F.D., Naus, C.C.G., Durand, M., Bloom, F.E., and Milner, R.J. (1987) *J. Cell Biol.* **105**, 3065-3073.
8. Cheng, C.Y., Ryan, R.F., Vo, T.P., and Hornsby, P.J. (1989) *Exp.Cell Res.* **180**, 49-62.
9. van Leeuwen, F., van der Beek, E., Seger, M., Burbach, P., and Ivell, R. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6417-6420.
10. Slagboom, P.E., de Leeuw, W.J.F., and Vijg, J. (1990) *Mech. Ageing. Dev.* **53**, 243-257.
11. Fujita, T. and Maruyama, N. (1991) *Biochem. Biophys. Res. Comm.* **178**, 1485-1491.
12. Hornsby, P.J., Hancock, J.P., Vo, T.P., Nason, L.M., Ryan, R.F., and McAllister, J.M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1580-1584.
13. Matus, A. (1988) *Ann. Rev. Neurosci.* **11**, 29-44.
14. Houliston, E. and Maro, B. (1989) *J. Cell Biol.* **108**, 543-551.
15. Lim, S.-S., Sammak, P.J., and Borisy, G.G. (1989) *J. Cell Biol.* **109**, 253-263.
16. Ginzburg, I., Teichman, A., Dodemont, H.J., Behar, L., and Littauer, U.Z. (1985) *EMBO J.* **4**, 3667-3673.
17. Wagner, A.P. (1989) *J.theor.Biol.* **138**, 132-139, 1989.
18. Wagner, A.P. (1990) *Gerontology* **36**, 132-139, 1990.
19. Cotman, C.W., and Scheff, S.W. (1979) *Mech. Ageing Dev.* **9**, 103-177.
20. Scheff, S.W., Bernardo, L.S., and Cotman, C. (1980) *Brain Res.* **199**, 21-38.
21. Coleman, P.D., Rogers K.E., and Flood, D.G. (1990) In: *Progress in Brain Research*, Coleman, G. Higgins and C.Phelps (Eds.) Vol.86, pp.75-87 Elsevier Science Publ. B.V., Amsterdam.
22. Scheff, S.W., Bernardo, L.S., and Cotman, C. (1978) *Science* **202**, 775-778.
23. Hoff S.F., Scheff S.W., and Cotman C.W. (1982) *J. Comp. Neurol.* **205**, 253-259.
24. Price, D.L., Koo, E.H., Sisodia, S.S. et al. (1990) In: *Progress in Brain Research*, Coleman, G. Higgins and C.Phelps (Eds.) Vol.86, pp.297-308, Elsevier Science Publ. B.V., Amsterdam.

25. Tucker, R.P. and Matus, A. (1988) *Dev. Biol.* **130**, 423-434.

26. Schoenfeld, T.A., McKerracher, L., Obar, R., and Valle, R.B. (1989) *J. Neurosci.* **9**, 1712-1730.

27. Viereck, C., Tucker, R.P., and Matus, A. (1989) *J. Neurosci.* **9**, 3547-3557.

28. Brugg, B. and Matus, A. (1988) *J. Cell Biol.* **107**, 643-650.

29. Hoffann, P.N. and Cleveland, D.W. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5430-5433.

30. Chuong, C.-M. and Chen, H.-M. (1991) *Am. J. Pathol.* **138**, 1-14.

31. Suzuki, M. and Choi, B.H. (1990) *Acta Neuropathol.* **80**, 355-361.

32. Goss, J.R., Finch C.E., and D.G. Morgan (1991) *Neurobiol. Aging* **12,** 165-170.

33. O'Callaghan, J.P. and Miller, D.B. (1991) *Neurobiol. Aging* **12,** 171-174.

34. Griffin, W.S.T., Stanley, L.C., Ling, C., White, L., MacLeod, V., Perrot, L.J., White, C.L., and Araoz, C. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7611-7615.

35. Vernadakis, A. (1988) *Int. Rev. Neurobiol.* **30**, 149-224.

36. Hoffamnn, P.N. (1989) *J. Neurosci.* **9**, 893-897.