DEVELOPMENTAL CHANGES IN THE BASIC PROTEINS OF NORMAL AND JIMPY MOUSE BRAIN

A.T. Campagnoni, C.W. Campagnoni, A.L. Huang and J. Sampugna

Division of Biochemistry, Department of Chemistry
University of Maryland, College Park, Maryland 20742

Received November 18, 1971

Summary

Basic proteins in brains of normal (Ta+/Y or ++/Y) and jimpy (Ta jp/Y or +jp/Y) mice were examined at several ages up to 23 days after birth. Total content of basic proteins in the brains of jimpy mutants was severely reduced during the developmental period of active myelination. Examination of the basic proteins by polyacrylamide gel electrophoresis revealed that at least one component which normally appeared during myelination was severely diminished in the jimpy mutant. The significance of these results is discussed.

Introduction

The availability of neurological mutants of inbred strains of mice has stimulated interest in their possible use in studies of central nervous system development (1-8). One of these, the "jimpy" mutant, has been shown to suffer from a severe lack of myelin. Most investigations have been concerned with the lipid composition of the mutant brain and it is now well established that the content of several lipids, particularly cerebrosides and sulfatides, is diminished (1,2,6,9,10,11).

Although some studies have been concerned with a few of the enzymes involved in lipid metabolism, no information is presently available on either myelin proteins or protein metabolism in general in these mutant mice. As part of a study to isolate and investigate myelin proteins from jimpy mice, we have examined a protein fraction obtained by acid extraction of a chloroform-methanol insoluble residue from whole brain. The present report

describes some initial findings.

Materials and Methods

Mutant mice were bred in our animal colony from stock obtained from The Jackson Laboratory, Bar Harbor, Me. Male jimpy (jp) mice used in this study were either C57BL/6J-A^{w-J}-- Ta jp/Y or C57BL/6J-A^{w-J}-- +jp/Y. In the former case the jimpy mutation is linked to the fur mutation 'Tabby', Ta, a marker gene in the same linkage group as the jp locus (12). This allows easy identification of jimpy mice prior to the onset of clinical systems (about 10-12 days) because males hemizygous for Ta are phenotypically different from littermates not bearing the mutation (13,14). Control animals were males of either C57BL/6J-A^{w-J}-- ++/Y or C57BL/6J-A^{w-J}-- Ta+/Y. Animals will henceforth be designated as Ta jp/Y, +jp/Y, Ta+/Y or ++/Y.

Basic proteins were extracted as described by Martenson and Gaitonde (15) and polyacrylamide gel electrophoresis was performed as described by Panyim and Chalkley (16). Protein bands were visualized by staining with fast green (17) and the gels were scanned at 600 nm using a gel scan attachment to a Gilford 2400 Spectrophotometer. Protein estimations were performed according to the method of Lowry et. al. (18).

Results and Discussion

Most experiments were performed with jimpy mice bearing the marker gene "Tabby" (Ta jp/Y) in order to conduct developmental studies including ages prior to the onset of clinical symptoms. Total brain protein content is plotted as a function of age in Figure 1. Within the limits of experimental error there was no observable difference in total protein per brain between the normal and mutant mice up to about 22 days.

Content of basic proteins from jimpy and normal brains at

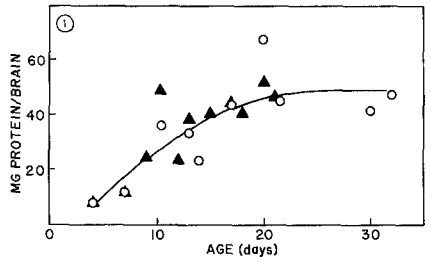


Figure 1: Total protein content of normal and jimpy brains as a function of age. Triangles: Ta jp/Y Circles: ++/Y.

various ages is shown in Figure 2. No difference was observed in the two groups of animals until after 11 days when, during the developmental period of rapid myelination, basic protein content in the mutant fell markedly and remained low until after 18 days at which time it increased but did not return to the level of the control.

In order to ascertain whether these results were due to the jimpy mutation or the Tabby mutation, basic proteins were isolated from +jp/Y and Ta+/Y animals. These data are summarized in Table 1 and it is clear that in jimpy animals of both genotypes basic proteins were severely reduced during the period of active myelination when compared to both normal (++/Y) and Tabby (Ta+/Y)

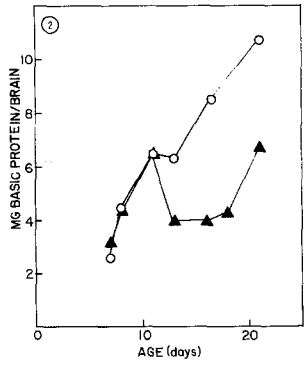


Figure 2: Basic protein content of normal and jimpy brains as a function of age. Triangles: Ta jp/Y Circles: ++/Y.

Table 1

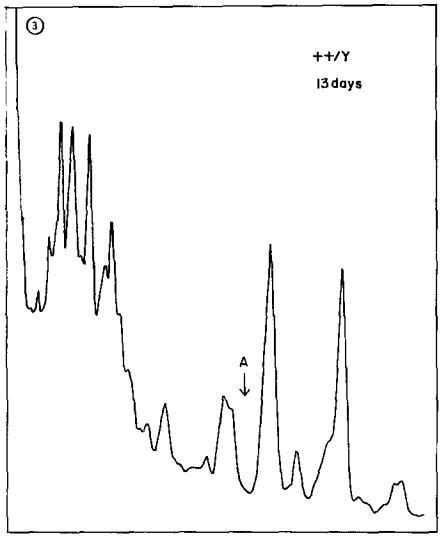
Basic Protein Content of Brains of Several Genotypes During Development

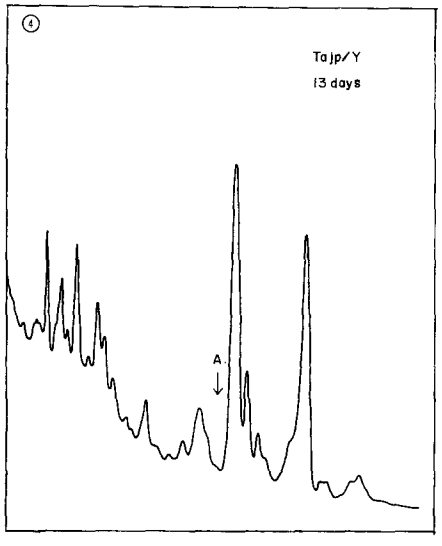
Genotype	mg/brain 8 Days	mg/brain 15-18 Days	mg/brain 20-22 Days	mg/g brain 8 Days	mg/g brain 15-18 Days	mg/g brain 20-22 Days
Ta jp/Y	(2)* 4.4	(6) 4.2	(1) 6.7	18.0	13.2	20.4
+jp/Y	(2) 4.4	(1) 4.1	(3) 8.5	15.2	10.4	23.2
++/Y	(2) 4.5	(2) 8.5	(3) 10.7	17.4	21.5	27.3
Ta+/Y	---	(3) 9.0	(2) 8.6	----	22.9	27.6

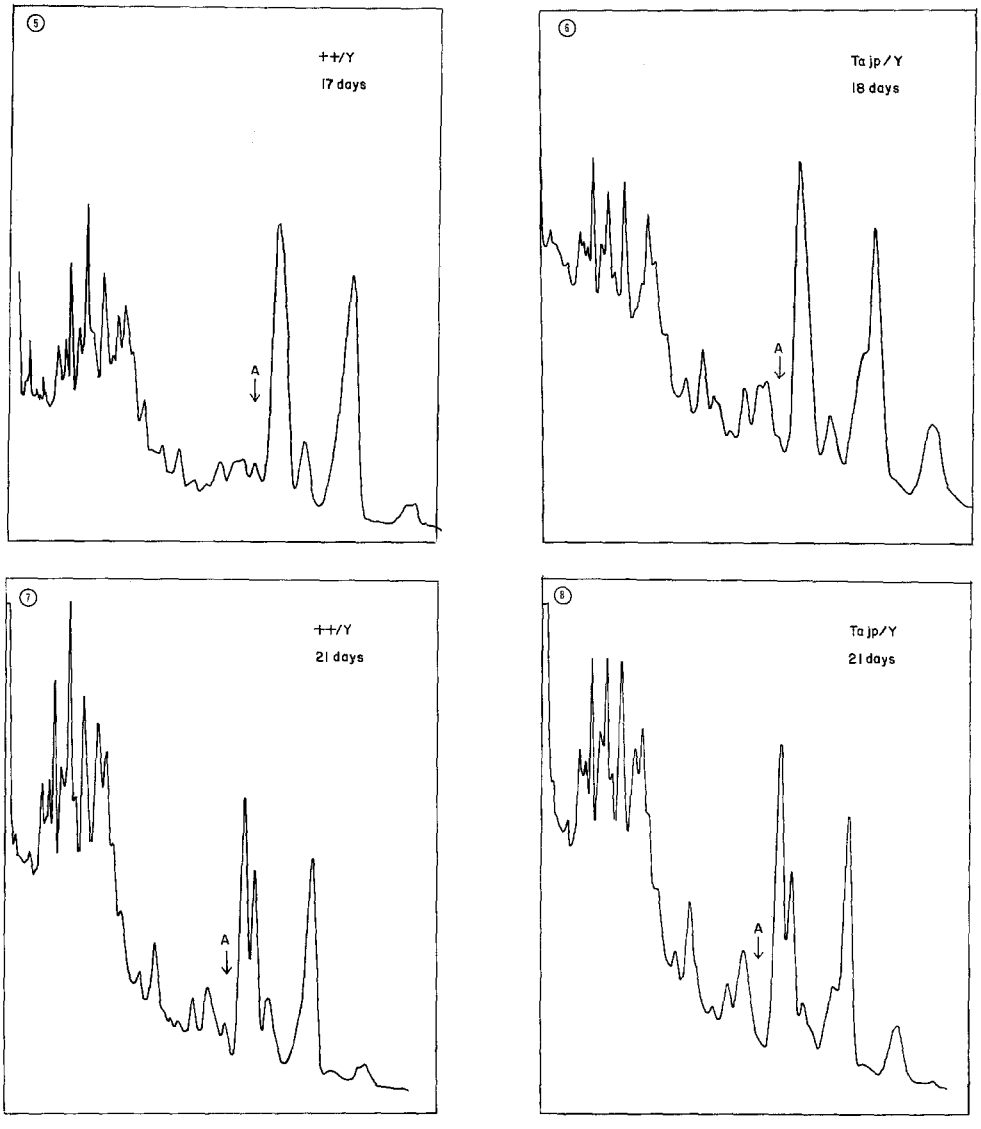
*Values in parentheses indicate number of brains analyzed.

controls. Thus, the large reduction in basic proteins during this developmental period appears to be due to the jimpy rather than the Tabby mutation.

The basic proteins isolated from the brains of mice at several ages were examined by polyacrylamide gel electrophoresis. As can be seen in Figures 3-8, approximately thirty three bands





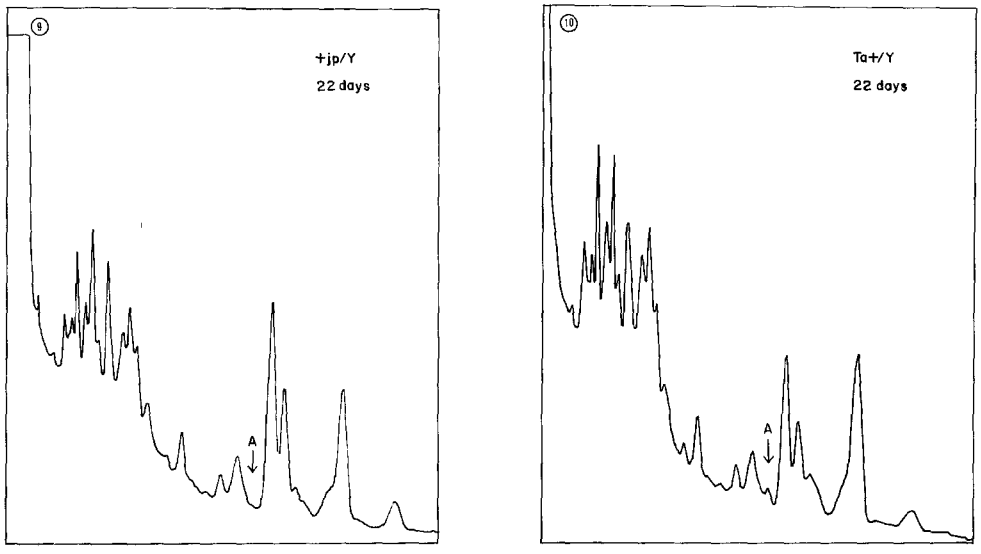


Figures 3-8: Polyacrylamide gel electropherograms of basic proteins isolated from the brains of 13, 17, and 21 day old normal (++/Y) and 13, 18, and 21 day old jimpy (Ta jp/Y) mice. 150-200 µg protein was applied to each gel. "A" denotes the band that is severely diminished in the mutant. Migration was from left to right.

were distinguished. Close inspection of the gel profiles indicated that the banding patterns at all ages were quite reproducible. The most obvious difference between normal and mutant mice was in the protein band labeled A in the figures. This protein was first evident in the normal mouse between 13 and 17 days. In the jimpy

mutant this band appeared to be either greatly diminished or absent.

Polyacrylamide gel electrophoresis of basic proteins isolated from +Ta/Y and jp+/Y at 22 days of age (Figures 9 and 10) confirmed that the observed difference was due to the jimpy mutation.



Figures 9-10: Polyacrylamide gel electropherograms of basic proteins isolated from the brains of 22 day jimpy (+jp/Y) mice and Tabby (Ta+/Y) controls.

Initial studies with jimpy mice have indicated that the metabolic defects in the mutant are all associated in some way with myelin components. The defect appears to be complex in that several enzymatic activities associated with lipid biosynthesis have been shown to be markedly reduced (3,4,8,19,20).

The present study has shown that in addition to the above defects, there is a marked reduction in the basic protein content of jimpy brain which coincides with the period of active myelination in these animals and in addition the presence of at least one protein is markedly reduced during this period. The fact that such

a large reduction in basic proteins in the mutant occured despite any evidence for differences between normal and mutant brains in total protein data (Figure 1) is probably related to the fact that the reduction in total protein could only amount to a maximum of about 10%. There was enough scatter in the total protein data to obliterate any differences of this magnitude.

The relationship between the synthesis of basic proteins and myelination is not clear. One tempting speculation is that the basic proteins may be involved in regulation of gene expression and that in these mutants a reduction of one or more crucial basic proteins might result in the failure of myelination to proceed normally. Alternatively, impaired myelin formation could be the result of reduced amounts of the basic encephalitogenic protein, a component of the myelin membrane, and one which is extracted by this procedure (15,21).

The nature of the protein representing the missing band is also unknown. Gaitonde and Martenson (21) have shown that in the rat the synthesis of the basic encephalitogenic protein is closely associated with myelination. It is possible, then, that the missing band represents this protein.

One consequence of these data is that whether or not the missing band is the myelin basic protein (or one of its subunits) it is likely that this protein is greatly reduced in the jimpy brain in so far as the total amount of basic proteins are reduced. This suggests that myelin proteins as well as myelin lipids may be involved in the jimpy defect. We are presently engaged in attempts to purify the myelin basic protein from these crude extracts in order to further clarify this point.

Acknowledgement

This work was supported by U.S. Public Health Service Research Grant NS-09944-01 from the National Institute of Neurological Diseases and Stroke.

References

1. Sidman, R.L., Dickie, M.M. and Appel, S.H., Science, 144, 309(1964).
2. Galli, C. and Galli, D., Nature 220, 165(1968).
3. Kandutsch, A.A. and Saucier, S.E., Arch. Biochem. Biophys. 135, 201(1969).
4. Kurihara, T., Nussbaum, J.L. and Mandel, P., J. Neurochem. 17, 993(1970).
5. Baumann, N.A., Harpin, M.L. and Bourre, J.M., Nature 227, 960(1970).
6. Hogan, E.L., Joseph, K.C. and Schmidt, G., J. Neurochem. 17, 75(1970).
7. Radin, N.S., Chem. Phys. Lipids 5, 178(1970).
8. Deshmukh, D.S., Inoue, T. and Pieringer, R.A., J. biol. Chem. 246, 5695(1971).
9. Galli, C., Kneebone, G.M. and Paoletti, R., Life Sci. 8, 911(1969).
10. Nussbaum, J.L., Neskovic, N. and Mandel, P., J. Neurochem. 16, 927(1969).
11. Nussbaum, J.L., Neskovic, N. and Mandel, P., J. Neurochem. 18, 1529(1971).
12. Sidman, R.L., Green, M.C. and Appel, S.H., Catalog of the Neurological Mutants of The Mouse, Cambridge, Harvard Univ. Press (1965) p. 27.
13. Wolf, M.K. and Holden, A.B., J. Neuropath. exp. Neurol. 28, 195(1969).
14. Green, E.L., Biology of the Laboratory Mouse. London, McGraw-Hill (1966) p. 118.
15. Martenson, R.E. and Gaitonde, M.K., J. Neurochem. 16, 333(1969).
16. Panyim, S. and Chalkley, R., Arch. Biochem. Biophys. 130, 337(1969).
17. Gorovsky, M.A., Carlson, K. and Rosenbaum, J.L., Anal. Biochem. 35, 359(1970).
18. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., J. biol. Chem. 193, 265(1951).
19. Neskovic, N.M., Nussbaum, J.L. and Mandel, P., F.E.B.S. Letters 3, 199(1969).
20. Neskovic, N.M., Nussbaum, J.L. and Mandel, P., Brain Res. 21, 39(1970).
21. Gaitonde,M.K. and Martenson, R.E., J. Neurochem. 17, 551(1970).