LINOLEIC- AND LINOLENIC ACID DEPENDENCY OF SOME BRAIN MEMBRANE-BOUND ENZYMES AFTER LIPID DEPRIVATION IN RATS

Bernsohn, J. and Spitz, F.J.

Neuropsychiatric Research Laboratory, VA Hospital,

Hines, Ill. and Department of Biochemistry and Biophysics,

Loyola University, School of Medicine, Maywood, Ill.

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SUMMARY

Brain monoamine oxidase and 5' -mononucleotidase activities were decreased to 57.0% and 37.6% of control values respectively in rats after a four month period of lipid deprivation. Glucose-6-phosphatase activity increased. On refeeding with either linoleic or linolenic acids, glucose-6-phosphatase values decreased to 35.3% and 58.7% of normal activity respectively, while monoamine oxidase levels returned to normal. However, only linolenic acid was effective in restoring 5' -mononucleotidase activities to normal while linoleic acid produced no change from the deficient values. The results would indicate that linolenic acid may have a biochemical function distinct from linoleic acid in 5' -mononucleotidase metabolism.

Investigations into the lipid requirements for activity of membrane-bound enzymes have been directed generally toward the nature of the lipid class involved. Information on the fatty acid specificity in lipids for enzymatic activity is meager and a few studies have indicated that phospholipids with lower melting point fatty acids tend to be more effective for enzyme reactivation (1-5). While it is recognized that linoleic acid is required for the maintenance of membrane integrity in mitochondria (6-11), erythrocytes (12) and lysosomal membranes (13) its role in the activation of membrane-bound enzymes is obscure. The role of linolenic acid appears to be unknown. While required for insect (14) and trout (15,16) development, linolenic acid does not appear necessary for rats (17), though the latter report has been criticized (18). We have studied the obligatory nature of both these fatty acids for the activities of monoamine oxidase (MAO) (EC 1.4.3.4), 5' -mononucleotidase (EC 3.1.3.5) and glucose-6-phosphatase (G-6-Pase) (EC 3.1.3.9) in brain, enzymes localized in various major

membranous structures of the central nervous system.

Pregnant female rats were placed on a fat-free diet (Nutritional Biochemicals, Cleveland, Ohio) at the 10-15 day of the gestational period and maintained on this diet throughout lactation. The offspring were weaned at 21 days of age and placed on the same diet for a subsequent period of 130-140 days. At that time, they evidenced roughness and thinning of the hair coat, scaliness or necrosis of the tail and erythema at mucocotaneous junctions, symptoms of essential fatty acid deficiency (19). About half the original litter of animals survived this regime. That the diet was otherwise adequate was demonstrated by the addition of menhaden oil to the deficient diet of some of the fat-deficient animals, whereupon all deficiency symptoms cleared up shortly, concommitant with a growth spurt.

At the end of this experimental period, about a third of the deficient rats, as well as controls at the same age who were fed a standard laboratory chow diet, were sacrificed and the brains removed and frozen at -60°C. until the following day when they were assayed. The remainder of the deficient animals were divided into 2 groups. One group was fed linoleic acid (>99% pure, Nu Chek Prep, Elysian, Minn.) by eye-dropper at a level of 0.2 ml/day. This provided about 4% of the dietary calories as the fatty acid compared to the 1% of calories said to be required for normal growth (20). At the end of 10 days, all fatty acid deficiency symptoms had disappeared. The second group was fed linolenic acid (>96% pure, Hormel Co., Minneapolis, Minn.) in a smilar manner. The deficiency symptoms appeared to be ameliorated but not to the extent that occured with linoleic acid. At the end of the 10-day period, the animals were sacrificed and the brains removed for analysis as described above. All rats were on an unrestricted water intake. Values reported are the averages obtained from 5-6 animals determined in duplicate.

Assays for MAO were performed on mitochondria, for G-6-Pase on microsomes and for 5' -mononucleotidase on brain homogenates since this last enzyme has been reported to be distributed in various subcellular membranes in the CNS

such as light and heavy myelin, synaptosomes, microsomes and nuclei (21).

The mitochondria were prepared after removal of nuclei and debris, by separation of the 14,800xg fraction from the homogenate and the purified mitochondria isolated by a combination and slight modification of the methods of Stahl et al. (22) and Eichberg et al. (23). The crude mitochondria were resuspended in 0.25M sucrose-6% Ficoll pH 7.6 with 10^{-3}M EDTA and centrifuged for 20 min. at 14,000xg. The pellet was layered on a discontinuous sucrose gradient from 0.8M-1.4M sucrose and centrifuged at 60,000xg for 2 hr. The mitochondria at the 1.2-1.4M sucrose interphase were diluted to 0.4M sucrose and centrifuged for 20 min. at 14,800xg. The purified mitochondria were recovered as the pellet. The microsomes were prepared by centrifugation of the supernatant derived from the isolation of the mitochondria at 125,000xg for 2 hr. and G-6-Pase activity determined on this fraction.

MAO was assayed by the method of Weisbach et al. (24) using kynuramine dihydrobromide as the substrate. 5' -mononucleotidase was assayed by the method of Heppel and Hilmoe (25). G-6-Pase was determined on the microsomal fraction as described by Swanson (26). The inorganic phosphate liberated by the enzymatic reactions was measured according to Bartlett (27). Protein was determined by the method of Lowry et al. (28). Values were calculated on the basis of specific activity (μM of phosphorus liberated (5' -nucleotidase G-6-Pase) or change in optical density (MAO) per mg. protein/hr.

The data are presented in Fig. 1 with the standard error of the means represented by the cross bars. There was a reduction in MAO and 5' -nucleotidase activities in the fat-deficient animals to 57.0% and 37.6% of control values respectively. These differences are statistically significant ($P<.01$). Upon supplementation of the diet of the deficient animals with either linoleic or linolenic acids, there was an increase in MAO activity to 109.6 and 123.7% of control values respectively. The significance of the increase above control values is uncertain since only one time period was observed and it is probable that different phases of a "rebound" effect are being

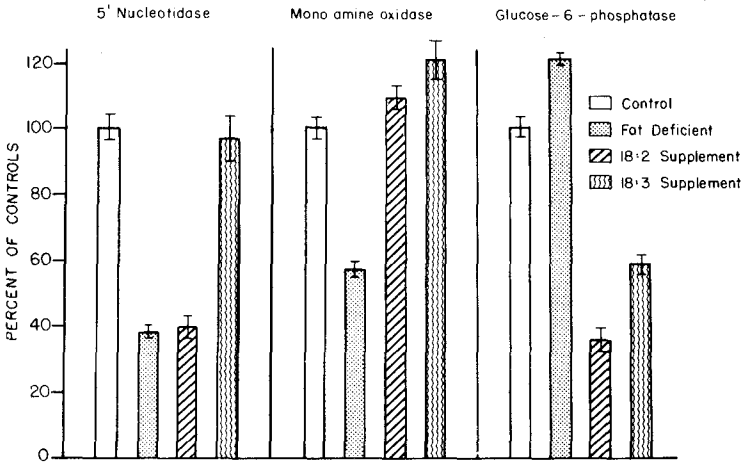

Fig. I. Effect of linoleic (18:2) and linolenic acid (18:3) supplementation on some membrane-bound enzymes in rat brain after fat deficiency. Data were calculated as specific activities of enzymes (μmoles P liberated or changes in optical density/mg protein/hr); cross bars represent S.E. mean.

measured. 5' -mononucleotidase activity was restored to control level (96.5%) only after linolenic acid supplementation. Linoleic acid was ineffective in this respect and 5' -nucleotidase activity after linoleic acid feeding remained at the level of that of the deficient animals (39.5% of control values).

In contrast to the effect on MAO and 5' -mononucleotidase, G-6-Pase activity increased to 121.7% of control levels, ($P<05$) in the deticient animals. Upon fatty acid supplementation, enzymatic activity fell to 35.3% and 58.7% of controls with linoleic and linolenic acid supplementation respectively ($P<.01$). Thus G-6-Pase appears to react differently than MAO and 5' -mononucleotidase activity on fat deprivation and refeeding.

That 5' -mononucleotidase is depressed during fat deprivation has been noted also for the enzyme in liver plasma membrane (29). It has been reported also that the enzyme from brain is not inactivated by delipidation or phospholipase treatment and apparently is independent of any lipid requirement for its activity in vitro (30,31). This does not appear to be the case

in vivo. A number of explanations for this apparent contradiction is possible. The rate of enzyme synthesis could be dependent on a member of the (n-3) fatty acid series either directly or via a prostaglandin mechanism. PGE_3 and $PGF_{3\alpha}$ are derived from 5,8,11,14,17 eicosapentaenoic acid (n-3). These prostaglandins have not been as extensively studied as those derived from 8, 11, 14 eicosatrienoic or arachidonic acids, but may play a role in modulating 5' -mononucleotidase activity either through the adenyl cyclase mechanism or in some unknown manner. An alternate possibility is that the binding of the enzyme to the membrane in brain requires the presence of some member of the (n-3) fatty acid family, probably docosahexaenoic acid, the principal (n-3) fatty acid in brain. The (n-6) fatty acids do not appear to function similarly. DePrury and Collins (32) have shown that the hydrophobic bonding between lipids and proteins in membranes may be influenced by the fatty acid composition of the phospholipids involved. This bonding involves penetration of unfolded areas of protein by the lipid (33) and in monolayers the degree at penetration is dependent on the fatty acid (34). Since fatty acids of the (n-3) and (n-6) series are not interconvertible one into the other (35), any unique function for one of tha fatty acids presents new data on the possible role of such a substance. This finding presents the first evidence for a biochemical function for linolenic acid independent from linoleic acid.

Both fatty acids tested affected MAO and G-6-Pase similarly though in opposite directions. The inhibitory effect on G-6-Pase produced would be consistent with the concept of an allosteric interaction of the enzyme with some metabolite, the availability of the latter being dependent on the presence of either linoleic or linolenic acid, or some member of the (n-6) or (n-3) fatty acid series. Again, the modulating effect of the prostaglandins may also be involved in this mechanism.

The technique employed in this study would appear to offer a promising approach in a study to determine fatty acid specificity requirements for the activity of membrane-bound enzymes.

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