

GESTATIONAL ETHANOL-INDUCED CHANGES IN PROTEIN EXPRESSION DURING THE MORPHOGENESIS OF THE RAT OPTIC NERVE

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Alcohol abuse during pregnancy results in a wide spectrum of adverse effects in the developing organism, which in its highest manifestation constitute the fetal alcohol syndrome (FAS), characterized by craniofacial dysmorphogenesis, central nervous system anomalies and growth deficits (1). The visual impairment and the frequent appearance of hypoplastic optic discs in children born to chronic alcoholic mothers (2, 3) called our attention to this important aspect of the development of the visual function. In spite of the theoretical criteria for alcohol abuse and dependence, important variations can be observed in the mother histories, ages-of-onset, outcomes, and the co-abuse of other drugs. Several years ago, our first goal was to obtain an animal model of FAS in which we were able to control some of the most critical factors, such as the ethanol administration, blood ethanol levels, gestational age and finally the nutritional status (4).

Using an experimental model of pregestational, gestational and postgestational ethanol exposure (ETOHG) in juvenile Wistar rats fed on a liquid diet, in which 36% of the daily total calories were administered by ethanol (with a constant concentration of 5% w/v), and maintaining a pair-fed group (PFG) in which the ethanol-derived calories were substituted by carbohydrates, we obtained the eyeballs and optic nerves from the offspring at key developmental stages [21 gestational day (GD), and 5, 7, 10, 12, 15 and 21 postnatal days (PD)] which were processed to morphological and morphometrical techniques, by using both light and electron transmission microscopes.

With the daily blood ethanol levels achieved in pregnant rats (90 mg/dl) and their offspring (120 mg/dl in newborns) during the course of the present work, we observed the reduced optic nerve size and multiple ultrastructural anomalies in the neuropil, macroglial cells, optic axons and myelin sheaths. Vacuolated areas, perinuclear blebs, damaged mitochondria, axonal swelling, periaxonal edema and degenerating and aberrant optic axons and myelin sheaths were found scattered all over the optic nerve cross-section in the ETOHG as compared to the PFG of the same age (fig. 1). In spite of this, the molecular basis of alcohol's action remains unclear.

These morphological findings stimulated our interest leading us to hypothesize that, as a result of alcohol injury, further changes in protein synthesis and expression might induce a failure in the molecular mechanisms of pre-patterning of the eyes and later might lead to alterations in the differentiation and proliferation of neurons and glial cells.

Therefore, the two questions are: what can be learned from the patterns of protein expression in the developing eyes? and, does gestational ethanol alter the expression of particular proteins during the development of the rat optic nerve?

To analyse these, we tested the most relevant developmental markers in the postnatal optic nerve by performing SDS polyacrilamide gel electrophoresis and immuno-

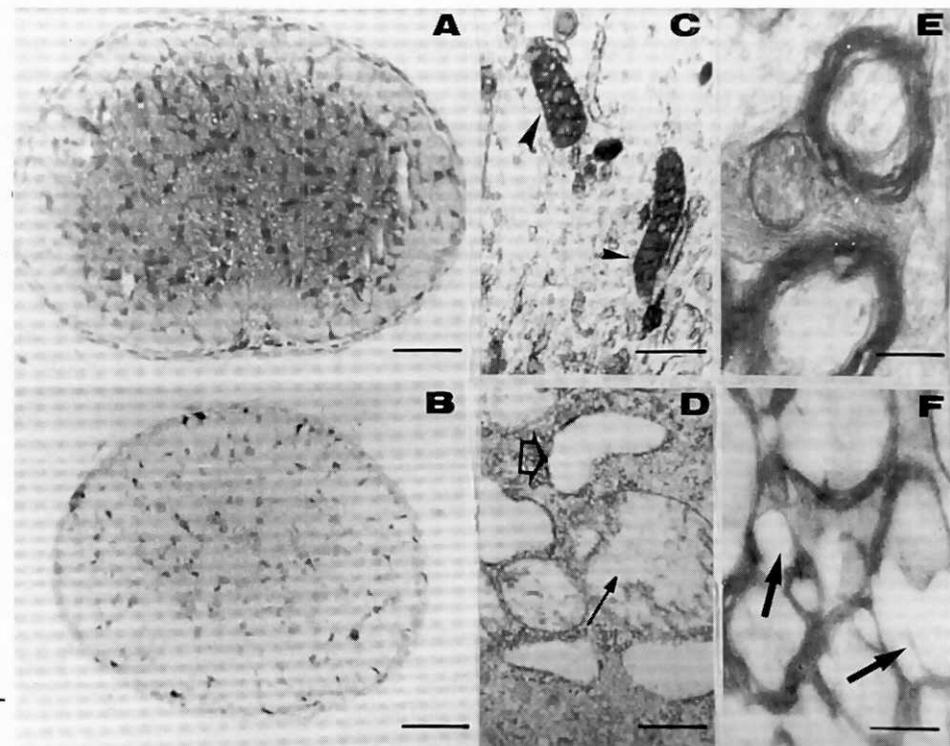


Figure 1 ... Morphological study of the rat optic nerve. PFG versus ETOHG. A,B) Micrographs of the semithin cross-section at 7 PD. Toluidine Blue. Scale bar 0.50 μ m. A smaller optic nerve is evident in the ethanol treated rats. C,D) Electron-micrographs of the ultrathin cross-section at 21 GD. Mitochondria in the astrocytic processes (arrowheads), Scale bar: 0.05 μ m. Damaged mitochondria (arrowhead) and vacuolated areas (arrow) in the ethanol-exposed group. Scale bar: 0.03 μ m. E,F) Electron-micrographs of the ultrathin cross-section at 21 PD. Comparative study of the myelinated axons. Arrows show the abundant vacuoles, and periaxonal edema. Scale Bar: 0.02 μ m.

Western Blot assays of the rat optic nerve showed the decreased pattern of VI expression at 5, 10 and 21 postnatal days. A detectable band (58 kDa), with a similar pattern to the controls was also observed in the ETOHG. No significant differences between groups were noted when the transparencies obtained from the nitrocellulose membranes were analysed and the data processed. Immunoblotting assays for the detection of GFAP in the rat optic nerve displayed a single band (54 kDa) of the above protein at 5, 10 and 21 postnatal days. Moreover, the densitometric analysis revealed increasing values throughout normal development, which were significantly smaller in the ethanol exposed rats. Finally, transfer blot procedures and antibody binding reactions showed high levels and increased expression patterns of the MBP (18 kDa) in the optic nerves from the PFG, from 5 to 21 postnatal days, where a quasi complete expression of this protein was observed. Significant delay in the onset of myelination and lower values from the densitometric analysis were observed in the ETOHG throughout development (Fig. 2).

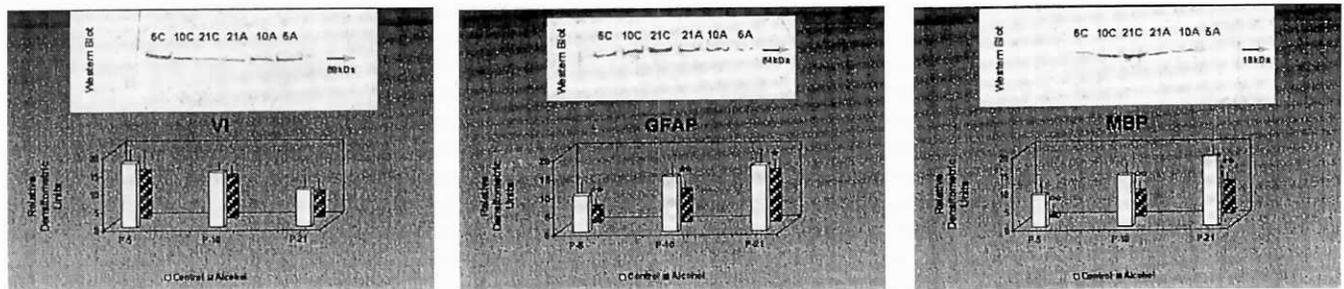


Figure 2 ... Immunoblotting assays of VI, GFAP and MBP in the rat optic nerve at key postnatal stages. The membranes were studied in an ultrascan laser densitometer and the data revealed the protein expression patterns during normal development, which were compared with those from the ethanol-exposed rats. Significant differences between groups were noted when comparing GFAP and MBP. (* $p < 0.001$)

In this point, we can summarize that VI expression decreased in the perinatal optic nerves whereas GFAP increased in parallel. MBP displayed an increased expression from 5 postnatal day to later in development. It is pertinent to consider that VI expression in the rat optic nerve did not reflect significant changes as a result of pre- and postnatal ethanol exposure. In contrast, GFAP and MBP expression underwent profound alteration in the treated rats, as a consequence of ethanol injury to the developing optic nerves.

With the rat experimental model of pre- and postnatal ethanol exposure which has been used during the present work, we described the delayed eye development and the altered neurogenesis, as shown before (5, 6, 7). A comparison of the protein expression patterns made in the course of the present work, firstly revealed important information on the disposition of proteins mediating in neuronal and neuroglial development, and secondly permitted to define the patterns of expression of all these proteins in the optic nerve from the pre- and postnatal ethanol-exposed rats.

Overall we interpret our results so that alcohol is a major teratogenic agent in the development of the eye and that alcohol injury to the developing visual system results in a delay in protein expression closely related to the altered neuronal and neuroglial development.

Because it is of particular interest to throw some light on the issue of craniofacial malformations and visual impairment in relation to alcohol abuse during pregnancy and yielding some type of social pressure, the study of the possible alterations induced by gestational alcohol on the molecular determinants of pre-patterning of the eyes, optic nerves and craniofacial structures, are now considered as a goal for our next investigations.

References

- (1) Jones, K. L., and Smith D.W. (1973). The recognition of the fetal alcohol syndrome in early infancy. *Lancet* 2: 999-1001
- (2) Strömmland, K. (1985). Ocular abnormalities in the fetal alcohol syndrome. *Acta Ophthalmol.*, suppl. 63-171.
- (3) Chan, T. Bowell R. O'Keefe M., and Lanigan B. (1991) Ocular manifestations in fetal alcohol syndrome. *Br. J. Ophthal.* 75: 524-526.
- (4) Sanchis, R. and Guerri, C. (1986). Acetaldehyde and alcohol levels in dams and fetuses at different gestation stages. *Alcohol* 2:267-270
- (5) Pinazo-Durán, M.D., Renau-Piqueras, J. and Guerri, C. (1993). Developmental changes in the optic nerve related to ethanol consumption in pregnant rats. *Teratology* 48: 305-322
- (6) Cook, C.S., Nowotny A.Z., and Sulik K.K (1987) . Fetal alcohol syndrome: Eye malformations in a mouse model. *Arch. Ophthal.* 105: 1576-1581.
- (7) Strömmland K., and Pinazo-Durán, M.D. (1994) . Optic nerve hypoplasia.. Comparative effects in children and rats exposed to alcohol during pregnancy. *Teratology* 50:100-111