Effects of chronic ethanol treatment on glial fibrillary acidic protein expression in adult rat optic nerve: an immunocytochemical study

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Abstract

Glial fibrillary acidic protein (GFAP) is used as a marker of astrocyte response to various central nervous system injuries. In the present study, the effects of chronic ethanol administration on GFAP immunoreactivity were evaluated in astrocytes of the adult optic nerve head. The results demonstrated that ethanol exposure significantly and dramatically increases GFAP immunoreactivity and the number of immunoreactive astrocytes (p < 0.001). In addition, GFAP immunoreactive cells in the optic nerve showed extensive hypertrophy (p < 0.001).

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1. Introduction

Ethanol is rapidly absorbed from the gastrointestinal tract and distributed throughout the body. Its concentrations in cerebro-spinal fluid, urine and pulmonary alveolar air bear a steady relationship to blood concentration (Slavson and Cooper, 1990). Ethanol metabolism produces acetaldehyde, which is much more toxic than ethanol itself (Vidal et al., 1998), and hydrogen peroxide is formed in the metabolizing cells. In the presence of oxygen metabolites, such as hydrogen peroxide and superoxide ion, polyunsaturated fatty acids in the membrane phospholipids undergo peroxidation, which leads to damage to membrane proteins and nucleic acids.

GFAP is an intermediate filament structural protein in astroglial cells. GFAP immunostaining is the most commonly used method for examining astrocyte proliferation and hypertrophy after various central nervous system (CNS) injuries (Satriotomo et al., 1999): transformation of normal astrocytes into so-called ‘reactive species’ by injury is associated with increased immunocytochemical staining for GFAP (Kraig et al., 1991). Astrogliosis is one of the most commonly recognized reactions to CNS damage or to neuronal degeneration in the neonatal brain (Goodlett et al., 1993).

The optic nerve (ON) consists mainly of ganglion cell axons, glial cells, blood vessels and mesodermic tissue. Among the neuroglia, astrocytes are particularly important for ON function since they perform many tasks necessary for axonal survival. Most importantly, they
maintain ionic equilibrium, regulate neuronal metabolism, contribute to the blood-optic nerve barrier and participate in the scarring and repair of the nervous system (Trivino et al., 1996). They extend processes to the nerve surface, forming a glial limiting sheath; to the blood vessels forming a perivascular sheath; and to the nodes of Ranvier (Raff, 1989). Two types of astrocytes have been identified, type-1 and type-2 (Ye and Hernandez, 1995). The astrocyte web is believed to play an important role in the mechanical and metabolic support of optic nerve axons and to protect them against various sources of damage (Elkington et al., 1990). Pre- and postnatal exposure to alcohol decreases the size of the developing optic nerve and induces many ultrastructural alterations (Pinazo-Duran et al., 1993).

The aim of the present study was to investigate the effects of chronic ethanol exposure on GFAP positive astrocytes in adult rat optic nerve.

2. Materials and methods

2.1. Chronic ethanol administration

The experiments reported here were carried out in accordance with the Declaration of Helsinki. Ethical approval was granted by Kocaeli University Ethics Committee (Kocaeli, Turkey). Adult male Wistar rats (220–260 g at the beginning of the experiments) were used. They were placed in a quiet, temperature- and humidity-controlled room (23 ± 1 °C, 60 ± 5% humidity) in which a 12 h light-dark cycle was maintained (08:00–20:00 hours light). The rats were individually housed in Plexiglas cages.

The rats 

The rats 

(mean ± SEM). Ethanol in the control groups was not measured. The body weights of the ethanol-treated rats were 3.8% less at the end of the 22-day exposure than at the beginning of the experiment. Administration as part of a totally liquid diet is obviously the best way to induce ethanol tolerance and dependence in animal models (Erden et al., 1999).

2.2. Immunocytochemistry

Each rat was deeply anesthetized with ether and transcardially perfused first with saline, then with 4% paraformaldehyde in 0.1 M phosphate buffer. The fixed optic nerves were removed and postfixed in the same fixative, first for 16–18 h at 4 °C, then overnight. The optic nerve heads (ONH) were dissected free of surrounding tissues and embedded in paraffin wax. Serial transverse sections (10 μm) were obtained, deparaffinized and rehydrated. Immunocytochemistry was performed by the avidin-biotin peroxidase method (Zymed®, San Francisco, CA, USA). The rehydrated sections were pre-treated with 3% hydrogen peroxide for 10 min to eliminate endogenous peroxidase activity, then washed in PBS-Triton X 100 (Tx). To eliminate nonspecific binding, they were pre-treated with normal goat serum before incubation with polyclonal GFAP rabbit antibody (prediluted, lot 80642168, Zymed, San Francisco, CA, USA) for 24 h at 4 °C in a humidified chamber. The sections were then washed in PBS-Tx, and biotinylated anti IgG secondary antibodies (Histostain plus kit, Zymed®) were applied for 15 min at room temperature. After a further wash in PBS-Tx, streptavidin-peroxidase conjugate (Histostain plus kit, Zyomed®) was applied for 15 min at room temperature, then a chromogenic solution of 0.6% hydrogen peroxide and 0.02% diaminobenzidine (DAB) was added for 5 min. Controls included omitting the primary antibody and replacing it with non-immune serum. Immunoreactivity was assessed by light microscopy (BX50F-3; Olympus, Tokyo, Japan).

2.3. Quantitative and statistical analysis

The number and the diameters of GFAP immunoreactive cells were quantified in 0.5 mm² fields of optic nerve tissue using a ×10 objective with an ocular micrometer system (Olympus). Counting of the immunostained cells in the optic nerve head were performed by blinded microscopic observation by three investigators. Data were presented as mean and standard deviation (SD). For statistical analysis, the means were compared by a Mann–Whitney U-test and differences at the p < 0.001 level were considered significant (Table 1).
3. Results

The daily ethanol consumption of the rats was in the range 12.3–18.2 g/kg. Blood ethanol concentrations were 293.6±5.2 mg/dl (mean ± SEM).

Numerous immunostained cell processes were seen throughout the transverse sections of the optic nerves. GFAP positive astrocytes in ONH sections from the control group (Fig. 1a,b) were distributed in all regions of the nerve. A similar distribution pattern was observed in the experimental (ethanol-exposed) group, but there was a general increase in GFAP immunoreactivity (IR) and a markedly stronger expression of GFAP-IR astrocytes throughout the OHN. These GFAP-IR astrocytes were characterized by hypertrophy of the cell bodies, with longer processes (Fig. 2a,b and Table 1). Also, the number of GFAP-IR astrocytes in the ONH was increased (Fig. 2a,b and Table 1). The immunocytochemical staining showed typically reactive astrocytes.

4. Discussion

Human optic nerve astrocytes present one of two basic characteristics: either thick or thin cell bodies. Thick-bodied astrocytes with prominent ovoid nuclei are even more strongly GFAP-positive than thin-bodied cells (Trivino et al., 1996). Ye and Hernandez (1995) investigated the regional heterogeneity of glial cells in the human optic nerve head. Their results suggest that at least two subpopulations of type 1 astrocytes form the glial-limiting membrane and the blood-nerve barrier, whereas type 2 (perinodal) astrocytes ensheath the nodes of Ranvier in myelinated nerves.

Our results, showing a general elevation of GFAP-IR in astrocytes after ethanol exposure, may indicate

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Table 1

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<th>Control group (n=10)</th>
<th>Experimental group (n=10)</th>
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<tbody>
<tr>
<td>Number of astrocytes</td>
<td>14.32±4.8</td>
<td>65.7±13.13*</td>
</tr>
<tr>
<td>Diameter of astrocytes (μ)</td>
<td>8.36±0.22</td>
<td>17.8±0.75*</td>
</tr>
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GFAP immunoreactive cells were counted in 0.5 mm² fields of optic nerve tissues from 10 animals in each group. *Significantly different from control value, p<0.001. n: number of animals.

Fig. 1. (a) Low power magnification of transverse section of control rat optic nerve immunostained for GFAP, arrow: GFAP immunoreactive astrocytes, bar: 100 μm. (b) High power view of the central area of the control rat optic nerve immunostained for GFAP, arrow: GFAP immunoreactive astrocytes, bar: 50 μm.

Fig. 2. (a) A section from chronically ethanol-exposed adult rat optic nerve. Morphological appearance of numerous positive star-shaped astrocytes and generalized increase of GFAP immunoreactivity, arrows: GFAP immunoreactive astrocytes, bar: 100 μm. (b) A high magnification photograph showing astrocyte morphology. Note the thick, intensely labelled fibrillary process and dense GFAP immunoreactivity, typical of reactive astrocytes, arrows: GFAP immunoreactive astrocytes, bar: 50 μm.
increases in both types 1 and 2 astrocytes. Astrocytes form webs between the vascular system and axons in adult rat optic nerve (Elkington et al., 1990). Chronic ethanol treatment has previously been shown to cause changes in oligodendrocytes in neonatal and very young animals (Phillips and Krueger, 1992). Pinazo-Duran et al. (1993) showed that pre- and postnatal exposure to alcohol altered the structure of cytoplasmic organelles and disorganized the cytoskeleton in developing optic nerve astrocytes. The same authors also found decreases in free ribosome density and nuclear membrane inclusions in oligodendrocytes (Pinazo-Duran et al., 1993). In contrast, the present study showed changes in GFAP synthesized in adult rat optic nerves.

Glia cells, particularly astrocytes, have many roles that are critical for normal brain function, including regulation of ions and transmitters in the microenvironment, production of growth factors, and regulation of water, energy and nutrient support for neurons. GFAP-IR is increased in various areas in the brains of chronic ethanol-treated adult rats (Dalpatrick et al., 2000). Satriotomo and colleagues (1999) showed that short-term ethanol exposure leads to strong expression of GFAP antigen in the suprachiasmatic nucleus of the hypothalamus. GFAP-immunoreactive astrocytes were more numerous and were hypertrophied, with longer than normal processes (Satriotomo et al., 1999). Franke and colleagues (1997) examined the effects of exposure to ethanol over periods of 4, 12 and 36 weeks on the adult rat hippocampus. Over 4 weeks, total GFAP immunoreactivity was increased and, concomitant with neuronal cell loss, behavioural impairments were found. Another group reported a dose-dependent decrease in GFAP-IR in rat cerebellum after life-long ethanol consumption (Rintala et al., 2001). The long-term ethanol protocol used in our study has been used by others (Uzbay et al., 1998; Erden et al., 1999; Yumuk et al., 2001).

The results of the present study demonstrate an increased number of GFAP immunoreactive astrocytes after long-term ethanol exposure. In addition, the GFAP-positive astrocytes exhibited hypertrophy. These responses to ethanol treatment over 31 days appeared to be the consequence of adaptive processes countering neurotoxic effects. The present study examined the effects of ethanol on adult rat optic nerve astrocytes immunocytochemically. Our results suggest that alcohol directly stimulates GFAP production in these astrocytes; the optic nerve is very sensitive to ethanol.

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References


