OPTIC NEUROPATHY INDUCED BY PRENATAL DRUG OR ALCOHOL EXPOSURE

ABSTRACT

Purpose: The main aim of this work was to analyze the cellular and molecular mechanisms involved in retinal and optic nerve development, and the consequences of methamphetamine «ice» (MA) or alcohol (EtOH) abuse during pregnancy on the developing visual system.

Material and methods: Wistar rats were exposed to MA or EtOH during gestation and lactation and their offspring studied. Control isocaloric rats were maintained in parallel. The eyes and optic nerves from pups (at 7, 14 and 21 postnatal days) were processed using morphologic, morphometric and western blot approaches using antibodies against glial fibrillary acidic protein (GFAP), myelin basic protein (MBP) and neurofilament protein (NFP).

Results: Statistically significant differences were observed between the methamphetamine-exposed and the alcohol-exposed rats, as compared to the controls. The optic nerve cross-sectional area was smaller in the drug or alcohol-exposed animals. The expression of developmental protein markers

RESUMEN

Objetivo: Nos proponemos analizar los mecanismos celulares y moleculares implicados en el desarrollo de la retina y el nervio óptico, y las consecuencias de un consumo abusivo de metanfetamina (MA) o alcohol (EtOH) durante la gestación sobre el sistema visual en desarrollo.

Material y métodos: Ratas Wistar fueron expuestas a MA o EtOH durante la gestación y lactancia para obtener su descendencia. Los globos oculares y nervios ópticos de neonatos (días 7, 14, 21 postnatales) fueron procesados para técnicas morfológicas, morfométricas y Western Blot, utilizando anticuerpos frente a la proteína fibrilar ácida de la glía (GFAP) y la proteína básica de la mielina (MBP) y la proteína de los neurofilamentos (NFP).

Resultados: Observamos diferencias estadísticamente significativas entre el grupo expuesto a MA y expuesto a EtOH frente a los controles. El tamaño de la sección transversa del nervio óptico fue inferior en relación a la exposición a drogas o alcohol. La expresión de GFAP y MBP está alterada en
PONS S, et al.

INTRODUCTION

The visual system is vulnerable to the action of xenobiotics, including several toxic agents such as alcohol and psychostimulants. This involves greater vulnerability in the case of abuse of these substances during pregnancy, causing a syndrome characterized by a pathognomic triad: malformations, stunted growth and ponderal development, central and peripheral nervous system alterations and mental retard. These expressions, which are known as «gestational toxic syndrome» (1) (GTS) are common to a diversity of agents such as those described for alcohol (2) and cocaine (3) abuse.

In 1985, Strömland described the presence of optic nerve hypoplasia and increased vascular tortuosity in the eye fundus of children born of chronic alcoholic mothers (4). Animal models have reproduced ocular involvement in rats prematurely exposed to EtOH (5), pointing out that the involved animals never recovered from their lesions, even after being deprived of the toxic agent (6). Animal models allow to control many variables which cannot be controlled in the human and perform experiments with the aim of clarifying the cellular and molecular bases of the action of said toxic agents on developing organisms.

In this work we endeavor to analyze the ethiopathogenic mechanisms which participate in development abnormalities in the retina and optic nerve in two experimental models exposed to psychostimulants or alcohol in rats.

SUBJECTS, MATERIAL AND METHODS

Design of experimental models and distribution of the animals

We utilized young Wistar rats (2 months old and body weight of 200-230 g), bred in the Cellular Pathology Laboratory of the Príncipe Felipe Research Centre of Valencia and the Molecular and Cellular Biology Research Institute of Porto (Portugal), under lab conditions and earmarking them for this and similar purposes (utilizing the same animal in different studies). We obtained the authorizations of the appropriate committees, and all the experiments complied with EU criteria for animal experimentation activity (86/609).

In the MA group, the drug was administered with subcutaneous injection at a dosage of 5 mg/kg of weight per day, dissolved in 3 ml of saline solution (0.9%) divided in three daily doses of 1 ml each with 1.6 mg of metamphetamine chlorhydrate in each. The administration began on gestation day 8 and was maintained until day 22. The gestation days were determined by washing of the vaginal fluid. The control group was subcutaneously injected with saline solution. The reason for administering the latter substance subcutaneously was the difficulty of administering it through the digestive pathway or mucous absorption. The control group of the metamphetamine model is isopair-fed vis-à-vis the treated group. To explain this it must be said that the consumption of this substance causes alterations in eating habits, reducing the intake of food in the experimental animals. Therefore, the control group animals were administered the same amount of food as the experimental ones in order to avoid

(GFAP and MBP) in the retina and optic nerve displayed striking alterations related to drug or alcohol abuse during gestation and lactation.

Conclusions: Psychostimulant and alcohol exposure alters the development of the retina and optic nerve (Arch Soc Esp Oftalmol 2007; 82: 21-26).

Key words: Optic neuropathy, methamphetamine, psychostimulants, alcohol, gestational toxic syndrome.

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Table I. Description of the number and distribution of rats for each model

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mother rats</th>
<th>Offspring 7 P</th>
<th>Offspring 14 P</th>
<th>Offspring 21 P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>MA group</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>EtOH group</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

MA: Metaamphetamine; EtOH: alcohol, 7P, 14P and 21P are postnatal days.
differences between both due to variations in the energy intake which could hinder or facilitate physiological processes which take place during gestation.

In the experimental group treated with EtOH, the mother rats were administered a liquid diet containing 5% of ethanol (weight/volume) for it to constitute 35% of daily calories. This diet was given for 6 weeks to make the animals dependent on alcohol. As of this time the animals were mated with Wistar males and the gestation days were determined by washing of the vaginal flux. After the birth of the offspring, both were administered the liquid diet with alcohol until the date of sacrifice. As regards the control group for the alcohol model, it must be pointed out that it is isocaloric vis-à-vis the treated group. In this way we avoid the differences which can be appreciated between both groups being influenced by different energy intake which could hinder or enhance the physiological processes which take place during development periods.

Obtaining and processing ocular samples

After completing the experiments with the animals of both models, we proceeded to obtain samples. The animals were sacrificed after perfusing with warm saline solution 0.9% and performing pre-fixation with formol. Subsequently the ocular globe and optic nerve of the animals were extracted. According to the procedure to be followed, some samples were allocated to fixation-inclusion methods and classified up to the time of their use while other samples were frozen and stored at –85ºC.

In the first case, by microsurgery techniques the retina was detached from the optic nerve and both fixed in a 2% glutaraldehyde solution and 3% formaldehyde in cacodilate tampon, 0.1 Mole and pH 7.4, performing dehydrations in growing concentrations of ethanol for final inclusion in EPON resin and performing semi-fine and ultra-fine slices which were examined by means of the specific dyes for optical and electronic microscopes as per previous descriptions (7) in order to study the morphometry of the optic nerve.

In the second case, each sample was admixed with 5 volumes of lysis tampon to facilitate cell rupture and homogenize the material. With this process we obtained a uniform suspension of which we needed to determine the amount of protein by utilizing the protein essay of bicinchoninic acid (BCA Sigma Aldrich), so as to carry out the Western Blot technique which allowed us to determine the proteins involved in neural development. This essay was useful to determine the amount of general protein in a given solution.

After determining the protein concentration of the samples, we were able to calculate the adequate volume to be utilized for the Western Blot technique.

The proteins we studied by means of the Western Blot technique and which were involved in neural development were:

— GFAP (Gyal Fibrilaric Acidic Protein 45-50KD) which marks the gyal cells in the optic nerve and in the retina the astrocytes, oligodendrocytes and Müller cells
— NFP (Neurofilament Protein 200KD) which marks the axons in the optic nerve and the retina
— MBP (Myelin Basic Protein 33KD) which signals the myelin sheath covering the optic fibers which make up the optic nerve

RESULTS

With the groups of treated animals and controls, and applying the methodology explained above, the following results were obtained.

Prenatal exposure to EtOH model

By analyzing the GFAP protein we observed the development of astrocytes. In the optic nerve we observed in all the days of the study (p7, p14, p21) a reduction of the astroglyal in the group treated with alcohol, but it became statistically significant only in day 14, whereas in day 21 it almost equaled the corresponding control. In addition, in the analysis of said protein, we observed that the reduction of gyal activity is highly significant in days 7 and 14. In day 21 the values for both groups became equal (fig. 1).

In the case of the NFP protein in the optic nerve, we have observed a growing expression during development, but with a reduction in the EtOH group compared to the control in all days of the study. Similarly, there is a considerable reduction of said protein expression in the treated group compared to the control group in the retina (fig. 2).
The expression of the MBP protein in the optic nerve increased during postnatal development. However, in the ethanol group it remained constant and did not increase throughout the study period. In addition, it was observed that the expression pattern of myelin in the alcohol group was different to that of the control group (fig. 3).

**Model of exposure to psychostimulating drugs, MA («ice»)**

The results of the measurements of the cross-section of the optic nerve show that in the metaamphetamine group the optic nerve cross-section area is smaller than the control group.

**Fig. 1: Expression of the GFAP protein in the optic nerve in rat retina in alcohol and control groups.**

**Fig. 2: Expression of the NFP protein in rat optic nerve retina in alcohol and control groups.**
However, the difference was not statistically significant.

The volumetric density of the nervous fiber packages was significantly lower in the control group (fig. 4).

**DISCUSSION**

The comparison of results between both experimental models allowed us to study the action mechanisms of both intoxicating substances and the variations between both in their interaction with ocular tissue.

Alcohol was administered orally, in liquid diet, following the model described by Lieber (8) which is proven to cause the same chronic alcoholism symptoms in rats and which can be extrapolated to humans. Several studies have confirmed this administration path both in animals (9,10) as in humans (2-11). Even though the MA administration path is different in humans, the concentration in rat blood and fetal tissue in this experimental model can be extrapolated to that in humans, overcoming the philogenetic scale. Said data have proved that subcutaneous MA induces abnormalities comparable to behavior alterations and lesions in nervous system development in children with the Gestational Toxic Syndrome (12).

The results obtained with alcohol intoxication on the GFAP protein must give rise to a new study to determine why in the last day of the study the levels reach those of the control group, both in the retina and the optic nerve. If we focus on the NFP protein, the results are clarifying in the sense that the alcohol group experiences a reduction in the amount of optic fibers both in the retina and in the optic nerve, thus serious impairing the visual system (13). In the last protein of the study, the MBP, it was seen that the abuse of alcohol causes changes in the expression pattern of said protein during the post-natal period. Said protein is myelin, which covers the optic fibers and is necessary for the proper functioning thereof. Accordingly, the reduction of myelin could also be due to the lower number of optic fibers (14).

The results obtained with metaamphetamine intoxication clearly show the differences between the control group and the drug group, which evidence and justify the large diameter of the optic nerve in the control group (15,16).

Finally, after this study it can be concluded that alcohol induces severe delays in the development of the retina and the optic nerve, in addition to causing ultrastructural alterations. For these reasons, it can be said that alcohol is a teratogenic agent for the retina and the optic nerve of rats, thus confirming the results of Pinazo-Durán (7).

After studying the metaamphetamine intoxication, it can be concluded that it induces delays in the development of rats optic nerves and abnormalities in the myelinization thereof, thus being a damaging substance for the development of the visual system.
Accordingly, both substances must be avoided during the gestation period to avoid undesirable effects.

REFERENCES


