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Research report

Comparative effects of alcohol and thiamine deficiency on the developing central nervous system

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ABSTRACT

The present study addresses the still unresolved issue of the character of alcohol–thiamine metabolic interferences in the developing central nervous system (CNS). Investigations compare developmental neurotoxicity evoked by three patterns of maternal thiamine deficiency (pre, peri and postnatal), with two patterns of maternal chronic alcohol intake (alcohol alone and alcohol + thiamine cotreatment), on seven neurodevelopmental abilities in the offspring. The three patterns of thiamine deficiency, pair-compared with controls, highlight four sequences of development: (1) embryonic–perinatal sequence; (2) perinatal–postnatal sequence; (3) "ontogeny in ontogeny out" sequence; (4) "off and on" developing sequence. The results suggest a temporally- and regionally emergence of structures and centers underlying functional maturation during CNS ontogenesis. Furthermore, both developmental thiamine deficiencies and ethanol exposure produce two waves of neurofunctional alterations, peaking at P15 (postnatal day 15) and P25, respectively. The first peak of vulnerability is a prenatal event; it may interfere with the periods of intense cellular proliferation and migration. The second peak represents both perinatal and postnatal events; it may interfere with the periods of cellular differentiation, synaptogenesis, axonogenesis and myelinogenesis. Alcohol + thiamine cotreatment fails to reduce the first peak, but neutralizes essentially the second peak. The results suggest that alcohol interferes with thiamine during cellular differentiation and membrane developmental processes mainly. Indeed, among the three conditions of thiamine-deficient diet, only perinatal thiamine deficiency exhibits a closer relationship with developmental alcohol exposure. Together, these observations suggest that the critical period for alcohol–thiamine antagonism occurs perinatally and affects primarily cellular differentiation.

1. Introduction

Normal development of the nervous system requires the concomitant and coordinated ontogeny of proliferation, migration, differentiation, synaptogenesis, myelinogenesis and apoptosis to occur in a temporally- and regionally dependent manner [1]. Interference of neurotoxicants, i.e. alcohol and related thiamine deficiency, with one or more of these developmental processes can lead to developmental neurotoxicity [2,3]. Accordingly, neurotoxicants-induced ontogenetic manipulations of behaviors can be used to draw maturation of specific brain structures or neural circuits in rodents [4].

Alcohol is the most common human teratogen. Fetal alcohol exposure produces a wide spectrum of developmental defects, including fetal death and growth retardation, fetal brain damage and subsequent neurobehavioral dysfunction [3,5,6]. Although much information has been learned about the clinical manifestations related to alcoholic diseases [7], the search continues for a better understanding of cellular and molecular mechanisms by which alcohol exerts its deleterious effects on neurodevelopmental events, such as apoptosis, differentiation, synaptogenesis and axonogenesis. Some molecular investigations indicated that chronic gestational exposure to alcohol impairs mitochondrial function and ATP production, increases oxidative stress and DNA damages [8,9]. Conversely, chronic maternal administration of antioxidant vitamins C plus E, together with alcohol, mitigates alcohol neurobehavioral teratogenicity [10]. Indeed, alcohol-induced oxidative damage to the fetus could be attenuated by a variety of antioxidants [11], including the B1 vitamin which acts as a scavenger of metabolically generated reactive oxygen species [12,13]. Thus, an important mechanism by which alcohol exerts its fetotoxic effects is causing B1 vitamin deficiency [3]. Previous studies reported that thiamine antagonizes alcohol-induced cytotoxic effects [14–16]. However, mechanisms underlying alcohol–thiamine metabolic interferences were not elucidated [18]. For further understanding of those interferences, fetal brains manipulations were ontogenetically induced by three pat-

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terns of maternal thiamine deficiency and two patterns of maternal chronic alcohol intake. The study compares five different patterns of developmental neurotoxicity, to explore similarities and differences between alcohol and thiamine deficiency, focusing on neurobehavioral measures in their offspring.

2. Material and methods

2.1. Animals

Nulliparous female of Wistar rats (bred in our colony at Cocody-Abidjan University), weighing 180–200 g, were single-housed in plastic cages (27 cm wide × 37 cm long × 18 cm high) with the floor covered by wood shavings. Experimental dams were subjected to three patterns of thiamine deficiency and two patterns of chronic alcohol intake. Each of the five experimental groups and *ad lib* control group includes six dams. For mating, females from the same treatment were grouped three per cage in which a Wistar male was introduced at 18:00 h daily. Presence of a vaginal plug indicated day 1 of gestation. Approximately 1 week prior to the parturition, the dams were returned in individual cages and checked daily for pups. The date of parturition was designated as postnatal day 1 (P1). Litter sizes were adjusted within 24 h following birth, so that each mother nursed 11 pups (6 males + 5 females), sampled at random in four out of every six litters subjected to the same treatment. After birth, offspring were left undisturbed until 10 days of age. Treatments effects were assessed on seven neurodevelopmental abilities in the offspring ($n = 11$). Testing sessions were performed at 10, 15, 20, 25, 30 and 45 days of age. Mothers remained with the pups at all times, except during testing sessions. At the weaning, those pups subjected to the same diet condition were housed in same sex groups of 3 by cage. The colony was bred in an aerated noiseless vivarium room subjected to diurnal daylight/night cycles, humidity (75%) and ambient temperature ($25 \pm 2\,^{\circ}C$).

On postnatal day 45, the pups were killed by decapitation, to assess any impact of diet conditions on brain weights. The brains were dissected and pooled from seven pups (4 males + 3 females) sampled at random in four out of every six litters from each diet condition. The brainstem was transversely sectioned posterior to the cerebellum and the brain was carefully removed, weighed and fixed in Bouin's fluid.

2.2. Thiamine deprivation

The method used for thiamine deprivation is the simple absorption of a synthetic diet no. 211 B1, lacking of B1 vitamin, manufactured by U.A.R. (Usine d'Alimentation Rationnelle, Epinay-Sur-Orge, France). The regular synthetic diet no. 210 B1, also supplied by U.A.R., is made up with the same nutrients like the diet 211 B1, but contains B1 vitamin. Briefly, thiamine deficiency was induced during three vulnerable periods of central nervous system (CNS) development: during fetal life (prenatal thiamine deficiency); from the end of fetal life to the 10th postnatal day (perinatal thiamine deficiency); from birth to the 25th postnatal day (postnatal thiamine deficiency). At the start of experiments, six dams were randomly assigned to one of the following experimental group.

2.2.1. Induction of prenatal thiamine deficiency

A 10-day thiamine deprivation is efficient to provoke anoestrus in the female rat [17], which exhibits a short gestation period (20–21 days). Thus, to obtain maximum deficiency days before parturition, dams were fed a thiamine-deficient diet beginning 3 days before mating. Females continued thiamine-deficient diet consummation from conception to parturition. At the day of parturition, females were returned to regular synthetic diet (U.A.R. no. 210 B1), which also was given to pups after weaning until the 45th postnatal day. The average length of this thiamine starvation period was about 26 ± 2 days.

2.2.2. Induction of perinatal thiamine deficiency

Females consumed thiamine-deficient diet 7 days after copulation, in order to reach the real thiamine-deficient status around the gestation day 17. This diet was kept on during gestation and the first 10 days of lactation. On the 10th postnatal day, females were given regular diet and pups received the same regimen after weaning until the 45th postnatal day. Again, this period of thiamine starvation averaged about 27 ± 1 day.

2.2.3. Induction of postnatal thiamine deficiency

Females received thiamine-deficient diet from birth to the weaning corresponding to the 25th postnatal day. At the weaning, the pups received the regular diet until the age of 45 days. All during the experimental induction of thiamine deficiency and the subsequent dietary reversion, all the groups were allowed access *ad libitum* to their diets and water.

2.3. Ad lib control

Dams were fed a regular synthetic diet (U.A.R., no. 210 B1, France) *ad libitum* through gestation and lactation. After weaning, pups received the same treatment until 45 days of age.

Ethical rules regarding in vivo experiments were observed in accordance with the Guidelines of the U.S. Public Health Service and NIH, regarding the care and use of animals for experimentation.

2.4. Alcohol treatment

2.4.1. Treatment of the dams

Induction of chronic alcohol intake in the dams was detailed elsewhere [14,18]. Nulliparous female rats were given alcohol as a 6% (v/v) aqueous solution, during a 1 week conditioning period. Then two types of treatment with ethanol were performed. The first group (alcohol, $n = 6$ females) was chronically ethanol-treated female rats which were given alcohol as a 12% ethanol in water, as the sole drinking fluid, 60 days before mating and continuing throughout gestation and lactation. During the 60-day period of chronic alcohol treatment, the mean daily intake of ethanol solution was 16.5 ± 3.6 ml/rat, corresponding to 9.17 g of ethanol/kg body weight/day. After birth, pups were exposed to ethanol uniquely trough maternal lactation which was stopped at weaning. At the weaning, offspring were fed a regular diet until 45 days of age. The second group (alcohol + thiamine cotreatment, $n = 6$ females) was also chronically ethanol-treated rats in which ethanol administration was rigorously identical with the first group (alcohol), except that 1 week before mating, mothers were given the same alcohol concentration as a 12% aqueous solution, mixed with thiamine hydrochloride at the pharmacological dose of 0.2 g/l, as the sole drinking fluid. The treatment continued throughout gestation and lactation. The mean daily intake of thiamine during chronic alcohol treatment was 14.35 mg/kg body weight. At the weaning, offspring were returned to the regular diet until 45 days of age.

2.4.2. Blood alcohol determination

Determination of blood alcohol levels from alcohol-treated dams was described previously [14]. Venous blood was taken from the tails of four unanaesthetized dams, within each of the two alcohol groups, at gestation weeks 1 and 3. The samples were centrifuged and serum ethanol levels were enzymatically determined, using a Sigma diagnostic kit (#332 UV) in a glycine buffer containing NAD+ and alcohol dehydrogenase. The highest blood alcohol levels averaged 135 + 6.6 mg/dl.

2.5. Neuronal development tests

Neuronal development was assessed by a battery of behavioral tests described previously [19]. Seven neurodevelopmental abilities were tested in the offspring, from the 10th to the 45th postnatal day.

2.5.1. Hole-board test

The apparatus used was an automatized version of the hole-board first introduced by Boissier and Simon [20]. It was a Plexiglas board with a 36 cm × 36 cm floor and 5.2 cm thick. The board was bored with 16 equidistant holes (4 × 4 holes), each 2.6 cm in diameter. Electric photocells, directly incorporated in the inner side of each hole, provided automated measurement of the number of head-dip responses by a microcomputer.

2.5.1.1. Head-dip responses. The hole-board test provides a relatively reliable measure of stimulus-directed exploratory behavior [21]. To begin the experiment, each rat was placed singly in the center of the hole-board and the number of head-dip responses was recorded. Only one 5 min trial was performed at every age.

2.5.1.2. Anxiety-induced droppings. The new situation evoked by the experimental context of the hole-board generates anxiety in the animal [22]. The number of emitted defecations was counted during a 5 min trial of exposure at every age. After each trial, the floor of the apparatus was wiped with dilute acetic acid and dried to remove traces of the previous path.

2.5.2. String test

The testing apparatus consisted of a piece of iron wire, 0.7 mm in diameter and 37 cm long, tied tightly between two vertical bars and suspended 35 cm over the ground.

2.5.2.1. Hind paws lifting reflex. The animal was left gripped by its fore paws at the middle of the wire. The time spent by the animal to retrieve its balance by bringing its hind paws upon the wire, was measured.

2.5.2.2. Wire-grasping time. The rat is compelled to get the grip in the middle of the wire by its fore paws and the observer counts time until the fall of the animal.

2.5.2.3. Crawling and leap execution latencies. The rat was compelled to get the grip on the middle of the wire. The time spent to reach one of the two vertical bars by crawling execution, or to leap on to the ground was timed.