THIAMINE ADMINISTRATION DURING CHRONIC ALCOHOL INTAKE IN PREGNANT AND LACTATING RATS: EFFECTS ON THE OFFSPRING NEUROBEHAVIOURAL DEVELOPMENT

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Abstract — A number of mechanisms may be involved in the pathogenesis of thiamine deficiency in the alcoholic. Among these mechanisms are inadequate dietary intake of thiamine, impaired intestinal transport of the vitamin and decreased conversion of thiamine to the active coenzyme. The present study was undertaken to further investigate the mechanism by which alcohol can interfere with thiamine deficiency in the brain. Thus, the neurobehavioural development of rat pups (E) nursed by 12% ethanol/water-drinking mothers, or pups (E-T) nursed by mothers drinking 12% ethanol/water + thiamine hydrochloride mixture, was monitored from the 1st to 45th postnatal days. Appropriate pair-fed saccharose (S) and ad libitum controls (C) were assessed. Histological studies were performed at the age of 45 days on the hippocampal CA3 pyramidal neurons of the offspring from each treatment. Exposing rat pups to ethanol during pregnancy and lactation showed a significant impairment of neurobehavioural development, more cornered pyramidal cells in the hippocampal field CA3, reduced cell number and cell size. The results point out long-lasting effects of maternal alcohol exposure in the offspring. Both functional and structural studies showed that neurotoxic effects of developmental alcohol exposure were not reversed by thiamine administration. However, adverse effects of undernutrition following developmental alcohol exposure were suppressed by thiamine administration. From this work, we suggest that inadequate dietary intake of thiamine and impaired intestinal transport of the vitamin are not critical mechanisms leading to thiamine deficiency in chronic alcoholism. The most prevalent mechanism contributing to ethanol-induced thiamine deficiency in chronic alcoholics would be the alteration of thiamine metabolism, and particularly the reduction of the vitamin conversion to its metabolically active form TPP (thiamine pyrophosphate).

INTRODUCTION

Human chronic alcoholism is frequently associated with thiamine deficiency, and thiamine deficiency is considered to be the cause of peripheral polyneuritis and of the central nervous system alterations of Wernicke encephalopathy in alcoholics (Hoyumpa, 1980; Leevy, 1982). Thiamine deficiency is also considered to be a causative factor of the intrauterine growth retardation (IUGR) in children born to alcoholic mothers (Recklin et al., 1985; Butterworth, 1993).

However, the mechanisms of thiamine deficiency in chronic alcoholism have not been completely elucidated. Preliminary attempts to explain these mechanisms have focused on the impairment of intestinal transport of thiamine in chronic alcoholics (Hoyumpa et al., 1978; Hoyumpa, 1980). However, the effects of chronic alcoholism on intestinal thiamine absorption are controversial, since both a reduction (Neville et al., 1968; Thomson and Leevy, 1972) and no change (Breen et al., 1985) have been reported. In addition, ethanol administration can reduce (Abe et al., 1979) or not affect (Balaghi and Neal, 1977; Chan et al., 1980; Inokuchi et al., 1981) brain thiamine content.

To further investigate the mechanism by which alcohol can interfere with thiamine metabolism in the brain, we undertook to evaluate, on the one hand, the neurobehavioural development of the offspring born to female rats drinking chronically an alcohol solution 2 months before mating and during gestation and lactation. This model theoretically would represent three most suitable levels for thiamine deficiency induction by chronic alcohol intake: (1) the impairment of intestinal

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transport of the vitamin in the alcoholic mothers (Balaghi and Neal, 1977; Butterworth, 1993); (2) the impairment of umbilical transfer of the vitamin (Fisher, 1988); (3) the alteration of the blood–brain barrier carrier sites for the vitamin in developing rats (Greenwood et al., 1985; Gastaldi et al., 1989).

On the other hand, we assessed the neurobehavioural development of neonates born to a second group of females drinking, during the same period, the same alcohol solution mixed with a pharmacological dose of thiamine hydrochloride. The central problem was to determine how thiamine administration during maternal chronic alcohol intake can counteract the deleterious effects of ethanol on the neurobehavioural development of the offspring.

To assess structural damages which underlie these functional changes, histological sections of the hippocampus were analysed. The hippocampal formation exhibits a clear and uncomplicated cytological organization (West and Pierce, 1986) and is commonly implicated in behavioural processes and learning capacities (O'Keefe and Nadel, 1978; Riley et al., 1986). Furthermore, during placental transfer and tissue distribution, the \([l-^{14}C]_{\text{ethanol}}\) accumulates to peak levels in the hippocampus (Ho et al., 1972).

**METHODS**

**Treatment of the dams**

Nulliparous female rats of a Wistar strain, weighing 170–200 g, 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 (E) was chronically ethanol-treated 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 9.174 g of ethanol/kg body weight/day. At the weaning, offspring were maintained on the same treatment until 45 days of age. The second group (E-T) was also chronically ethanol-treated rats in which ethanol administration was rigorously identical with the first group (E), 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 14.347 mg/kg body weight. At the weaning, the same treatment was delivered to the offspring until 45 days of age.

Pair-feeding experiments (group S) were initiated to control for any alcohol-related undernutrition. Using this procedure, food and fluid intake of alcohol-treated dams and pups were measured every other day and amounts consumed were calculated. Control animals were given food and fluid equivalent to that consumed on the previous day by animals given alcohol. In the drinking fluid, saccharose replaced ethanol isocalorically. The percentage of saccharose in the drinking water was calculated taking into account the calorific values of both ethanol and saccharose [1 g (7 kcal) and 1 g (4 kcal), respectively].

The second control group (C) was non drug-treated dams and was allowed *ad libitum* access to food and water. At the weaning, pups born from saccharose-treated dams and from untreated dams were maintained respectively on maternal regimen until the age of 45 days.

Body weights of six females in each treatment group were determined on gestation weeks 1 and 3.

**Subjects**

Wistar rat pups born from dams submitted to different treatments were used. Approximately 1 week prior to parturition, the dams were housed individually in plastic cages (27 × 37 × 18 cm) with the floor covered by wood-dust. The dams were checked daily in the morning for pups. The colony was bred in an aerated noiseless vivarium room subjected to diurnal daylight/night cycles and ambient temperature (25 ± 2°C).

Litters were randomized within 24 h following birth so that every mother nursed 9–11 pups coming from three or four litters subjected to the same treatment. The date of parturition being designated as postnatal day 1 (P1). After birth, offspring were left undisturbed until 10 days of age. 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. The pups were brought from their nest.
each time and run through all appropriate tests before being returned to the nest. At weaning, rat pups were housed in same-sex groups of three by cage. Pups' weights were routinely determined at every age.

Neuronal development tests

Neuronal development was assessed as described previously (Ba and Seri, 1995). The same pups were used from the 10th to 45th postnatal day in all treatments.

Hole-board test. The apparatus used was a Plexiglas board with a 36 × 36 cm floor and 5.2 cm thick. The board was bored with 16 equidistant holes (4 × 4), each 2.6 cm in diameter. Electric photocells directly located in the inner side of each hole provided automated measures of the number of head-dip responses by a microcomputer. This apparatus allowed the measurement of exploratory activity, habituation, and emotional defecation.

- Measure of exploratory activity: Each animal was placed singly in the centre of the board and the number of head-dip responses was recorded. Only one 5 min trial was performed at every age.
- Measure of habituation: The novelty of the experimental context of the hole-board induces in the rat a response of exploration (Boissier and Simon, 1962). The decrement of this exploratory response following a prolonged exposure of the animal to the same situation was measured at the 1st, 2nd, 3rd, 4th and 5th min of exposure to new surroundings. Only one trial of 5 min was carried out at every age.
- Measure of emotional reaction: The new situation evoked by the experimental context of the hole-board generates anxiety in the animal (Boissier et al., 1964). The number of emitted defecations was counted during a 5 min trial of exposure at every age.

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. This apparatus was used to measure the wire grasping time, and the crawling execution latency along wire.
- Measure of the wire grasping time: The rat was held by the tail and the forepaws were placed in contact with the wire near its midpoint. When the rat grasped the wire with its forepaws, the investigator released the tail and began timing.
- Crawling execution latency: The rat was compelled to get a grip on the middle of the wire. The time spent to reach one of the two vertical bars by crawling execution was recorded with a stopwatch.

Tail-flick test. Baseline pain responsiveness was assessed as described previously (Ba and Seri, 1993). Briefly, the tail-flick was evoked using a feedback-controlled projector lamp (24 V, 100 W) focused on the dorsal surface of the tail, at the half-length. The projector lamp emitted a high intensity light beam, the temperature of which reached 55°C.

At the beginning of a trial, a latency timer and the radiant heat were activated simultaneously. The heat source and timer automatically stopped as soon as a flick of the tail out of the path of the emitted heat was obtained.

All behavioural testing was conducted between 09:00 and 11:00 h. For assessing baseline pain responsiveness, three tail-flick trials were conducted at 5-s intervals, and baseline was defined as the mean latency of the three trials. A 10-s latency limit to respond was employed to prevent tissue damage.

Blood ethanol determination

Blood ethanol levels were measured routinely from dams in the E and E-T groups. Ethanol freely traverses the placental barrier and the concentrations of ethanol in maternal and fetal blood are comparable (Ho et al., 1972; Guerri and Sanchis, 1985). Therefore, the blood alcohol concentration of pregnant females and not fetuses were examined in the morning (09:00 h) and in the evening (19:00 h), because of differences in circadian drinking rhythms (Aalto, 1986).

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.

Tissue preparation

At day 45 postnatally, the pups were killed by decapitation. The brains were dissected and pooled from six pups from at least three dams
within each of the experimental groups. The brainstem was transversely sectioned posterior to
the cerebellum and the brain was carefully
removed, weighed and fixed in Bouin's fluid. The
brains were then dehydrated through successive
washes of alcohol solutions of increasing con-
centration and embedded in paraffin. Serial par-
affin sections, 10 μm thick, were cut with a
microtome, mounted on glass slides, and stained
with a combination of haematoxylin–eosin
(nucleus and cytoplasm staining respectively) and
indigo carmine (nucleus staining in particular).
The sections were parasagittal, through the left
cerebral hemisphere. The slides were observed
under oil immersion (×250).

Cell counts and measurements

The slides were photomicrographed and printed
at a final magnification of ×1125. Cell counting
was done directly on the photomicrographs, in
areas measuring 40 by 31.7 mm, corresponding to
1000 μm² on the histological section. Neuronal
populations were counted from the hippocampal
field CA3c described in previous studies (Bilkey
and Schwartzkroin, 1990; Bonthius and West,
1991). The cell counting concerned solely 130 μm
of the histological portion jutting-out in the den-
tate gyrus. The results given by six rats in each of
the four treatment groups were expressed as a
mean number of cells per 1000 μm² of the field
CA3.

Because neurons in the field CA3 exhibited a
pyramidal-shaped morphology, soma height and
soma width were measured for each cell body, by
an eyepiece micrometer.

Data analysis

Two-way analysis of variance (ANOVA) was
performed on behavioural data with four factors
of treatment, and either six or four factors of age.
The same factorial analysis was used to assess
between-treatment group differences. One-way
ANOVA was used to assess habituation, food
consumption, body and brain weights, cell num-
ber and size. Post hoc testing was by Scheffé test
for specific between-group differences (Wayne,
1987).

RESULTS

Maternal weight gain and pup growth

During the 60-day pre-mating period, food con-
sumption was 62.2 ± 0.9 g/kg body weight/day in
control dams, and was significantly reduced to
50.9 ± 1.3 g/kg body weight/day in ethanol-
treated dams F(1, 62) = 88.585, P = 0.0001.

Administration of 12% alcohol solution in the
drinking water to rat dams during pregnancy
and lactation was able to achieve blood alcohol
levels ranging from 56 ± 0.3 mg/dl at 09:00 h to
123 ± 5.2 mg/dl at 19:00 h.

There was no treatment effect on maternal
weight gain (Table 1) at gestation week 1
[F(3, 15) = 0.405, P = 0.7517]. However, signif-
icient differences appeared on gestation week 3
between treatment groups [F(3, 15) = 7.147,
P = 0.0033]. Average weight gain during gestation
by ethanol-ingesting dams and by the saccharose-
treated dams were significantly lower (P = 0.05)
than that exhibited by control dams [F(1, 5) =
4.579 and F(1, 5) = 3.39, respectively]. Alcohol
and saccharose-treated dams did not differ from
one another in gaining weight during the gestation
period [F(1, 5) = 0.089]. However, thiamine
administration during maternal alcohol ingestion
significantly reversed the adverse effects of alcohol on maternal weight gain \(F(1,5) = 3.6122\).

There was no treatment effect on the mean body weight of the offspring (Table 1) at postnatal days 10 \(F(3,15) = 0.541, P = 0.6618\) and 45 \(F(3,15) = 0.388, P = 0.763\).

**Exploratory activity**

Overall analysis of exploratory activity (Fig. 1) yielded the main effect of treatment \(F(3, 210) = 14.3, P < 0.001\), a significant change over days \(F(5, 210) = 24.5, P < 0.001\) and a reliable treatment \times age interaction \(F(15, 210) = 3.1, P < 0.001\).

The appropriate control for the alcohol groups in these studies is the saccharose group. Therefore, to establish if adverse effects of malnutrition induced by maternal chronic alcohol intake influenced exploratory activity of developing offspring, data from the C and S groups were subjected to treatment \times age ANOVAs. These ANOVAs indicated that saccharose offspring did not differ from control offspring on any of the days \(F(1,108) = 0.4, P = 0.553\).

However, the development of exploratory activity was significantly lower in ethanol-exposed offspring than in saccharose-treated controls \(F(1, 96) = 23.2, P < 0.001\), indicating a specific effect of ethanol treatment, with a reliable treatment \times age interaction \(F(5, 96) = 3.1, P = 0.013\). This interaction was due to lower activity of alcohol offspring on days 25 \(F(1, 8) = 14.929, P = 0.005\) and 30 \(F(1, 8) = 5.735, P = 0.0435\). This effect of ethanol was not reversed by thiamine administration during maternal chronic alcohol intake, when E offspring were compared to E-T offspring \(F(1, 102) < 0.01, P = 0.931\).

**Habitation**

While the typical feature of habituation, showing high initial activity with rapid decline during prolonged exposure to the same situation, appeared at 20 days of age in control offspring, the ethanol-exposed pups failed to habituate until 45 days of age (Table 2). Indeed, while control pups exhibited at the age of 20 days a clear and significant decrement of exploratory activity, from the 1st to 5th min of exposure \(F(1,11) = 6.791, P < 0.01\), a similar decrement of exploration following a 5 min exposure time was not exhibited by alcohol-treated offspring until the age of 45 days \(F(1,8) = 4.663, P < 0.01\).

Likewise, the delay of the ontogenetic appearance of habituation was clearly displayed in the saccharose-treated offspring in which habituation appeared at 45 days of age, when exploratory activity decreased significantly from the 1st to 5th min of exposure \(F(1,8) = 5.274, P < 0.01\).
Table 2. Mean frequency of head-dip responses over exposure time to new surroundings in the offspring from different treatment groups

<table>
<thead>
<tr>
<th>Postnatal day of test</th>
<th>Control (n = 12)</th>
<th>Alcohol (n = 9)</th>
<th>Saccharose (n = 9)</th>
<th>Alcohol + thiamine (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.011 ± 0.246</td>
<td>1.444 ± 0.475</td>
<td>2.222 ± 0.547</td>
<td>1.7 ± 0.367</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>2.25 ± 0.279</td>
<td>2.444 ± 0.338</td>
<td>1.778 ± 0.364</td>
<td>2.1 ± 0.482</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>4.917 ± 0.645</td>
<td>2.111 ± 0.539</td>
<td>4.033 ± 0.289</td>
<td>3.9 ± 0.233</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>7.022 ± 0.707</td>
<td>2.044 ± 0.236</td>
<td>4.222 ± 0.547</td>
<td>4.5 ± 0.269</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>5.833 ± 0.613</td>
<td>1.444 ± 0.294</td>
<td>4.011 ± 0.928</td>
<td>1.5 ± 0.224</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>2.25 ± 0.524</td>
<td>2.667 ± 0.913</td>
<td>2.111 ± 0.351</td>
<td>1.6 ± 0.427</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Number of subjects in each experiment is noted in parentheses. Scheffe test: *P < 0.05 versus 1st min; tP < 0.01 versus 1st min.

Thiamine administration during chronic alcohol treatment reversed this effect of ethanol on habituation. The 20-day-old rats in E-T group exhibited a significant decrement of exploration from the 1st to 5th min of exposure \(F(1,9) = 17.63, P < 0.01\).

**Emotional reaction**

The results of the overall two-way ANOVA on excretion data (Fig. 2) showed the main treatment effect \(F(3, 210) = 11.8, P < 0.001\), the substantial increase over ages \(F(5, 210) = 102.4, P < 0.001\) and the reliable treatment × age interaction \(F(15, 210) = 1.9, P = 0.027\). The same factorial analysis established that saccharose offspring defecated more than control offspring \(F(1, 108) = 16.8, P < 0.001\), with no treatment × age interaction, \(F(5, 108) = 0.9, P = 0.505\), indicating the adverse effect of malnutrition induced by maternal chronic alcohol intake. Ethanol-exposed offspring did not defecate more than the saccharose-treated controls \(F(1, 96) = 1.184, P = 0.081\), showing that this effect of ethanol was not dissociable from the adverse effect of malnutrition induced by ethanol treatment. Nevertheless, this effect of ethanol was reversed by thiamine administration during maternal chronic alcohol intake as noted in E vs E-T comparisons \(F(1, 102) = 15.8, P < 0.001\). The interaction of these last treatments with age was also
Fig. 2. Mean (±SEM) number of fear-induced boli excretions over days in control pups (n = 11), ethanol-treated (n = 9), saccharose-treated (n = 9), and ethanol + thiamine-treated (n = 10) pups.

Fig. 3. Mean (±SEM) grasping time as a function of days in offspring exposed through maternally ingested water (n = 11), ethanol (n = 9), isocaloric saccharose (n = 9), or ethanol + thiamine solutions (n = 10).

significant \([F(5, 102) = 4.2, P = 0.002]\). Post hoc comparisons showed that the E offspring defecated more than E-T offspring on days 30 \([F(1, 9) = 5.211, P = 0.0484]\) and 45 \([F(1, 9) = 16.568, P = 0.0028]\).

To control the efficiency of thiamine administration during chronic alcohol intake, E-T pups compared to C pups did not show any difference \([F(1, 114) = 0.764, P = 0.384]\), whereas a significant difference was found between E-T and S pups \([F(1, 102) = 9.402, P = 0.003]\), with no reliable treatment \(\times\) age interaction \([F(5, 102) = 0.761, P = 0.580]\).

Grasping times

Analyses of grasping times (Fig. 3) indicated an overall difference among treatment groups \([F(3, 140) = 26.696, P < 0.001]\), as well as a significant increase over days \([F(3, 140) = 110.476, P < 0.001]\). The results of two-way
ANOVA on the grasping times did not show any difference between groups in C versus S comparisons \([F(1, 72) = 1.241, P = 0.096]\), whereas ethanol-treated offspring averaged reliably shorter grasping times than the saccharose controls \([F(1, 64) = 25.179, P < 0.001]\), with a reliable treatment \(\times\) age interaction \([F(3, 64) = 8.0172, P < 0.001]\). Subsequent analyses of the main effects of this interaction, using the Scheffé test, established that grasping times were shorter in ethanol offspring than in saccharose controls on days 20 \([F(1, 8) = 17.201, P = 0.0032]\) and 25 \([F(1, 8) = 10.769, P = 0.0112]\).

However, no significant difference was noted in the grasping times when E pups were compared to E-T offspring \([F(1, 68) = 0.1008, P = 0.752]\).

Crawling latency
The results of treatment \(\times\) age ANOVA on these data indicated that latency of crawling
Table 3. Neuronal counts and measurements in the hippocampal formation of 45-day-old rats from different treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Brain weight (g) (n = 6)</th>
<th>Cell number/1000 μm² (n = 15)</th>
<th>Soma height (μm) (n = 24)</th>
<th>Soma width (μm) (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.543 ± 0.043</td>
<td>19.333 ± 0.667</td>
<td>6.159 ± 0.169</td>
<td>4.296 ± 0.168</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.563 ± 0.039</td>
<td>11.607 ± 1.046*§</td>
<td>5.726 ± 0.254</td>
<td>3.489 ± 0.176*‡</td>
</tr>
<tr>
<td>Saccharose</td>
<td>1.581 ± 0.031</td>
<td>17.133 ± 0.878</td>
<td>6.107 ± 0.128</td>
<td>3.868 ± 0.133</td>
</tr>
<tr>
<td>Ethanol + thiamine</td>
<td>1.618 ± 0.019</td>
<td>12.867 ± 0.991†</td>
<td>6.215 ± 0.187</td>
<td>4.734 ± 0.185</td>
</tr>
</tbody>
</table>

Values represent means ± SEM of brain weight, number and sizes of cells located in the subregion CA3c. Three sections from the left hippocampus of each animal were examined. The number of brains, 1000 μm² areas, and cells sampled are shown in parentheses. Scheffé test: ethanol versus control, *P < 0.01; ethanol versus saccharose, §P < 0.01; ethanol + thiamine versus control, †P < 0.01; ethanol versus ethanol + thiamine, ‡P < 0.01.

execution (Fig. 4) varied as a function of both treatment \(F(3, 140) = 4.1, P = 0.008\) and age \(F(3, 140) = 44.9, P < 0.001\). There was no treatment \(\times\) age interaction \(F(9, 140) = 0.9, P = 0.536\). The same factorial analysis on data showed no effect of malnutrition in C vs S comparisons \(F(1, 72) = 0.4236, P = 0.517\), whereas a reliable effect of ethanol was exhibited in E vs S comparisons \(F(1, 64) = 5.6382, P = 0.020\). This effect of ethanol was not reversed by thiamine administration during chronic alcohol intake as noted in E vs E-T comparisons \(F(1, 68) = 2.1, P = 0.110\).

Nociception

Overall analysis of the tail-flick latencies (Fig. 5) yielded a reliable treatment effect \(F(3, 210) = 24.041, P < 0.001\) as well as a significant decrease over days \(F(5, 210) = 62.8798, P < 0.001\) and treatment \(\times\) age interaction \(F(15, 210) = 4.515, P < 0.001\). The results of two-way ANOVA on data showed that the ontogenetic reduction of tail-flick latencies during development was significantly delayed in saccharose offspring than in controls \(F(1, 108) = 34.7040, P < 0.001\), whereas no difference was found in ethanol vs saccharose comparisons \(F(1, 96) = 4.054, P = 0.051\). Reduction of the mean tail-flick latency was also delayed in E and S offspring compared to E-T offspring \(F(1, 102) = 24.475, P = 0.001,\) and \(F(1, 102) = 7.23, P = 0.008,\) respectively], whereas no difference was found in C vs E-T comparisons \(F(1, 114) = 3.508, P = 0.072\). Post-hoc analyses of the main effects of treatment \(\times\) age interaction established that nociceptive responses were significantly delayed in E and S offspring compared to E-T pups on day 20 \(F(1, 9) = 63.534, P = 0.0001,\) and \(F(1, 9) = 18.132, P = 0.0021\), and on day 25 \(F(1, 9) = 29.694, P = 0.0004\) and \(F(1, 9) = 10.124, P = 0.0112\).

Hippocampal histology

The one-way analysis of variance showed no treatment effect on the mean brain weight (Table 3) at the age of 45 days \(F(3, 15) = 0.723, P = 0.554\), suggesting that the ethanol-exposed offspring did not exhibit any significant microencephaly. In 45-day-old rats, there were no statistical differences \((P > 0.05)\) in the mean brain weight of the control compared to either the ethanol \(F(1, 5) = 0.046\) or the saccharose \(F(1, 5) = 0.165\) groups. In addition, there was a non-significant increase in the mean brain weight of the E-T offspring \((1.618 ± 0.019 g)\), more than that observed in ad libitum controls \((1.543 ± 0.043 g)\) \(F(1, 5) = 0.666\).

However, an ANOVA on the hippocampal CA3 pyramidal cell number of 45-day-old rats (Fig. 6A) showed significant between-group differences \(F(3,42) = 17.86, P = 0.0001\). Cell number in the field CA3 (Table 3), was significantly reduced \((P = 0.01)\) in alcohol (Fig. 6C) compared with either saccharose (Fig. 6D) \(F(1,14) = 6.971\) or control (Fig. 6B) \(F(1,14) = 13.616\) offspring, showing a specific effect of ethanol treatment. Thiamine (Fig. 6E) did not counteract this effect of ethanol when E and E-T pups were compared \(F(1,14) = 0.365\). Cell number was more reduced in E-T than in C offspring \(F(1,14) = 9.521\), confirming the last result.

There was no effect of treatments on the
Fig. 6. Parasagittal sections of the left hippocampus in 45-day-old rats. Paraffin 10 μm sections were stained with a combination of haematoxylin-eosin and indigo carmine. (A) The arrows denote the subregion CA3C (x112.5). Cells were counted directly in photomicrographs in boxes outlined 31.7 × 40 mm corresponding to 1000 μm² of histological area as the one depicted in panel B (x1125). (B) Normal control. (C) Ethanol-exposed. (D) Pair-fed saccharose. (E) Ethanol + thiamine-treated (x1125).

average soma height of the hippocampal CA3 pyramidal cells \[ F(3,69) = 1.283, \ P = 0.287 \]. However, the average soma width was significantly altered by the treatments \[ F(3,78) = 9.402, \ P = 0.0001 \]. Whereas the soma width was significantly reduced \( P = 0.05 \) in the ethanol-exposed offspring compared to the controls \( F(1,26) = 3.528 \), no differences in the soma width of E and S offspring were noted \( F(1,26) = 0.777 \). This effect of ethanol was significantly reversed by thiamine administration when comparing E and E-T offspring \( F(1,26) = 8.399 \).

Finally, photomicrographs of hippocampal sections showed more cornered than pyramidal-shaped cells in the hippocampal CA3 field of alcohol-exposed rats (Fig. 6C) compared to the control (Fig. 6B) or saccharose (Fig. 6D) groups. This breaking in cell membrane curvature was counteracted by thiamine administration during developmental alcohol exposure (Fig. 6E).
DISCUSSION

The present study showed a significant impairment of neurobehavioural development of the offspring born to severely alcoholic mothers. Comparison of ethanol and saccharose groups showed specific effects of maternal chronic alcohol intake on the neurobehavioural development of the offspring. Such effects were not reversed by thiamine treatment during chronic ethanol administration and were designated as neurotoxic effects of ethanol. In contrast, comparison of the findings in the control and saccharose groups showed the non-specific effects of maternal chronic alcohol intake on the neurobehavioural development of the offspring. Such effects were suppressed by thiamine treatment during chronic ethanol administration, whereas adverse effects of undernutrition caused by chronic alcohol intake are suppressed by thiamine treatment during long-term alcohol consumption.

In the literature, several mechanisms theoretically leading to thiamine deficiency in heavy drinkers have been suggested (Butterworth, 1989): (1) inadequate dietary intake (Poupon et al., 1990); (2) intestinal malabsorption of the vitamin (Thomson et al., 1970; Hoyumpa et al., 1978; Gastaldi et al., 1989); (3) a toxic effect of alcohol on the rate of transformation of thiamine into thiamine pyrophosphate (TPP) (Rindi et al., 1986, 1987). Following our experimental procedures, thiamine administration during chronic alcohol intake could suppress the first two mechanisms, since this treatment reversed adverse effects of undernutrition caused by chronic alcohol intake. Indeed, intestinal thiamine transport depends on two mechanisms: active transport at physiological concentrations and passive diffusion at higher concentrations. Consequently, in our experiments, chronic administration of ethanol mixed with a pharmacological dose of thiamine promotes the non-saturable component of thiamine transport due to passive diffusion. These conditions would provide an elevated blood concentration of thiamine and might facilitate placental diffusion of the vitamin, so that repletion of tissue thiamine stores and decreases in blood levels of the vitamin are not implicated in this model of chronic alcohol administration. Ethanol-induced neurotoxic effects persisted in this model, despite elevated blood concentrations of thiamine. Therefore, the likely explanation for the reversal of the adverse effects of undernutrition, by thiamine administration in the alcoholic group, is that poor intake of nutrients in the alcoholic results in impaired thiamine availability and absorption.

Indeed, it has been shown that thiamine deficiency is unusual in well-nourished patients with or without alcoholic disease (Dancy et al., 1984; Baines et al., 1988). It has been noted also that dietary thiamine supplementation can maintain normal thiamine levels in the animal, despite excessive, long-term alcohol consumption (Shaw et al., 1981). Recently, Poupon et al. (1990) reported that thiamine deficiency is either slight or absent in chronic drinkers. Consequently, it is reasonable to assume that the critical mechanism contributing to thiamine deficiency-induced neuropathy in chronic alcoholics is the alteration of thiamine metabolism and particularly the decrease in the vitamin conversion to its metabolically active form (TPP).

Structural data support the results obtained in functional studies. Thus, histological studies showed a decrease in the hippocampal CA3 pyramidal cell number and size in ethanol-exposed pups. A loss of the hippocampal CA1 pyramidal neurons has been reported in a previous study (Bonthius and West, 1991). Here, neuronal deficits persisted into adult life, as a consequence of developmental alcohol exposure, while no effect of treatments on brain weights of 45-day-old rats was noted. Consequently, permanent neuronal deficits can exist following developmental alcohol exposure without any microencephaly (Henderson and Schenker, 1977).

Administration of thiamine during maternal chronic alcohol intake counteracted in the offspring the reduction in cell size, but not the reduction in cell number. It is concluded that cell loss during developmental alcohol exposure is the result of toxic effects of ethanol-induced neuronal death, whereas inhibition of cellular growth by ethanol would be due to adverse effects of malnutrition. These data support previous studies in which long-term maternal consumption of moderate doses of ethanol adversely affected cellular development (DNA and protein content) and cell...
size in the offspring, even in the absence of differences in maternal weight gain or in fetal and newborn body weights (Gallo and Weinberg, 1986). So, these inhibitory effects of a non-specific undernourishment accompanying developmental alcohol exposure, on cellular growth, would contribute to the intrauterine growth retardation described in the fetal alcohol syndrome (Butterworth, 1993).

Histological studies showed also an increased number of cornered pyramidal cells in the hippocampal field CA3 of alcohol-exposed pups. These abnormalities might be explained by the non-specific effects of ethanol on biophysical properties (fluidity) of biological membranes (Topel, 1985). After chronic ethanol ingestion, there is a change in the properties of discrete regions of neuronal membranes, or in lipid–protein interactions (Tabakoff et al., 1987). Ethanol probably interacts with phospholipid bilayers at the lipid–water interface, disorders the lipid chains by desaturation (Villanueva et al., 1994), and fluidizes the membrane lipids (Barry and Gawrisch, 1994). These actions might explain chronic ethanol-induced breaking of cell membrane curvature. In this paper, thiamine would prevent chronic ethanol-induced breaking of cell membrane curvature. Thiamine may be involved in nerve conduction or transmission and might act directly on neuronal membranes (Berman and Fishman, 1975; Elder et al., 1976; Cooper and Pincus, 1979). Therefore, we must assume that thiamine can act as a protecting mechanism against ethanol-induced membrane fluidity, and can play a major role in regulating membrane stability.

Further work is required to determine the incidence and significance of thiamine deficiency in the alcoholic, but the evidence suggests that impaired absorption occurred solely during poor dietary intake. In addition, the pattern of the effects of thiamine administration during developmental alcohol exposure — compensatory effects on non-specific undernourishment and inability to counteract neurotoxic effects — is similar in both functional and structural studies. Therefore, it appears that future research must be more focused on the ethanol-induced reduction of TPP content in nervous tissues, rather than assessment of thiamine concentrations in blood and other tissues.

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