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Effect of Acute Ethanol Exposure During Pregnancy on Dentate Gyrus Synaptic Plasticity in 45-Day-Old Rats

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GÓMEZ, R. A., S. FULGINITI AND O. A. RAMÍREZ. *Effect of acute ethanol exposure during pregnancy on dentate gyrus synaptic plasticity in 45-day-old rats.* PHARMACOL BIOCHEM BEHAV 42(1) 85–89, 1992.—Pregnant Wistar rats were treated during the eighth day of gestation (GD8) with two IP injections, spaced by an interval of 4 h, of either ethanol (2.9 g/kg in saline solution) or saline. At 45 days of age, rats prenatally exposed to alcohol showed an improved hippocampal synaptic plasticity in granule cells layer of dentate gyrus. This was assessed measuring the threshold to generate long-term potentiation (LTP) on hippocampal slices. We propose that this result might account for the good performance in some learning tasks observed in animals prenatally exposed to alcohol during short periods.

Prenatal ethanol intoxication LTP Synaptic plasticity Dentate gyrus Hippocampus slices

PRENATAL ethanol exposure can cause profound, long-lasting physical, physiological, and behavioral abnormalities in both human and laboratory animals (8,10,40,41,42). The pattern of defects associated with prenatal ethanol exposure was first termed "fetal alcohol syndrome" (FAS) by Jones et al. in 1973 (20). Although adverse effects of chronic alcohol consumption throughout gestation have been extensively investigated in different animal models, relatively few experimental designs deal with the consequences of acute exposure to alcohol during a critical period of fetal development. Acute alcohol intoxication during the period of gastrulation in mice has been found to produce craniofacial dismorphologies and severe brain malformations in the offspring (42,47) similar to those often observed in children born to alcoholic mothers. For the rat, acute intraperitoneal treatment with alcohol during this short period of gastrulation is sufficient to induce dose-dependent morphological, behavioral, and developmental alterations in the offspring (30,31,45,46), as well as an altered reactivity to pharmacological agents (16,17,31). These findings may support a useful rat model of fetal alcohol effects since clinical evidences of developmental abnormalities have been described in children born to women who consume ethanol in restricted episodes during early pregnancy (18,25).

Many lines of evidence suggest the particular sensitivity of the hippocampus to the toxic effects of ethanol during development (43). Exposure to high alcohol concentrations reduce the number of pyramidal cells in offspring hippocampus (2,49). There is also a decrease in the density and arrangement of dendritic spines (1). Hoff (19) demonstrated that prenatal ethanol exposure has no effect on the appearance of synapses in the dentate gyrus during early postnatal life. However, prenatal ethanol exposure appears to affect synapse turnover, suggesting an altered synaptic plasticity on hippocampal formation, which in turn could disrupt the animals' ability to acquire and process various forms of information (19). Learning alterations implicated in FAS have been extensively reported. While many authors described that adult offspring exposed to ethanol in utero exhibit deficits in a variety of learning tasks (1,3,13,26,39), others demonstrated no effects (30) or even an improvement (35).

Brief high-frequency stimulation of a monosynaptic excitatory hippocampal pathway produces an enhancement of synaptic transmission that can last for several days (5). This phenomenon, called long-term potentiation (LTP), is considered a useful model of learning and memory. Recently, we reported that the rat learning ability in a shuttle-box avoidance paradigm is correlated with dentate gyrus synaptic plasticity (36). Taking into account the important role of hippocampus in learning and memory and the particular sensitivity of hippocampal formation to the toxic effects of prenatal alcohol exposure, in the present study we examined the effect of acute ethanol intoxication on gestational day 8 (GD8) on hippocam-

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pal synaptic plasticity in adult offspring to correlate the hippocampal synaptic plasticity with the behavioral alterations observed in adult rats prenatally exposed to alcohol.

METHOD

Subjects

The procedure used to expose rats to ethanol during GD8 has been already described (16). Parent animals were male and nulliparous female Wistar rats 90–120 days old born and reared in our laboratory. They were provided with food and water ad lib on a 12 L : 12 D cycle (lights on at 0700) and under constant temperature conditions (22 ± 1°C). In the evening of the day of proestrus, females were housed overnight with male rats in a relation of 3 to 1. The presence of spermatozoa in the vaginal smears the following morning was taken as an index of pregnancy and was referred to as gestational day 1. Females were weighed and housed individually in breeding cages in a separate nursery. Pregnant females throughout gestation were fed a lab chow maternity diet (Nutrimentos S. A., pregnancy lab chow).

On GD8, dams received two IP injections spaced by an interval of 4 h (1000 and 1400) of ethanol (2.9 g/kg in 24% v/v saline solution) or the same volume of saline. Injections were made with considerable care to avoid trauma to the uterus. Mothers injected with saline were kept without food and water during approximately 12 h since alcohol-treated rats exhibited an inhibition of feeding habits during that same period of time.

Maternal blood ethanol levels had been already reported (16). Briefly, they were determined by gas chromatography (22) immediately before and after 30, 60, 120, and 180 min of the second dose of alcohol. Ethanol levels were at their highest level 60 min after the second injection (457 ± 12 mg/dl, n = 9).

From GD20 until birth, breeding cages were checked three times daily for newborn pups. Within 12 h after birth [postnatal day (PD) 1], all pups were weighed, inspected for gross physical anomalies, and litters were culled to four males and four females whenever possible. Offspring were weaned at PD25 and grouped 8–10 per cage according to prenatal treatment and sex.

Hippocampal Slice Preparation and Electrophysiological Technique

Electrophysiological experiments were performed using the in vitro hippocampal slice essentially as described by Yamamoto et al. (51). Briefly, 45-day-old male offspring (one animal from each litter) were sacrificed between 11:00 a.m. and 12:00 p.m. (1-h period) to prevent variations determined by circadian rhythms or nonspecific stressors (11).

The hippocampal formation was dissected and transverse slices, approximately 400 μm thick, were obtained and placed in a recording chamber perfused with a standard solution saturated with 95% O_2 and 5% CO_2. Rate of perfusion was 2–3 ml/min; the temperature of the bathing solution was kept at 30°C. Under visual control, a stimulating electrode made of two insulated twisted wires except for the cut ends (diameters 50 μm) was placed in the perforant path (PP) and a recording microelectrode made with a micropipette (tip 10–20 μm) was inserted in the dentate granule cell body layer (36,37). Only one slice per animal was included in this study for control and experimental animals. Evoked field potentials were amplified and photographed. Ten field potentials, in response to the stimuli, were sampled at 0.2 Hz averaged on-line using a Max PC XT microcomputer and stored on diskettes for further analysis. Once a stable evoked field response, including its characteristic population spike, had been obtained, the intensity of the electrical stimulus to the PP was set at the value that would elicit spikes approximately at 30% of the maximum. The threshold frequency to obtain LTP was determined as described (36). Tetanus consisting of a train of pulses (0.5 ms) of 1-s duration and with increasing frequency was delivered to the slice at 20-min intervals or more (up to 45 min) starting with a tetanus at 5 Hz and increasing with each train to 10, 25, 50, 100, and 200 Hz. Fifteen to 20 min after a tetanus had been delivered, a new averaged response was recorded; if LTP was not observed, another tetanus at the next higher frequency was applied. LTP was considered to have been produced when the amplitude of the evoked population spike (PS; Fig. 1) recorded 20 min after the tetanus had been increased by at least a 30%.

RESULTS

No differences were observed in maternal weight gain on GD8 and GD18 nor during gestational period, total litter size, and body weight of pups at birth. Body weight gain (g) between GD1 and GD8 was (mean + SE): CG = 19.9 + 1.0 (n = 11), EG = 18.8 + 1.4 (n = 10); weight gain between GD8 and GD18 was: CG = 39.2 + 2.7, EG = 33.7 + 4.3; length of gestational period (days): CG = 22.8 + 0.1, EG = 23.1 + 0.1; total litter size: CG = 11.0 + 0.4, EG = 10.9 + 0.6; and body weight of pups at birth: CG = 6.3 + 0.1, EG = 6.2 + 0.12.

Figure 1 shows a typical example of a field potential response recorded in the granule cell body layer of the dentate gyrus evoked by stimuli (0.2 Hz) given to PP. The right panel

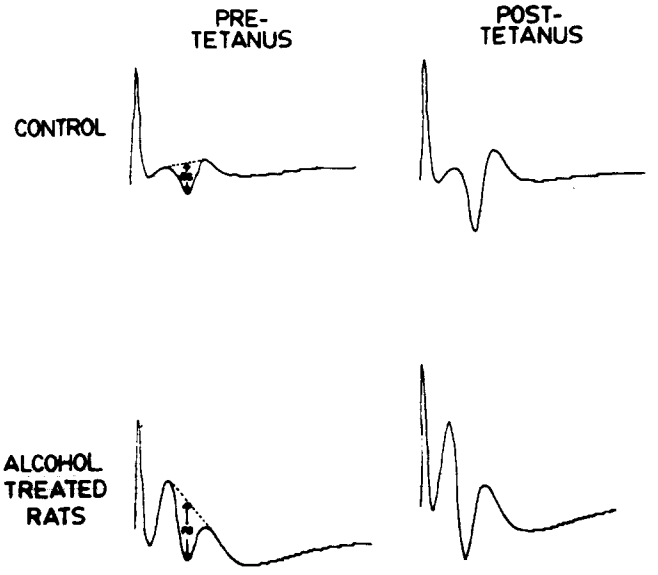

FIG. 1. Field potentials recorded of responses from granulle cells layer of the dentate gyrus evoked by stimulation of the perforant path in a hippocampal slice. Pretetanus was recorded delivered at 0.2 Hz; posttetanus shows the responses recorded after a train of high-frequency stimulation. Calibration bars represent: 6.3 ms and 305 μV for control rats and 610 μV for alcohol-treated rats. PS, population spike.

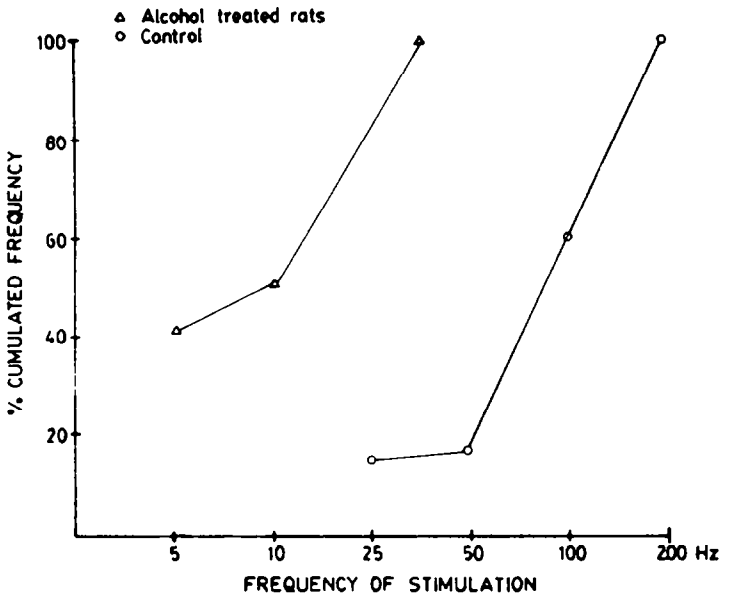

FIG. 2. Cumulated frequency curves indicate the number of experiments in which LTP was induced by a tetanus at the corresponding frequency (Hz) in slices from alcohol-exposed offsprings and controls rats. Mean frequency for induction of LTP was 12.14 ± 3.7 Hz for alcohol-treated rats and 110.7 ± 27.5 Hz for controls rats. Significance level was $p < 0.01$ (Student's *t*-test).

shows the increased amplitude of population spike after an effective tetanus. The potentiation magnitude obtained was greater in alcohol-treated rats ($n = 7$) than in controls ($n = 7$) (mean ± SEM: 157.28 ± 30.07% vs. 88.7 ± 22.04%, $p < 0.05$).

Hippocampal slices from alcohol-exposed offsprings required lower-frequency trains (12.14 ± 3.70) than those from control rats (110.7 ± 27.5) for LTP induction (Fig. 2). All slices from alcohol-pretreated rats ($n = 7$) displayed LTP with 25 Hz or less, while control rats required ($n = 7$) up to 100 Hz. Threshold to induce LTP on slices from animals of our colony did not differ from control saline-injected animals (112.44 ± 32.7). The increased amplitude of PP was maintained until the end of experiment, in some cases up to 3 h after tetanus.

DISCUSSION

The present study demonstrated that 45-day-old offspring from mothers exposed to a single ethanol intoxication during GD8 showed a decreased threshold to induce LTP on hippocampal dentate gyrus. Gastrulation was reported to be a sensitive period for the production of fetal alcohol effects. Since this period involves the mesoderm formation and mesoderm is responsible for induction and maintenance of the neuroepithelium, an adverse effect on the mesoderm can result in deficient neural development. Likewise, a number of different periods during gestation has also been described as especially vulnerable to alcohol exposure. There is profuse literature concerning the effects of prenatal alcohol exposure on neuronal and synaptic maturation in the CNS. However, there appears to be little or no profound alteration in the process of synaptogenesis [see (49)]. Moreover, Hoff (19) reported subtle alterations of synaptogenesis in the male hippocampal dentate gyrus after prenatal alcohol exposure. Our present finding related to the increased hippocampal synaptic plasticity can be explained by means of other effects of prenatal alcohol exposure than those reported for synaptogenesis of dentate gyrus. Moreover, chronic prenatal exposure to ethanol decreases physiological plasticity in the CA1 area of rat hippocampus (44). This dissimilar result may be accounted for, at least in part, by the different areas of the recording or even the time of alcohol administration.

The mechanisms generating LTP are little understood; available evidence suggests that both pre- and postsynaptic mechanisms are involved (4,24,28), including increase of Ca^{+2} levels in pre- and postsynaptic neurons (7,21), enhancing release of excitatory aminoacids (12), and opening more channels associated with NMDA receptors (9). Besides, it has been demonstrated that antagonists of GABA transmission facilitate the generation of LTP on hippocampus (50). On the other hand, Mott et al. recently reported that baclofen, a $GABA_B$ receptor agonist, facilitates the development of LTP in the dentate gyrus (33). The increased synaptic plasticity in the dentate gyrus reported in this study might reflect an enhanced release or decrease in glutamatergic binding sites, if they are located presynaptically, effects that have been described as induced by prenatal alcohol exposure (15,38). A decrease in GABA levels in hippocampus (23) may account for the enhanced dentate gyrus excitability reported here. It is important to consider that following the second injection of ethanol intoxicated mothers showed respiratory depression; thus, deleterious effects of hypoxia on the developing CNS may occur, altering one or more of the proposed mechanisms involved in LTP induction.

The relationship between hippocampal synaptic plasticity and the performance in different kinds of learning tasks has been demonstrated by many authors (27,32,36,37,48). The in-

creased hippocampal synaptic plasticity observed in rats prenatally exposed to a brief period of ethanol may account for the good performance of rats preexposed to alcohol in some tasks that have been previously reported. Osborne et al. (35) described that offspring of rats treated with ethanol during days 10-14 of gestation performed more avoidance responses and correct discriminations in the Y-maze at 65 days than controls. We recently observed that rats of 45 days of age prenatally exposed to acute intoxication with ethanol on GD8 displayed an improvement in avoidance acquisition in a shuttle box compared with offspring of control mothers (unpublished data). Molina et al. (30) stated that both acquisition and retention of active avoidance responses had not been affected in offspring of 65- to 70-day-old rats treated in utero with a similar alcohol scheme of administration. Behavioral alterations induced by prenatal administration of high doses of alcohol during the gastrulation period have been reported: overactivity, deficits in passive avoidance (30,45), as well as deficits in multiple operant performance (46). Similarly, chronic prenatal alcohol treatment may induce comparable alterations (13,26,41).

The deleterious effects of prenatal administration of alcohol on the behavior of the offspring may subserve on structures beside hippocampus since subtle alterations induced by alcohol in this structure have been described.

Moreover, it is possible that prenatal alcohol noradrenergic-induced alterations previously described (14,34) may cause a failure in selective attention (29) responsible for the deleterious effects in the learning performance tasks described after prenatal alcohol exposure in adult animals. It is also possible that some effects of prenatal exposure to alcohol are a function of offspring age and timing of alcohol administration.

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