

Fetal Alcohol Effects: Mechanisms and Treatment

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This article represents the proceedings of a symposium at the 2000 ISBRA Meeting in Yokohama, Japan. The chair was Edward P. Riley. The presentations were (1) Does alcohol withdrawal contribute to fetal alcohol effects? by Jennifer D. Thomas and Edward P. Riley; (2) Brain damage and neuroplasticity in an animal model of binge alcohol exposure during the "third trimester equivalent," by Charles R. Goodlett, Anna Y. Klintsova, and William T. Greenough; (3) Ganglioside GM1 reduces fetal alcohol effects, by Basalingappa L. Hungund; and (4) Fetal alcohol exposure alters the wiring of serotonin system at mid-gestation, by F. Zhou, Y. Sari, Charles Goodlett, T. Powrozek, and Ting-Kai Li.

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IN ITS RECENT portfolio review of fetal alcohol syndrome, the NIAAA indicated that "basic and clinical studies designed to assess individual and/or environmental characteristics that mitigate the effects of prenatal alcohol exposure need to be conducted" (NIAAA, 1997). The NIAAA also reported that to intelligently develop potential treatments or interventions, we needed to improve our understanding of the mechanisms that underlie the abnormalities which result from prenatal alcohol exposure. This symposium was driven from these two topics of high priority. The mechanisms and treatments discussed here are not the only ones being pursued (see NIAAA, 2000, for a review), but they provide examples of how mechanistic studies impact ideas about intervention. Our purpose was to present research on both mechanisms and treatment for fetal alcohol effects and to demonstrate how the two are intimately intertwined. Identifying mechanisms and understanding how alcohol exerts its varied effects on the developing organism will help to identify more effective interventions in these processes and will help mitigate the devastating effects of gestational alcohol exposure.

DISCUSSION

Withdrawal and Fetal Alcohol Effects

One novel mechanism for damage of the central nervous system (CNS) of the fetus involves an indirect rather than a direct effect of the alcohol. Clinically, it appears that binge drinking produces more severe outcomes in offspring than more chronic exposure. Although there are other explanations for this, one possibility is that the offspring of women who binge during pregnancy have experienced episodes of in utero withdrawal and the concomitant effects of such withdrawal. It is suggested that periods of alcohol withdrawal may lead to NMDA-receptor-mediated excitotoxicity, a process of cell death, which may contribute to fetal alcohol effects.

The NMDA receptor is activated by the excitatory amino acid glutamate during normal neurotransmission. This receptor plays a critical role in neuronal plasticity both during development and later, during associative learning (McDonald and Johnston, 1990). The NMDA receptor also plays a role in cell death. If the receptor is overactivated, it can lead to a cascade of events that ultimately kills the cell, a process called excitotoxicity. Excitotoxicity can occur after a number of insults, such as hypoxia/ischemia, and in a number of neurodegenerative disorders, such as Alzheimer's disease (Choi, 1988). Alcohol directly interacts with the NMDA receptor. Acutely, it inhibits, or antagonizes, the receptor (Hoffman, 1995). This action, however, may lead to a neuroadaptive response, which increases the number or sensitivity of NMDA receptors. When alcohol is removed from the system there is a rebound excitability of NMDA receptors, an effect that may contribute to the seizure-like activity associated with withdrawal and may cause excitotoxic cell death (Lovinger, 1993).

The possibility that this mechanism contributes to fetal alcohol effects is intriguing because of the role of the NMDA receptor during development. During periods of

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synaptogenesis and neurite outgrowth, there is a transient increase in the number of NMDA receptors. For example, around postnatal day (PD) 6 and 7 in the rat, there is a transient increase in the number of NMDA receptors in the hippocampus and cortex (McDonald and Johnston, 1990). Coincidentally, at this same time there is an abrupt increase in vulnerability both to excitotoxic cell death (Ikonomidou et al., 1989) and to alcohol-induced reductions in forebrain weight and deficits on behavioral tasks that rely on the functional integrity of the hippocampus and cortex (Thomas et al., 1995).

Drs. Thomas and Riley hypothesized that if NMDA receptor-mediated excitotoxicity occurs during withdrawal in the developing organism, then blocking the NMDA receptor during withdrawal should reduce the severity of ethanol's effects. MK-801 is a noncompetitive NMDA receptor antagonist that protects against excitotoxicity. The effects of administering MK-801 during ethanol (EtOH) withdrawal were assessed by using a rat model of EtOH treatment during a portion of the third-trimester-equivalent "brain growth spurt." The brain growth spurt that occurs in the third trimester in humans occurs during the first week or so of postnatal life in rats (Dobbing and Sands, 1979). Alcohol treatment was accomplished via an artificial rearing procedure.

The researchers first examined the consequence of administering 0.1 mg/kg MK-801 during withdrawal from a heavy binge-like alcohol treatment on postnatal day (PD) 6 (Thomas et al., 1997). There were five treatment groups: EtOH exposure, EtOH exposure + MK-801 during withdrawal, MK-801 only, an artificially reared gastrostomy control, and a normally reared suckle control. On PD 6, during the first two daily feedings, EtOH was added to the diet of the EtOH subjects for a total dose of 6.0 g/kg. Twenty-one hours after the completion of EtOH treatment, all artificially reared subjects were injected intraperitoneally with either 0.1 mg/kg MK-801 or saline. On PD 40 to 42, subjects were tested on a serial spatial discrimination reversal-learning task.

One day of alcohol treatment on PD 6 impaired performance on this task; however, treatment with MK-801 during the withdrawal phase significantly attenuated EtOH's effects. EtOH subjects were impaired in their ability to discriminate position and to reverse the discrimination. For example, only 70% of EtOH subjects were able to reverse on the first day of testing. In contrast, more than 90% of the EtOH + MK-801 subjects were able to reverse the discrimination on Day 1 of testing, which was significantly better than EtOH subjects and not different from controls. Examination of the types of errors suggested that the EtOH subjects had particular difficulty in inhibiting responses. EtOH produced a robust increase in the number of within-trial, perseverative errors. Again, MK-801 administration during withdrawal reduced the severity of this effect. Although the EtOH + MK-801 group still committed a

greater number of errors than controls, they made fewer errors than the EtOH group (Thomas et al., 1997).

The dose-dependency of MK-801 to attenuate EtOH's effects then was examined. Five groups were treated with 6.0 g/kg EtOH in a manner as described previously and then were treated with either 0, 0.05, 0.1, 0.5, or 1.0 mg/kg MK-801, 21 hr after the EtOH treatment. On PD 18 and 19, activity level was measured, and on PD 40 to 42, reversal learning was assessed. EtOH alone significantly increased activity, but the three lower doses of MK-801 during withdrawal attenuated this effect. In contrast, administration of the 1.0 mg/kg MK-801 dose did not reduce the alcohol-induced overactivity. A similar dose-response pattern was observed on the reversal-learning task. EtOH increased perseverative-type errors, which was attenuated by the three lower doses of MK-801 but not by the high dose.

Finally, this hypothesis is fairly specific. These protective effects should be seen only when the antagonist is given during the withdrawal phase. MK-801 was administered at varying times relative to the EtOH treatment. Four groups were treated with 6.0 g/kg EtOH on PD 6 and then were injected with 0.5 mg/kg MK-801 either during EtOH treatment (0 hr) or at 9 hr, 21 hr, or 33 hr post-EtOH. A fifth EtOH-treated group was injected with saline vehicle. Survival rates varied as a function of treatment. Administration of MK-801 concurrent with EtOH was highly lethal (25% survival). MK-801 at later time points did not significantly affect survival rates. Behaviorally, EtOH subjects treated concurrently with MK-801 were significantly more active compared with the EtOH group. Administration of MK-801 9 hr after EtOH did not affect activity; however, administration of MK-801 at 21 and 33 hr after EtOH significantly attenuated EtOH-induced overactivity. Thus, MK-801 administration at the time of EtOH treatment exacerbated EtOH's effects, whereas administration during withdrawal, as blood alcohol levels approached 0, mitigated EtOH's effects.

These data demonstrate that blockade of the NMDA receptor with MK-801 during EtOH withdrawal reduces the severity of EtOH-induced alterations in activity level and reversal learning. The ability of MK-801 to attenuate EtOH's effects is both dose- and time-dependent. Administration of low, but not high, doses of MK-801 during withdrawal attenuated EtOH's effects on behavioral development. Importantly, for normal development, an optimal level of NMDA receptor activation is necessary. Both underactivation and overactivation can have adverse effects on the developing CNS. Perhaps the lower MK-801 doses allowed the NMDA receptors to achieve a more optimal level of activation, whereas the high dose in and of itself may have induced further CNS damage. This damage may have occurred as a result of excessive inhibition, an effect that produces apoptotic cell death (Ikonomidou et al., 2000), or as a result of enhanced rebound excitability. The ability of MK-801 to attenuate EtOH's effects is also spe-

cific to the period of alcohol withdrawal. Administration of MK-801 at the same time as EtOH was highly toxic and exacerbated EtOH's effects in survivors, whereas administration during withdrawal reduced the severity of EtOH's effects. These data are consistent with the hypothesis that EtOH acutely inhibits the receptor, which produces a rebound overexcitation during withdrawal.

EtOH, the Cerebellum, and Remediation

The cerebellum appears to be especially susceptible to the effects of prenatal alcohol exposure, and cerebellar abnormalities have been noted in autopsy and recent magnetic resonance imaging studies of fetal alcohol syndrome (Roebuck et al., 1998). Consistent with this, deficits have been reported for coordinated motor performance and in tests of balance after prenatal alcohol exposure (Mattson and Riley, 1998). Animal studies also demonstrate that the developing cerebellum is especially vulnerable, particularly when the exposure occurs during the early postnatal period. Again, this is probably because the stage of brain development comparable to that of the human third trimester occurs in rats during the period of the neonatal brain growth spurt (Dobbing and Sands, 1979). For example, the stages of development of the Purkinje cells in rats and humans progress from neurogenesis and migration to outgrowth of the properly oriented dendritic tree accompanied by rapid synaptogenesis. Although the stages are similar, the timing relative to birth is quite different. The changes in morphology that occur from fetal week 26 to birth in the human are roughly equivalent to that which occurs during PD 4 to 12 in the rat.

To establish how the extent of cell loss varies with dose, blood alcohol concentration (BAC), timing, and duration of exposure, three-dimensional cell counts were performed (Goodlett et al., 1998). Also, the behavioral consequences were evaluated by using tests of motor performance and learning known to depend on the functional integrity of the cerebellum and brain stem. One particularly sensitive motor test is parallel bar traversal, in which rats are tested for their ability to traverse elevated, parallel bars of increasing gap widths. More recently, classical conditioning of eye-blink responses has been used to evaluate behavioral consequences of cerebellar damage induced by neonatal alcohol exposure.

As summarized in a recent review (Goodlett and Johnson, 1999), it is now well established that neonatal alcohol exposure in rats produces a significant, dose-dependent loss of cerebellar Purkinje cells. There are proportional losses of cell populations that form direct connections to Purkinje cells, which include granule cells, deep cerebellar neurons, and inferior olive neurons. There are striking temporal windows of vulnerability to neonatal alcohol-induced cerebellar Purkinje cell loss, with the period of greatest vulnerability occurring on PD 4 to 6, compared with either prenatal exposure or exposure that occurs after PD 6

(Goodlett and Johnson, 1999; Goodlett et al., 1998; Thomas et al., 1998). Recently completed studies also indicate that exposure during PD 4 to 6 produces significantly more severe Purkinje cell loss than similar exposure on PD 1 to 3. The conclusion that PD 4 to 6 constitutes a temporal window of vulnerability of alcohol-induced cerebellar damage is supported by numerous replications across several labs.

Behavioral studies also indicate that binge-like neonatal alcohol treatments that include the period of PD 4 to 6 result in deficits in motor performance (parallel bar traversal) and motor learning (eye-blink classical conditioning). The severity of the deficits in parallel bar traversal depends on the dose and timing of exposure (Goodlett and Lundahl, 1996; Thomas et al., 1998). Exposure during the period of PD 4 to 6 produces the most severe deficits in performance, and the deficits are correlated with the extent of Purkinje cell loss (Thomas et al., 1998).

For eye-blink classical conditioning, the alcohol-induced deficits are particularly clear. Weanling rats given binge alcohol exposure on PD 4 to 9 (peak BACs, 330 mg/dl) had severe impairments in acquisition (Stanton and Goodlett, 1998). In recent replications, it was found that the effect is a severe acquisition impairment (rather than a complete failure), and that the impairment is evident only with relatively high doses that produced high peak BACs. The acquisition impairments observed in weanlings were also evident in adult rats (Green et al., 2000). The deficits in eye-blink classical conditioning with heavy binge neonatal alcohol exposure reflect deficits in associative learning and cannot be attributed to nonspecific performance deficits. Preliminary evidence gathered with extracellular single-unit recording in the deep interpositus nucleus shows reduced neural activity of these neurons during the CS period during acquisition, compared with controls. This suggests that there are underlying deficits in training-related neuroplasticity in one or more sites critical for this form of associative learning.

In terms of remediation, studies that used acrobatic motor training initiated in adulthood have assessed whether training that involves complex motor learning can stimulate formation of new brain synapses, and whether it ameliorates deficits in motor performance induced by alcohol exposure. In these rehabilitation studies, adult rats assigned to the acrobatic training group were given 10 to 20 days of training to traverse an obstacle course of 10 difficult motor tasks each day (e.g., traversal of chains, rope ladders, etc.). Posttraining assessment involved testing on three tasks not part of the acrobatic training—parallel bar traversal, rotating barrel traversal, and rope climbing. Controls included groups given comparable handling and enforced locomotor activity without complex motor learning and groups of normal socially housed rats. The studies of training-induced morphological synaptic plasticity were performed in groups separate from those tested on the behavioral outcomes, in which the paramedian lobule of the cerebel-

lum was processed for electron microscopic analysis. Quantitative stereological morphological studies of the electron microscopic sections evaluated the number of parallel fiber-to-Purkinje cell synapses in the paramedian lobule and the synapse-to-neuron ratios of neurons in layers II to III in motor cortex.

The results indicate that even with the permanent, severe cerebellar cell loss and functional disruption, intensive behavioral intervention that demands complex motor learning can be an effective rehabilitation treatment. The adult acrobatic motor training stimulated training-related synaptogenesis in the cerebellum and motor cortex (Klintsova et al., 1997, 1999). Alcohol-exposed rats given acrobatic training had significantly larger volumes of the molecular layer and more parallel fiber synapses (per Purkinje cell) in the paramedian lobule than did the neonatal alcohol-exposure rats maintained in standard social cages. In the motor cortex, the acrobatic training increased the number of synapses per neuron in layer II/III to a comparable extent (about 18–20%) in both the alcohol-treated and the control groups. Because the two alcohol-exposed groups had comparable loss of Purkinje neurons in the paramedian lobule, and of layer II/III neurons in motor cortex, the training-induced increases in synapses indicate that the alcohol-damaged brain retains significant potential for experience-driven neuroplasticity.

The 20 day acrobatic motor training eliminated motor performance deficits in parallel bar traversal, rope climbing, and rotating drum tests. As measured by these tests, the complex motor training resulted in a full rehabilitation effect. Alcohol-treated rats in both the social housing condition and the locomotor activity control condition showed deficits compared with either the normally reared or artificially reared controls (not exposed to alcohol). In contrast, the alcohol-treated rats given 20 days of acrobatic training performed with very few errors even with manipulations that increased the difficulty of each motor test across trials; these trained rats were entirely comparable to the neonatal treatment controls given the acrobatic training.

This research supports several conclusions about the consequences of neonatal alcohol exposure. There is a dose-dependent neuronal loss in the cerebellum, with a critical period of enhanced vulnerability to Purkinje cell loss on PD 4 to 6. Binge exposure increases the risk for cerebellar damage, and even a single binge on PD 4 can produce significant Purkinje cell death. Binge exposures during the period of vulnerability produce long-lasting deficits in behavior known to depend on cerebellar-brain stem circuitry. In terms of implications for treatment of alcohol-affected children, training in adulthood that involves complex motor learning can stimulate synaptic morphological plasticity and ameliorate motor performance deficits.

Gangliosides and Prenatal Alcohol

One of the primary sites for EtOH's action is on neural membranes, of which gangliosides, known to be affected by EtOH, are a predominant component (Goldstein et al., 1983; Morgan et al., 1976). Gangliosides undergo characteristic changes during various stages of cellular development and differentiation, which include pattern formation, synapse formation, and cellular migration. EtOH exposure during development could have a profound effect on CNS ganglioside synthesis and turnover and hence development. Exogenous gangliosides currently are used in experimental therapy to treat a variety of neuronal disorders, which include peripheral neuropathies and injuries caused by stroke, ischemia, and spinal cord injury (Hungund and Mahadik, 1993). It has been reported that pretreatment with ganglioside GM1 reduced the accumulation of fatty acid ethyl esters, toxic metabolites of EtOH, and EtOH-induced cytosolic phospholipase A2 in the brain membranes of chronic EtOH-exposed mice (Hungund and Gokhale, 1994; Hungund et al., 1994). In the present study, the distribution of plasma membrane GM1 in cortical cells derived from gestational day (GD) 15 and GD 20 offspring of EtOH-exposed dams with and without GM1 treatment was assessed. The goal was to determine whether cellular growth and maturational changes that underlie fetal EtOH effects would be evidenced and whether GM1s would be neuroprotective.

On GD 6, dams were divided into four groups. Group GM1 received ganglioside GM1 (10 mg/kg, intramuscularly) 24 hr and then again 1 hr before EtOH. EtOH was administered in two doses of 2.9 g/kg intraperitoneally, spaced 4 hr apart. A similar regimen was followed on GD 8, but only the 1 hr GM1 treatment was given. Group EtOH received only EtOH after vehicle instead of GM1. Group C received only vehicle, and group C-GM1 received GM1 and then saline. Male offspring were tested for sleep time. A selected number of mothers from groups GM1 and EtOH also received additional doses of EtOH on GD 13 and 14 and GM1 treatment on GD 12 (24 hr) and GD 13 and 14 1 hr before EtOH administration, respectively. The control groups (C and C-GM1) received vehicle as described previously. Fetuses were removed on GD 20, and brains were collected and analyzed for ganglioside composition.

At 45 days of age, male offspring were given a hypnotic dose of EtOH (3.5 g/kg intraperitoneally). Rats were monitored for the loss of righting reflex ("sleep time") as defined as the time between the loss and regaining of the righting reflex. BACs were determined at the time of righting. Gangliosides were extracted and purified, and *N*-acetylneuraminic acid (NANA) content in ganglioside fraction was determined. The percentage distribution of individual gangliosides was determined, and the results were expressed as percentage of total gangliosidic NANA. Neuraminidase activity was assayed and enzyme activity expressed as nanomoles of NANA liberated per minute per

milligram of protein (Prasad, 1992). For tissue culture studies, cerebellar neocortices from either 15- or 20-day-old rat fetuses were pooled, and mixed neuronal cortices were grown. After 24, 48, and 96 hr and 7 days in culture, the cell surface GM1 was labeled with cholera toxin/antitoxin/fluorescein labeled immunoglobulin, and cultures were assessed for cell survival, attachment, clustering, arborization, synaptic formation, and extent of GM1 staining.

At PD 45, when the animals were tested for sensitivity to the EtOH challenge dose (3.4 g/kg), there were significant differences in sleep time. The controls had similar sleep times, which averaged about 130 ± 28 min, whereas the EtOH-treated group exhibited shorter sleep times (81 ± 19 min). The group that received GM1 before EtOH had a sleep time similar (131 ± 21 min) to controls. No differences in waking blood EtOH levels were noted. However, when the rate of elimination of ingested EtOH over a period of 3 hr was determined, no significant difference between different treatment groups was observed.

Because gangliosides play an important role in early CNS development, the effect of in utero EtOH exposure and GM1 treatment on the fetal brain ganglioside was studied. Fetal exposure to EtOH had a profound effect on brain ganglioside composition. EtOH-exposed fetal brains had higher levels of ganglioside GM1 compared with controls. By contrast, maternal pretreatment with GM1 resulted in normalization of this aberrant ganglioside profile: The ganglioside profile was more similar to the controls. The levels of polysialogangliosides GD1a, GT1b, and GQ1b were also lower in EtOH-exposed fetal brains. GM1 pretreatment appeared to mitigate this decrease in polysialogangliosides.

The behavioral effect coincided with the altered brain ganglioside composition, especially the increased accumulation of endogenous ganglioside GM1 in GD 20 fetal brain. This may result from EtOH-induced increases in sialidase activity, an enzyme responsible for removal of sialic acid moieties of polysialogangliosides. GM1 is resistant to sialidases. An increase in sialidase activity indeed was seen in the crude synaptosomal membranes of the brains of fetuses exposed to EtOH in utero. GM1 pretreatment reduced the EtOH-induced activation of this enzyme. Thus, GM1 may produce its neuroprotective effect by preventing this increase in EtOH-induced sialidase activity.

The cell culture studies complement and extend the behavioral and biochemical observations and demonstrate the deleterious effects of EtOH exposure at a cellular level. The effect on cellular integrity was determined by labeling the surface-exposed ganglioside GM1 with cholera toxin antibodies, which bind specifically to GM1. The cultures derived from EtOH-exposed GD 15 fetuses consistently evidenced poor cell attachment, reduced survival, and marginal growth, which included nominal levels of arborization, cell clustering, and synapse formation. The control and GM1 cultures exhibited more robust growth characteristics, which included good attachment, high cell survival, and early arborization, followed by synapse formation. Overall

morphology of cells taken from fetuses exposed to both EtOH and GM1 was very similar to that seen in controls. The control cultures exhibited continuous GM1 staining at all time points, which became more pronounced as the cells developed processes and synaptic formations. The same staining pattern was observed in both EtOH + GM1 and GM1 groups. There was clear evidence of intact plasma membrane structures. In contrast, the EtOH group's cells rarely exhibited continuous staining along the membrane. When present, the intensely stained cell soma often resembled cell ghosts or debris. The cultures derived from GD 20 fetuses exhibited similar growth patterns and GM1 staining as described for GD 15 fetuses except for magnitude of cellular growth and maturity. In both GD 15 and GD 20 derived cultures, there was clear evidence that the effects on the cellular plasma membrane caused by in utero EtOH exposure were largely reversed by GM1 treatment.

The abnormal staining of surface-exposed GM1 suggests significant changes in ganglioside distribution and content. These changes in ganglioside composition could profoundly affect various surface events and subsequently could affect developmental, growth, and maturation processes. The correct developmental profile of gangliosides may be imperative for normal CNS development.

One of the mechanisms of the neuroprotective effect of GM1 may be to maintain the normal profile of CNS gangliosides by inhibition of EtOH-induced increase in brain sialidase activity, which is responsible for abnormal accumulation of GM1. Alternatively, the GM1 may be incorporated into cell membrane, which thereby would stabilize the leaky membrane produced by EtOH. Gangliosides also are known to modulate the action of nerve growth factors, and thus their synergistic action may lead to neuroprotective effects. These in vivo and in vitro studies indicate that ganglioside GM1 can enhance maturation of CNS and, thus, therapeutic intervention with GM1 at critical periods during gestation before an EtOH insult may reduce/repair cellular damage and prevent neurobehavioral abnormalities.

Prenatal Alcohol and Serotonin

Recently, it was reported that a mild form of neural tube defect, incomplete neural tube closure (iNTC), occurred in C57BL/6J mouse fetuses when pregnant dams were under the influence of alcohol from embryonic age (E) 8 to E 14 (Zhou and Zhang, 2000). The iNTC at E 15 occurred intermittently along the neural axis with missing floor and/or roof plates and compromised midline structures. It also was accompanied by smaller brain weight and enlarged ventricles. Among the midline structures, the raphe is the home of serotonin (5-HT) neurons, which are known to be critical in maintaining healthy mental function. It has been reported that in the offspring of dams treated with alcohol, a subpopulation of cells in the raphe failed to develop into 5-HT neurons, or developed into 5-HT neurons but failed to migrate from their birth site. They also had fewer nerve

fibers and smaller cell bodies at E 15. The alcohol-exposed groups also had fewer 5-HT neurons than their pair-fed and chow-fed controls.

In this symposium, Dr. Zhou reported that alcohol exposure during E 8 to E 14 impedes the development of the efferent fiber projections of the 5-HT neurons along their trajectory through the midbrain to the forebrain primordium. C57BL/6J dams were divided into three groups. Beginning on E 8, one group received EtOH via a liquid diet that provided 25% EtOH-derived calories as the sole source of nutrients. The second group was pair-fed the liquid diet with maltose-dextrin isocalorically substituted for EtOH. The third group was fed ad libitum mouse chow. Dams were sacrificed on E 15, and fetal brains were processed for immunocytochemical staining of 5-HT. The immunostaining fibers, along with the brain regions, then were quantified.

At E 15, the 5-HT fibers that emerge from cell bodies in the raphe first gather and form a solid bundle within a large nerve cable, the medial forebrain bundle (MFB). The ascending 5-HT fibers use the MFB as a throughway to reach most of the forebrain. In fetuses from the alcohol-exposed group, the MFB was reduced not only in number of 5-HT fiber density but also in diameter. No difference was found between the controls. Among the first group of 5-HT fiber projections to the forebrain by E 15 is the septal nucleus. The density of 5-HT-immunostaining fibers in the septal nucleus was lower and the size of the septal nucleus was smaller in alcohol-exposed subjects compared with controls. At E 15, the 5-HT fibers reach as rostrally as far as the prefrontal cortex, and a dual entry to the frontal cortex is evident in normal development. In the alcohol-exposed embryonic brain, the dual entry apparently is altered compared with controls.

5-HT neurons are known to innervate and communicate with almost the entire population of neurons in the brain. The MFB is the sole nerve bundle that carries all ascending 5-HT fibers that innervate the forebrain. The reduction in size of the MFB, as well as the number of 5-HT axons in MFB, predicts less 5-HT innervation in the forebrain. The observation of 5-HT fibers in the adjacent rostral structure and septal nucleus agrees with our hypothesis about reduction of 5-HT innervation in forebrain.

What happens to the compromised 5-HT fibers in the major trajectory pathway during early development? Can an adult offspring actually have less 5-HT innervation in the brain as a consequence of fetal alcohol exposure? If so, it is plausible that fetal alcohol-exposed babies are prone to functional disorders related to deficient 5-HT in the brain. In another spectrum, during development, 5-HT repeatedly has been implicated in the regulation of functions as diverse as cell division, differentiation, migration, growth, and synaptogenesis (Lauder, 1990; Whitaker-Azmitia et al., 1996). In rodent cerebral cortex, 5-HT recently was shown to play a role in the development of the primary somatosensory map (Cases et al., 1996; Osterheld-Haas and Hor-

ning, 1996). Not only does a less developed 5-HT system possibly delay or hinder normal cortical development, but if persistent to adulthood, it has the potential of predisposing an individual to psychiatric disorders. Thus, the defected serotonin system may have a profound cascaded consequence on normal development.

SIGNIFICANCE

The work presented at this symposium demonstrated the adverse consequences that prenatal exposure to alcohol can have on the developing embryo and fetus. The findings also showed that there is hope for effective interventions. Drs. Thomas and Riley provided evidence that NMDA receptor-mediated excitotoxicity during periods of withdrawal is one mechanism by which EtOH alters behavioral development. They suggested that withdrawal from alcohol may be particularly damaging and suggest that abrupt alcohol withdrawal should be avoided both by women who drink during pregnancy and by newborns who undergo withdrawal.

The work presented by Dr. Goodlett has a long history, and clinically these data have important implications. First, severe cerebellar structural damage may be linked to alcohol abuse that extends into and beyond fetal week 24. Second, even limited episodes of heavy binge drinking during fetal weeks 26 to 32 may constitute a risk for permanent cerebellar damage. Third, deficits in coordinated motor performance and motor learning such as eye-blink classical conditioning are likely to accompany severe prenatal alcohol-induced damage to cerebellar circuits, which in turn may depend on exposure during the critical period in the early third trimester. Fourth, intensive rehabilitation programs that involve active complex motor learning may stimulate synaptic morphological plasticity and help ameliorate at least some of the motor performance and coordination deficits in alcohol-affected children.

Dr. Hungund was among the first to demonstrate that one could intervene and mitigate the effects of in utero EtOH exposure. His work demonstrates that prenatal EtOH exposure, even for a short duration during critical gestational periods, leads to behavioral deficits, altered ganglioside composition, abnormal cell morphology, and delayed maturation. Ganglioside GM1 pretreatment reduces these EtOH-induced anomalies probably through its action on ganglioside synthesizing and catabolizing enzymes, and thus ganglioside GM1 may have a therapeutic potential in treatment of a variety of illnesses caused by alcohol abuse, which include fetal alcohol syndrome.

Finally, the new data presented by Dr. Zhou are intriguing. Mice that consumed alcohol in early gestation had offspring with 5-HT neuron deformation and also reduced 5-HT nerves in the major 5-HT nerve cable that projects to forebrain. This abnormality has two potential consequences. It has been reported that the serotonin neurons send 5-HT to signal the growth of brain cortex. Lessening

of this signal has been found to affect the formation of forebrain. Reduced 5-HT in the brain also is known to contribute to affective (e.g., depression, anxiety), sleeping, and eating disorders. If the shortage of 5-HT or 5-HT neurons in the embryonic stage continues in the adult brain, it may contribute to the hyposerotonin syndromes and related mental deficits.

Certainly, the importance of developing interventions, both during pregnancy and for the affected offspring, cannot be overstated. We hope that the work presented here, and similar ongoing work, will open new avenues of intervention and treatment.

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