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Long-Term Effects of Fetal Ethanol Exposure on Pituitary-Adrenal Response to Stress[1]

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TAYLOR, A. N., B. J. BRANCH, S. H. LIU AND N. KOKKA. *Long-term effects of fetal ethanol exposure on pituitary-adrenal response to stress.* PHARMAC. BIOCHEM. BEHAV. **16**(4) 585–589, 1982.—Pregnant female rats were fed either a 5.0–5.5% w/v ethanol-containing liquid diet ad lib or pair-fed the isocaloric control diet during gestation weeks 2 and 3. At 75–105 days of age, female offspring of the ethanol-treated dams showed significantly greater corticosterone responses than pair-fed- or normally-derived offspring to the stress of cardiac puncture or of noise and shaking, while pituitary-adrenal responses to exposure to a novel environment, cold or 2–3 days of fasting were normal. Adrenal sensitivity to ACTH in dexamethasone-suppressed adult offspring was unaffected by the prenatal treatment. The results demonstrate that fetal ethanol exposure enhances adult pituitary-adrenal responses to certain stressors, including alcohol as demonstrated previously, and suggest that the long-term effects may be mediated by developmental actions of alcohol on central neural mechanisms involved in the regulation of this neuroendocrine system.

Ethanol Prenatal alcohol Fetal alcohol Rats Stress Hypothalamic-pituitary-adrenal system Corticosterone

MATERNAL ethanol consumption has long-lasting effects on responsiveness of offspring to acute challenges with ethanol as adults. Depending upon method of ethanol administration to pregnant rats, blood ethanol levels attained and/or strain of rat, their offspring have been found to be either more sensitive [22] or more tolerant [1] to the hypothermic effects of ethanol as adults. Fetal ethanol-exposed adult rats which show enhanced sensitivity to ethanol-induced hypothermia are also more sensitive to ethanol-induced activation of the hypothalamic-pituitary-adrenal (HPA) system than pair-fed control offspring [22].

Prenatal factors, such as maternal stress, alterations in HPA function or malnutrition during pregnancy, are known to exert long-lasting effects on HPA function of offspring as adults [13, 16, 26]. Ethanol is a stressor which activates the HPA axis in adult organisms [6, 15, 17]. Maternal ethanol consumption affects HPA function in the newborn rat [12,20], and if the ethanol-containing liquid diet is not supplemented with protein, malnutrition, as indicated by reduced body weights of newborn pups, may occur [22,27]. Thus, prenatal exposure to ethanol may likewise be a factor which exerts long-lasting effects on HPA function of adult offspring. Since our previous studies indicated that maternal ethanol consumption affects ethanol-induced HPA activity in adult offspring [22], the present studies were undertaken to determine whether the hypersensitivity of the HPA system is specific to alcohol or if there is a generalized increase in responsiveness to other stimuli. Thus a variety of stimuli were selected, each thought to have different afferent inputs to the central nervous system (CNS), but having in common the hypothalamus as a nodal point. A preliminary report has appeared [21].

METHOD

Four shipments of timed-pregnant nulliparous Sprague-Dawley rats (40/shipment) were received from Charles River Breeding Laboratory (Wilmington, MA) when the rats were 6 days pregnant. On day-8 of gestation, the dams were randomly divided into three weight-matched groups, each to be fed a different diet: (a) In each of 3 shipments, 10 rats were fed a liquid diet (Bio-Serv Inc., Frenchtown, NJ) containing 5.0% w/v ethanol supplemented with casein [27], ad lib, and in one shipment 10 rats were fed the casein-supplemented diet containing 5.5% w/v ethanol, ad lib; (b) In each shipment 8–10 rats received the casein-supplemented liquid diet without ethanol, but containing instead an isocaloric amount of maltose-dextrin, pair-fed to the volume consumed by the ethanol dams; and (c) In each shipment the remaining dams received normal rat chow and water diet, ad lib. All pregnant

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dams were housed individually under constant environmental conditions (lights on 0300 hr, off at 1700 hr, 22±1°C). Fresh diet was presented at 0900 hr daily from day-8 of gestation to parturition. Dams were weighed daily and the volume of diet consumed was recorded daily.

On the day of birth, pups were culled and randomized within each diet group and cross-fostered in litters of 5 males and 5 females each to normal (chow-fed) dams which had given birth within the same 24-hr period. The pups were weighed at weekly intervals until weaning on day-21, when pups were group-housed by sex under the environmental conditions described above. As adults, female offspring were tested for their pituitary-adrenal responses to various stressors described below. Only females were studied because of their greater pituitary-adrenal responsiveness [13]. Postnatal body weights of similarly treated pups have been reported [22].

Pituitary-Adrenal Response to Stress

Between 0900 and 1200 hr, blood samples (0.5 ml) were drawn by cardiac puncture from 75–100 day-old female rats. Each experiment utilized randomly selected subjects each representing separate litters of ethanol-derived, appropriate pairfed-derived or normal rats. Different animals were used for each stress.

1. Cardiac puncture. A basal blood sample was obtained by cardiac puncture from manually restrained rats within 2 min after removal of the animal from its cage. Thirty min later a second sample was taken to reflect the stress of the first sample. Fetal ethanol-derived subjects were the offspring of dams fed the 5.0% w/v ethanol-containing diet.

2. Noise and shake. Metal cages housing rats individually were shaken for 30 sec every 2 min for 30 min, at which time blood samples were drawn. Ethanol-derived subjects were the offspring of dams fed the 5.5% w/v ethanol diet.

3. Cold. Rats in individual metal cages were placed in a cold room (4°C) for 60 min, at which time blood samples were drawn. Ethanol-derived subjects were the offspring of dams fed the 5.5% w/v ethanol diet.

4. Novel environment. Rats were removed from their metal home cages, placed in a plastic cage (17 × 8.5 × 8 cm) for 2 min and returned to the home cage. Blood samples were drawn at 10, 20, 30 and 60 min after moving the rats to the new cage. Different animals were sampled at each time-point. Ethanol-derived subjects were the offspring of dams fed the 5.0% w/v ethanol diet.

5. Fasting. Rats were deprived of food for 72 hr. Blood samples were drawn at 48 and 72 hr after onset of fasting and at 24 hr after refeeding. Different animals were sampled at each time-point. Ethanol-derived subjects were the offspring of dams fed the 5.0% w/v ethanol diet.

Blood samples were centrifuged and the plasma frozen for subsequent fluorometric analysis of its corticosterone content [8].

Corticosterone Response to ACTH

In order to assess adrenal sensitivity, 75–105 day-old rats that had not been used for other experiments, were injected with dexamethasone (Elkins-Sinn, Inc., 400 μg/kg bwt, SC) followed 3 hr later by saline or ACTH (Parke-Davis, 0.2 or 2.0 U/100 g bwt, IP). Blood samples were drawn by cardiac puncture 30 min after the saline or ACTH injections for corticosterone analysis, as described above. Ethanol-derived subjects were the offspring of dams fed the 5.0% w/v ethanol-containing diet.

Statistical Analyses

Mean plasma corticosterone levels were subjected to analysis of variance (ANOVA) prior to *t*-test analysis. All nominal significance levels were corrected for the number of multiple comparisons performed [9]; p values <0.05 were considered significant.

Blood Ethanol Levels

Blood ethanol levels were monitored by a modification of the gas chromatographic method of Jain [10]. Estimates of maternal blood ethanol levels were made on samples of tail vein blood obtained at 0900 and 2000 hr on day-18 of gestation. The offspring of these dams were not used for any further studies.

RESULTS

Maternal daily consumption of the 5.0% w/v ethanol diet supplemented with casein and the resulting blood ethanol levels were similar to those we reported previously [22]. Maternal consumption of the 5.5% w/v ethanol-containing casein-supplemented diet, as with the 5.0% diet, averaged 12.0 g/kg/day over the 2-wk period. After 11 days on the 5.5% diet (gestation day-18), maternal blood ethanol levels were similar to those attained with the 5.0% diet i.e., 48.5±15.0 (SEM) mg/100 ml (N=4) at 0900 hr and 129.2±18.6 mg/100 ml (N=5) at 2000 hr. Maternal consumption of the 5.5% diet did not affect litter size or body weights of pups at birth, which were similar to those reported with the 5.0% casein-supplemented diet [22], i.e., average litter size, 10 pups/litter, and average pup weight, 6.9 g/pup. Since all these measures were similar for the 5.5 and 5.0% diets, results from experiments with the two diets are considered together in this report. All pups grew normally, as described previously [22] and at the time of testing as adults, body weights of all groups were similar.

Basal, non-treatment corticosterone levels were similar in adult female offspring of ethanol-treated (E), pair-fed (P) or normal (N) dams (Fig. 1). These data have been reported previously [22]. The corticosterone responses at 30 min after cardiac puncture differed among the groups, $F(2,17)=9.82$, $p<0.01$. Figure 1 shows that the plasma corticosterone levels 30 min after cardiac puncture were significantly higher in E than in P rats, $t=4.24$, $p=0.002$. P and N levels were similar.

Corticosterone responses to noise and shake stress differed among the groups, $F(2,19)=10.24$, $p<0.01$. Figure 1 indicates that corticosterone levels after 30 min of intermittent noise and shaking of the cages were significantly higher in E than in P rats, $t=4.526$, $p=0.001$. P and N levels did not differ.

Corticosterone responses to cold stress did not differ among the groups. Following 60 min of exposure to cold, corticosterone levels were similarly elevated in E, P and N rats (Fig. 1).

E, P and N rats all responded maximally at 20 min after brief placement in a novel environment, and there were no differences in corticosterone levels between any of the groups at this time (Fig. 2). Similarly, there were no differences in corticosterone levels at 10, 30 and 60 min after stress onset in E, P or N rats (Fig. 2).

Plasma corticosterone levels tended to be higher after 3

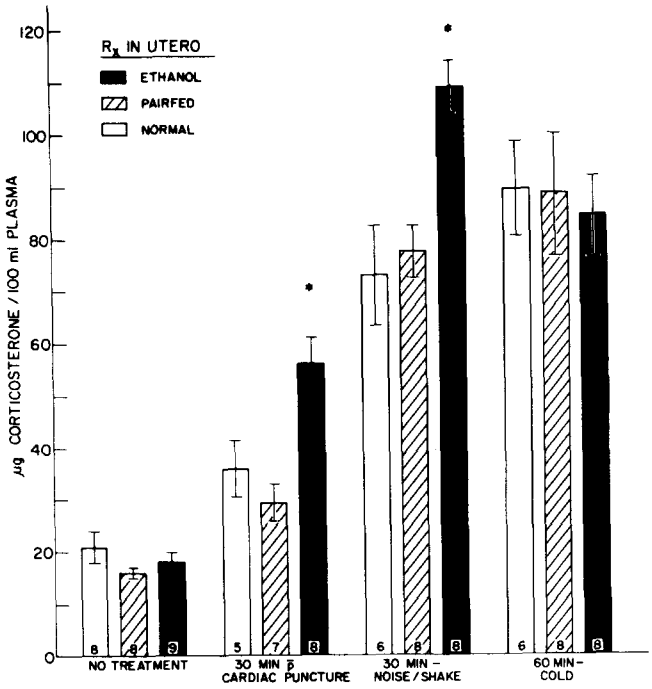

FIG. 1. Basal, nontreatment plasma corticosterone levels and corticosterone responses to various stressors of 75–105 day old female offspring of dams fed a 5.0–5.5% w/v ethanol-containing liquid diet or pair-fed the isocaloric control diet during weeks 2 and 3 of gestation or normally fed (lab chow and water ad lib). The histograms indicate mean corticosterone levels±SEM in blood samples obtained in untreated animals (reported previously, [22]) and 30 min after cardiac puncture or after 30-min of intermittent noise and shaking or after 60-min of cold exposure. The number of animals studied is given at the bottom of each histogram. The asterisks indicate significant differences between ethanol and pairfed groups ($p \leq 0.002$).

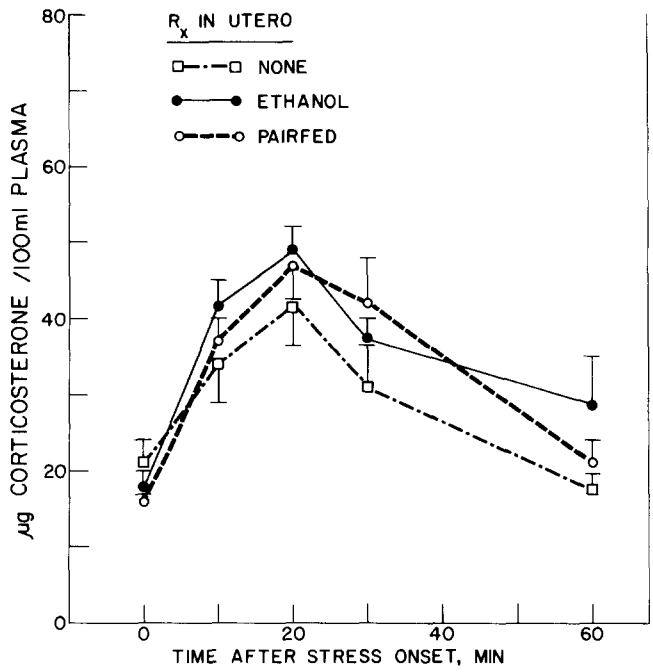

FIG. 2. Plasma corticosterone levels before and at various times after onset of 2-min exposure to a novel environment in adult female offspring of dams fed a 5.0% w/v ethanol-containing liquid diet or pair-fed the isocaloric control diet during weeks 2 and 3 of gestation or normally fed (lab chow and water ad lib). The vertical bars indicate±SEM. Seven to ten animals from each group were sampled at each time point. The 0-time data are the same as the no treatment data of Fig. 1.

days than after 2 days of food deprivation in P and N rats, while steroid levels in E rats appeared to peak at 2 days after fasting onset and did not increase further on the 3rd day (Fig. 3). Nevertheless, there were no significant differences between corticosterone levels in E, P or N rats at 2 or 3 days. Corticosterone levels declined to baseline after 24 hr of refeeding in all animals and there were no differences between the groups (Fig. 3).

Plasma cortiocosterone levels were similarly suppressed in E, P and N rats by the one dose of dexamethasone tested (Table 1). ACTH produced similar dose-dependent elevations in plasma corticosterone in all 3 groups (Table 1).

TABLE 1

PLASMA CORTICOSTERONE RESPONSES TO ACTH IN DEXAMETHASONE-TREATED (400 μg/kg), ADULT, FEMALE OFFSPRING OF DAMS FED ETHANOL-CONTAINING, PAIR-FED AND NORMAL DIETS DURING GESTATION

	Plasma Corticosterone, μg/100 ml		
	Dose of ACTH, units/100 g bodyweight		
R_x In Utero	0	0.2	2.0
Ethanol	7.0 ± 1.2 (7)*	19.1 ± 3.3 (7)	67.2 ± 3.4 (6)
Pair-fed	6.0 ± 0.8 (8)	22.5 ± 2.6 (8)	66.4 ± 3.6 (6)
Normal	7.1 ± 1.2 (8)	23.6 ± 4.8 (7)	61.0 ± 5.3 (6)

*Values are means ± SEM; number of animals is in parentheses.

DISCUSSION

The present experiments demonstrate that HPA activation induced by certain stressors in adult rats is enhanced by fetal exposure to ethanol. Thus the hypersensitivity of the HPA system to challenge doses of ethanol demonstrated by fetal ethanol-exposed adult females [22] is not specific to alcohol, but appears to represent a generalized increase in HPA responsiveness to stress. A confirmatory report demonstrating enhanced stress-induced corticoid responses in pubertal female fetal ethanol-exposed offspring has recently appeared [24].

While other aspects of adult HPA function, i.e., basal and circadian variations, are unaffected by the prenatal treatment [20,22], our findings indicate that stress-induced HPA activity shows enhanced responsiveness in a rather selective manner in fetal ethanol-exposed adult rats. Thus, significant differences were observed with 2 of the 5 stimuli tested.

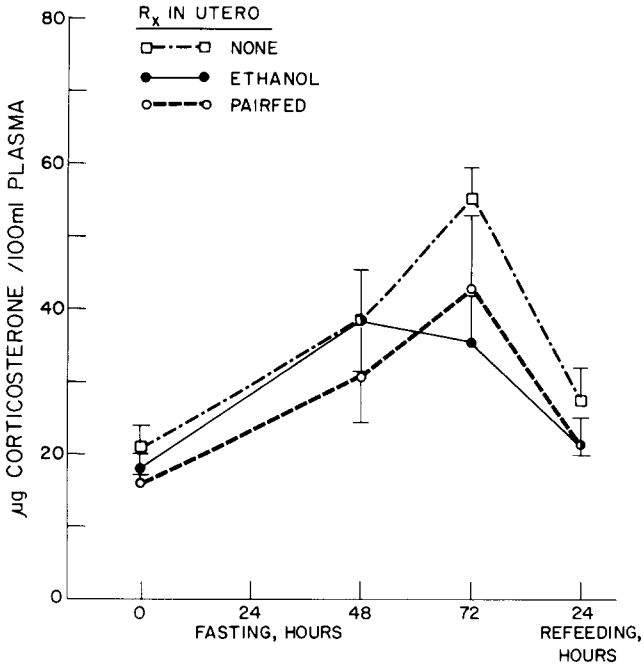

FIG. 3. Plasma corticosterone levels before, at 48 and 72 hr of food deprivation and at 24 hr after refeeding of adult female offspring of dams fed a 5.0% w/v ethanol-containing liquid diet or pair-fed the isocaloric control diet during weeks 2 and 3 of gestation or normally fed (lab chow and water ad lib). The vertical bars indicate±SEM. Seven to nine animals from each group were sampled at each time point. The 0-hr data are the same as the no treatment data of Fig. 1.

namely cardiac puncture and noise and shake. In considering the nature of these stressors, the main components of cardiac puncture are restraint, pain and fear, whereas noise and shake has primarily only the last component. While novelty stress may be considered to induce anxiety more than fear, its failure to differentiate between fetal ethanol-exposed and control animals may be due to insufficient intensity and/or duration of this stressor. In contrast to the enhanced HPA activity induced by sensory/emotional stimuli and alcohol [22], corticosterone responses to environmental or metabolic stimuli, such as cold or fasting, remain essentially unaffected in fetal ethanol-exposed adult rats. Since the latter stimuli were of longer duration than the others, it is also possible that HPA hyperresponsiveness in fetal ethanol-exposed adult rats may be induced by acute rather than chronic stressors.

In order to determine whether altered adrenal sensitivity may explain the augmented corticosterone responses in the fetal ethanol-exposed rats, adrenal activation by ACTH was examined in steroid-suppressed rats. The results indicate that the enhanced HPA response is not due to altered adrenal sensitivity to ACTH. It, therefore, appears that maternal ethanol consumption exerts long-term effects on the hypothalamus and/or pituitary or on central processes which mediate HPA activation in adult offspring. Although dexamethasone suppressed HPA function to a similar extent in prenatally treated and control rats, the possibility of altered set-points for steroid feedback sensitivity is not excluded by these experiments since only one dose of the steroid was examined.

Two perinatal experimental conditions have been described which produce augmented stress-induced HPA responses in adult offspring, i.e., malnutrition [26] and maternal adrenalectomy [14,23]. Although neither duplicates our experimental conditions, there may be some commonalities. The pair-feeding procedure controls for diet composition and quantity of maternal food consumption, and in all cases stress responsiveness was similar for pair-fed-and normally-derived adults. Nevertheless, effects of alcohol ingestion on maternal nutrient absorption cannot be excluded. Maternal ethanol consumption, like maternal adrenalectomy [14,23], stimulates pituitary-adrenal activity in the fetus, as evidenced by enlarged adrenals and elevated brain and plasma corticosterone levels at birth [12,20]. However, unlike maternal adrenalectomy, maternal ethanol consumption would be expected to augment maternal HPA activity, assuming that pregnant rodents respond like nonpregnant animals to chronic ethanol consumption [6, 11, 19]. If the latter factor were acting alone, fetal HPA function should be suppressed, as others have observed when maternal ACTH and steroid levels are elevated; and offspring of such dams show suppressed HPA responses to stress as adults [16]. Thus, it appears that maternal ethanol consumption may produce long-term effects on HPA function in offspring by combined actions on the HPA systems of mother and fetus. Further studies are needed to determine whether the long-term effects of fetal alcohol exposure are indeed due to elevated corticosterone levels in the fetus or neonate.

The augmented stress-induced corticosterone responses appear to result from the effects of fetal ethanol exposure on the development of neural mechanisms that regulate HPA function. Fetal ethanol exposure has been shown to exert long-term morphological alterations in hippocampus [2,25], a limbic structure which inhibits HPA activity [3,5]. In this connection, it is interesting that others have observed a lack of inhibition of behavioral responses in fetal ethanol-exposed adult rats [18]. Likewise, long-term effects of fetal ethanol exposure have been observed on levels of brain catecholamines [4], in particular, norepinephrine which has been proposed as an inhibitory neurotransmitter for the HPA system [7]. These and other yet to be defined CNS mechanisms may mediate the long-term effects of fetal ethanol exposure on stress-induced HPA activity.

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