

Fetal alcohol exposure attenuates interleukin-1 β -induced fever: neuroimmune mechanisms

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Abstract

Central mechanisms for the attenuating effects of fetal alcohol exposure (FAE) on interleukin-1 β (IL-1)-induced fever were studied in adult male offspring of dams fed a liquid diet supplemented with ethanol (E), in pair-fed (P) control and in normal (N) offspring. Hypothalamic levels of IL-1 were significantly lower in E than in N rats at 2 h, but not at 4 and 6 h, after intraperitoneal administration of lipopolysaccharide. Fever induced by intracerebroventricular (icv) IL-1 was significantly lower in E than in N and P rats. In contrast, E rats showed a normal febrile response to icv prostaglandin-E₂. Thus, whereas FAE does not affect central thermoregulatory mechanisms, per se, FAE alters the kinetics of hypothalamic IL-1 production/appearance and decreases the responsiveness of central mechanisms which mediate the febrile response to IL-1. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Fetal alcohol exposure (FAE) is associated with profound alterations in the development and function of the immune system. Children exposed to alcohol in utero exhibit serious impairments in both cellular and humoral immunity (Gottesfeld and Abel, 1991; Chiappelli and Taylor, 1995) which may be responsible for the increased morbidity from infections and increased incidence of malignancies associated with the fetal alcohol syndrome (FAS). Deleterious effects on cellular immunity have also been described in animal models of FAE (reviewed in Chiappelli and Taylor, 1995).

During infection, the initial host defense response is elicited by bacterial endotoxin or other pathogen products which stimulate macrophages and monocytes to release endogenous pyrogens, such as the cytokines interleukin-1 β (IL-1), tumor necrosis factor- α (TNF α), and interleukin-6

(IL-6), into the circulation (Kluger, 1991; Rothwell et al., 1996). These cytokines provide a sensory-like signal to the nervous system, alerting it to pathogen-induced immune activation (Schobitz et al., 1994). Cytokines, in general, and IL-1 in particular, affect neural, neuroendocrine and behavioral functions via specific receptors located throughout the central nervous system (Hopkins and Rothwell, 1995; Rothwell et al., 1996; Maier and Watkins, 1998). The interaction of IL-1 with its receptors, particularly within the hypothalamus, produces an increase in body temperature by raising the thermoregulatory set point (Kluger, 1991; Blatteis and Sehic, 1998). During infections, IL-1 also elicits a complex set of behavioral patterns which promote conservation of heat and body resources, while inhibiting goal-directed behavior. This behavioral syndrome, which has been termed “sickness behavior” (Hart, 1988; Kent et al., 1992), includes retardation of motor activity (Otterness et al., 1988; Avitsur et al., 1995), increased slow wave sleep (Krueger et al., 1984), and marked suppression of ingestion (Mrosovsky et al., 1989), exploration (Dunn et al., 1991), social activity (Crestini et al., 1991), and sexual behavior (Yirmiya et al., 1995).

We have demonstrated that FAE attenuates the febrile response to systemic administration of endotoxin, i.e.,

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lipopolysaccharide (LPS) (Yirmiya et al., 1993), and the febrile and anorectic responses to IL-1 (Yirmiya et al., 1996). FAE is associated with age-related biphasic effects on stress-induced hypothalamo-pituitary–adrenal (HPA) function (Taylor et al., 1982, 1986; Weinberg, 1992) and recent reports indicate that in response to various immune challenges, FAE attenuated the secretion of ACTH in weanling rats and enhanced the release of ACTH in young adult rats (Lee and Rivier, 1993, 1994, 1996). Given that glucocorticoids are potent inhibitors of many actions of LPS and IL-1, including their effects on thermogenesis, body temperature and behavior (Strijbos et al., 1993; Johnson et al., 1996), FAE-induced alterations in HPA activation may be involved in modulating the effects of LPS and IL-1 on body temperature and sickness behavior.

The blunting effect of FAE on the febrile response following ip administration of LPS or IL-1 may reflect either altered central and peripheral levels of pyrogenic or cryogenic cytokines and/or decreased responsiveness of central mechanisms which mediate the febrile response. In order to distinguish between these alternatives three experiments were performed. In the first, we assessed the effects of LPS on levels of IL-1 and related cytokines, as well as ACTH and corticosterone, in the hypothalamus and blood of fetal alcohol-exposed and control rats. In the second, IL-1 was administered intracerebroventricularly (icv) in order to assess the responsiveness of the central elements mediating fever and sickness behavior. In the third experiment, we administered prostaglandin- E_2 (PGE $_2$), a known pyrogen (Scammell et al., 1996; Sehic and Blatteis, 1996), icv in order to determine the functional status of thermoregulatory effector mechanisms in fetal alcohol-exposed rats.

2. Materials and methods

2.1. Subjects

Subjects were adult male offspring of timed-pregnant nulliparous Sprague–Dawley rats, which were received from Charles River Breeding Laboratory (Portage, MI) when the dams were 6 days pregnant. Commencing on day 8 of gestation, the dams were fed one of three diets: an alcohol-containing liquid diet (No. 1265, Bio-Serv, Frenchtown, NY; 5% w/v ethanol, 35% ethanol-derived calories) ad libitum; the liquid diet containing an isocaloric amount of maltose-dextrin in place of ethanol (No. 1264), pair-fed (P) by volume to the corresponding ethanol (E) dam; or normal (N) rat chow and water ad libitum. All pregnant dams were individually housed under constant environmental conditions (lights on at 0400 h, off at 1800 h, $22 \pm 1^\circ\text{C}$). On day 21 of gestation, all dams were replaced on normal rat chow and water. Within 24 h of birth, pups were pooled and randomly distributed in litters of 10 among the nursing dams of each prenatal diet group. We

have shown previously (Taylor et al., 1981) that with this procedure, intake of ethanol averages $12.0 \text{ g kg}^{-1} \text{ day}^{-1}$ over the 2-week period and blood alcohol levels in the dams average 80 mg/100 ml at 0900 h and 127 mg/100 ml at 2000 h on day 18 of gestation. Similar paradigms of ethanol administration during the last 2 weeks, or even the last week, of gestation in rat dams have been demonstrated to produce profound, and remarkably consistent, effects on postnatal neuroendocrine and immune function in the offspring (Weinberg and Gallo, 1982; Weinberg and Jerrells, 1991; Taylor et al., 1982; Redei et al., 1989; Lee et al., 1990; Norman et al., 1991).

Pups were weighed weekly until weaning at postnatal day 21 when, in order to avoid litter effects, pups from all dams within each prenatal treatment group were pooled and randomized prior to housing five per cage under the conditions described above. All experimental procedures were approved by the UCLA and VA institutional animal care and use committees.

2.2. Experiments and procedures

2.2.1. Effect of LPS on hypothalamic and blood levels of IL-1, TNF α , IL-6, and IL-10 and on ACTH and corticosterone

Sixty-day-old male E, P and N rats were injected between 0800 and 1200 h with LPS (500 $\mu\text{g/kg}$, ip) or saline ($n = 4\text{--}11/\text{group}/\text{injection}$). At 2, 4 or 6 h postinjection, rats were decapitated and brains and blood were collected. In a preliminary study (data not shown) we determined that this dose produced optimal effects on hypothalamic IL-1 levels at these time points. Brains were removed, placed on ice, and a hypothalamic block (extending from optic chiasm anteriorly to mamillary bodies posteriorly, between the hypothalamic sulci bilaterally and to the third ventricle dorsally) was dissected and placed in 500 μl cold RPMI 1640. After transfer to 500 μl cold RPMI 1640 containing 100 KIU Aprotinin, hypothalami were homogenized and centrifuged at 10,000 rpm for 15 min at 4°C . Supernatants were collected and stored at -70°C until assayed for IL-1 β and TNF α by ELISA. Aliquots of blood samples were centrifuged at 2000 rpm for 15 min at 4°C and serum was stored at -70°C until assayed by ELISA for IL-1 β , TNF α , IL-6 and IL-10. A second blood aliquot was collected in EDTA-containing vacutainer tubes to which Aprotinin (200 KIU/ml) was added and plasma was processed and stored as above until assayed by RIA for ACTH and corticosterone.

IL-1 levels of brain and serum were determined using a rat IL-1 β ELISA kit (R&D Systems, Minneapolis, MN). The reported detection limit of this assay is 5 pg/ml with a recovery of exogenous IL-1 β of 100% and intra- and interassay coefficients of variation of 3.9% and 4.4%, respectively. TNF α levels of brain and serum were assessed with a rat TNF α ELISA kit (BioSource International, Camarillo, CA). The reported detection limit of this

assay is 4 pg/ml, the recovery of exogenous TNF α is 100%, and intra- and interassay coefficients of variation are lower than 2.7% and 3.9%, respectively. IL-6 was assessed with a rat IL-6 ELISA kit (BioSource International, Camarillo, CA). The reported detection limit of this assay is 8 pg/ml, the recovery of exogenous IL-6 is 96%, and intra- and interassay coefficients of variation are lower than 4.9% and 9.9%, respectively. IL-10 was assessed with a rat IL-10 ELISA kit (BioSource International, Camarillo, CA). The reported detection limit of this assay is less than 5 pg/ml, the recovery of exogenous IL-10 is 95%, and intra- and interassay coefficients of variation are lower than 7.5% and 9.3%, respectively. The results are expressed as pg/hypothalamus and as ng/ml serum.

Plasma corticosterone levels were assessed with a rat corticosterone ¹²⁵I RIA kit (ICN Biomedicals, Costa Mesa, CA). The reported detection limit of this assay is 8 ng/ml, the recovery of exogenous corticosterone is 100%, and intra- and interassay coefficients of variation are lower than 10.3% and 7.2%, respectively. The results are expressed as ng/ml plasma.

Plasma ACTH levels were assessed with a human ACTH ¹²⁵I RIA kit (Inctar, Stillwater, MN). The reported detection limit of this assay is 15 pg/ml, the recovery of exogenous ACTH is greater than 78%, and intra- and interassay coefficients of variation are lower than 12.5% and 6.7%, respectively. The results are expressed as pg/ml plasma.

2.2.2. Effect of icv administration of IL-1 on the febrile response, locomotor activity and food / water consumption

At approximately 60 days of age, E, P and N rats were anesthetized (50 mg/kg sodium pentobarbital) and stereotaxically implanted (1.0 mm posterior to bregma, 1.5 mm lateral to the midline) with a 26-gauge guide tube (Plastics One, Roanoke, VA) lowered 4 mm below the skull surface to position the tip 1 mm above the lateral ventricle (Paxinos and Watson, 1986). The guide cannula was secured to the skull with stainless steel screws and dental cement. A 33-gauge wire attached to a cap was used to seal the guide tube. At the end of this procedure, a battery-operated biotelemetric transmitter for continuous recording of body temperature and motor activity (Mini Mitter, Sunriver, OR) was implanted in the peritoneal cavity. Following the operation, rats were housed individually. Rats were habituated daily to the icv injection procedure for two weeks when a 33-gauge injection cannula that protruded 1 mm beyond the tip of the guide cannula was inserted into the latter and either saline or IL-1 β (20 ng/rat, dissolved in 10 μ l of saline) was administered at a rate of 10 μ l/min. Recombinant human IL-1 β (sp act of 18,000 units/ μ g) was a gift from Dr. Craig C. Reynolds (Biological Response Modifiers Program, NCI, USA). The dose selected was based on our previous studies which showed that, with this preparation of IL-1 β , 10–20 ng/rat are required to obtain reliable behavioral effects (Avitsur et al., 1997).

Injections occurred between 0800 and 0900 h, and body temperature and activity were monitored continuously for 8 h. Food and water consumption were measured 24 h after the injection. Cannula placement was verified by injection of Trypan blue prior to euthanization.

2.2.3. Effect of icv administration of PGE₂ on the febrile response

At approximately 60 days of age, E, P and N rats were implanted with a cannula for icv injection and a telemetric transmitter for recording body temperature as described above. At 2 weeks later, either saline or PGE₂ (200 ng in 10 μ l of saline) was administered at a rate of 10 μ l/min. Injections occurred between 0800 and 0900 h, and body temperature was monitored continuously for 90 min after the injection. Cannula placement was verified by injection of Trypan blue prior to euthanization.

2.3. Statistical analysis

To compare brain and blood cytokine and hormone content after injection of LPS or saline, the data were analyzed by two-way analyses of variance (ANOVAs) across prenatal treatment groups for the factors of injection (LPS or saline) and time. Subsequently, the LPS-induced brain and blood cytokine and hormone data were analyzed by two-way ANOVA for the factors of prenatal treatment and time, and means were contrasted by *t*-tests with Bonferroni corrections for multiple comparisons.

Body temperature and activity data were recorded at 5-min (Experiment 3) or 10-min (Experiment 2) intervals and the latter were averaged for every 30 min prior to analysis. The data were analyzed by three-way ANOVAs with prenatal treatment and injection as between-subjects factors and the time intervals as repeated measures. In Experiment 2, to examine further the febrile response in each prenatal group, for each IL-1 injected animal, we computed the difference in its body temperature from the mean body temperature of the corresponding saline-injected group, at each time point. Planned comparisons were used to test for specific differences among the IL-1-injected E, P and N groups. The effects on food and water consumption were analyzed by two-way ANOVAs, with prenatal group and injections as between-subjects factors, followed by post-hoc *t*-tests with Bonferroni corrections for multiple comparisons.

3. Results

3.1. Experiment 1: effect of LPS on hypothalamic and blood levels of IL-1 β , TNF α , IL-6, and IL-10 and on ACTH and corticosterone

Hypothalamic and blood levels of IL-1 β and TNF α and blood levels of IL-1 β , TNF α , IL-6 and IL-10 at were

assessed at 2, 4 and 6 h after injection of LPS (500 $\mu\text{g}/\text{kg}$) (Fig. 1). Across treatment groups and at all time points, brain levels of IL-1 β and TNF α were significantly stimulated by LPS in comparison to the effect of saline injection [$F(1,75) = 22.49$, $p < 0.0001$ and 5.36 , $p < 0.05$, respectively]. Our assays did not permit us to detect any stimulatory effects of LPS on hypothalamic IL-6 or IL-10 (data not shown).

Two-way ANOVA of LPS-induced hypothalamic IL-1 β indicated a significant effect of time [$F(2,59) = 25.38$, $p < 0.0001$] and a significant time \times prenatal treatment interaction [$F(4,59) = 2.57$, $p < 0.05$], reflecting the significantly lower IL-1 β levels in E than in N rats at 2 h ($p < 0.05$). Across all groups, brain levels of IL-1 β were significantly lower at 2 than at 4 or 6 h postinjection ($p < 0.001$).

Hypothalamic levels of TNF α were significantly affected by time [$F(2,59) = 18.15$, $p < 0.0001$]. The interaction of time \times prenatal treatment was marginally significant [$F(4,59) = 2.16$, $p = 0.085$], reflecting significantly lower TNF α levels in P hypothalami at 2 h postinjection than in E and N hypothalami ($p < 0.05$). Across all groups,

TNF α levels were significantly higher at 2 than at 4 or 6 h postinjection ($p < 0.001$).

Serum levels of all cytokines (Fig. 1) were significantly stimulated by LPS across all treatment groups and at all time points in comparison to the effect of saline injection [IL-1 β : $F(1,70) = 15.10$, $p < 0.001$; TNF α : $F(1,70) = 9.22$, $p < 0.01$; IL-6: $F(1,72) = 16.63$, $p = 0.0001$; IL-10: $F(1,70) = 5.71$, $p < 0.05$]. There were no differential effects of prenatal treatment on LPS-induced serum levels of any of the cytokines, however, in each case, there was a significant effect of time [IL-1 β : $F(2,58) = 3.20$, $p < 0.05$; TNF α : $F(2,58) = 35.92$, $p < 0.0001$; IL-6: $F(2,45) = 6.03$, $p < 0.01$; IL-10: $F(2,45) = 9.90$, $p < 0.001$]. Across all groups, serum IL-1 β was significantly lower at 2 than at 4 h postinjection ($p < 0.05$); TNF α and IL-10 were significantly higher at 2 than at 4 or 6 h postinjection ($p < 0.01$); and IL-6 was significantly lower at 6 than at 2 or 4 h postinjection ($p < 0.05$).

Plasma levels of ACTH and CORT were significantly stimulated by LPS across treatment groups and at all time points in comparison to the effect of saline injection [ACTH: $F(1,73) = 4.64$, $p < 0.05$; corticosterone: $F(1,75)$

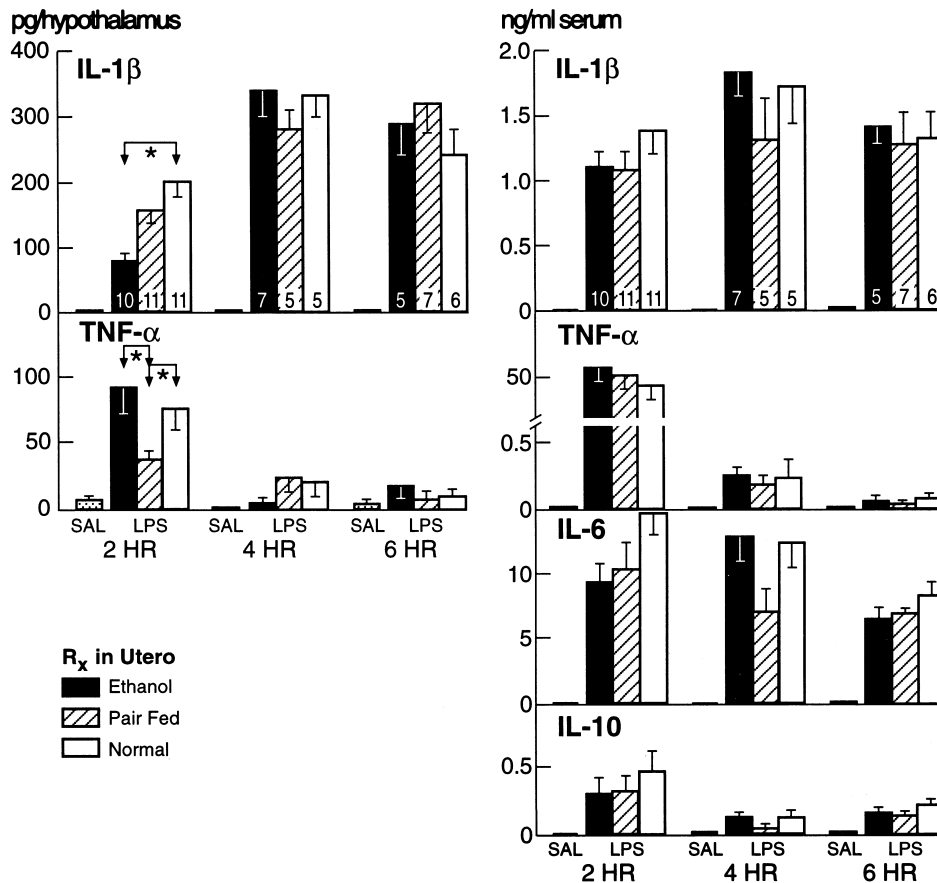


Fig. 1. Effect of fetal alcohol exposure on LPS-induced hypothalamic levels of IL-1 β and TNF α and serum levels of IL-1 β , TNF α , IL-6 and IL-10. Mean (\pm SEM) hypothalamic and serum levels of IL-1 β , TNF α , IL-6 and IL-10, determined by ELISA, in rats exposed prenatally to ethanol (E), pair-feeding (P), or normal (N) diets. Rats within each of the prenatal treatment groups were injected ip with either LPS (500 $\mu\text{g}/\text{kg}$) or saline (SAL) early in the light period, and hypothalami and blood were obtained 2, 4 and 6 h later. The numbers in each LPS histogram indicate the number of animals sampled. SAL histograms represent the combined data from two E and two P animals at each time point.

Table 1

Mean \pm SEM plasma ACTH and corticosterone at 2, 4 and 6 h after injection of LPS (500 μ g/kg, ip) or saline

Prenatal group	Time post injection	2 h	4 h	6 h
<i>ACTH (pg/ml)</i>				
Ethanol	LPS	1131.1 \pm 206.8 (10) ^a	255.7 \pm 94.5 (7)	465.2 \pm 184.1 (5)
Pair-fed	LPS	1023.1 \pm 153.6 (11)	163.4 \pm 19.1 (5)	271.1 \pm 140.7 (7)
Normal	LPS	1441.5 \pm 246.4 (11)	265.7 \pm 72.3 (6)	177.1 \pm 77.0 (6)
All	Sal	320.7 \pm 39.0 (3)	46.3 \pm 12.6 (4)	95.7 \pm 18.5 (3)
<i>Corticosterone (ng/ml)</i>				
Ethanol	LPS	416.2 \pm 26.1 (10)	354.6 \pm 31.3 (7)	296.8 \pm 39.2 (5)
Pair-fed	LPS	410.9 \pm 26.0 (11)	338.4 \pm 48.2 (5)	314.0 \pm 22.8 (7)
Normal	LPS	407.3 \pm 24.1 (11)	294.7 \pm 37.4 (6)	324.3 \pm 34.0 (6)
All	Sal	51.3 \pm 18.7 (4)	65.4 \pm 31.0 (4)	118.0 \pm 46.2 (4)

^aNumber of animals is in parentheses.

= 32.55, $p < 0.0001$] (Table 1). There were no differential effects of prenatal treatment on either hormone, however, in both cases, secretion was significantly affected by time [ACTH: $F(2,59) = 28.11$, $p < 0.0001$; corticosterone: $F(2,59) = 10.01$, $p < 0.001$]. Across all groups, both hormones were significantly higher at 2 than at 4 or 6 h postinjection ($p < 0.01$).

3.2. Experiment 2: effect of icv administration of IL-1 β on the febrile response, locomotor activity and food/water consumption

There were two replications of this experiment, and since both produced similar results, data from the two were combined for the analysis of temperature and activity. Fig. 2 shows the effect of icv administration of IL-1 or saline at 0800–0900 h on body temperature. After an initial injection-induced hyperthermia at 30 min in all groups, the

IL-1-injected animals continued to develop fever while fever subsided in the saline-injected animals. Three-way repeated measures ANOVA of the data at half-hourly intervals during the period between 90 and 480 min postinjection revealed significant effects of prenatal treatment [$F(2,33) = 4.151$, $p < 0.05$] and the injection [$F(1,33) = 80.487$, $p = 0.0001$]. In addition, there was a significant prenatal treatment \times injection \times time interaction [$F(26,429) = 2.087$, $p < 0.01$], reflecting the differential effect of IL-1 in the prenatal groups at the time points of maximal fever. Planned contrasts of the differences in body temperature of the IL-1-injected animals from the mean body temperature of the corresponding saline-injected group showed significant differences between the E- and P-IL-1 groups [$F(1,17) = 4.685$, $p < 0.05$], and between the E- and N-IL-1 groups [$F(1,17) = 13.09$, $p = 0.001$]. The P- and N-IL-1 rats did not differ significantly in their hyperthermic responses [$F(1,17) = 1.70$, $p = 0.20$].

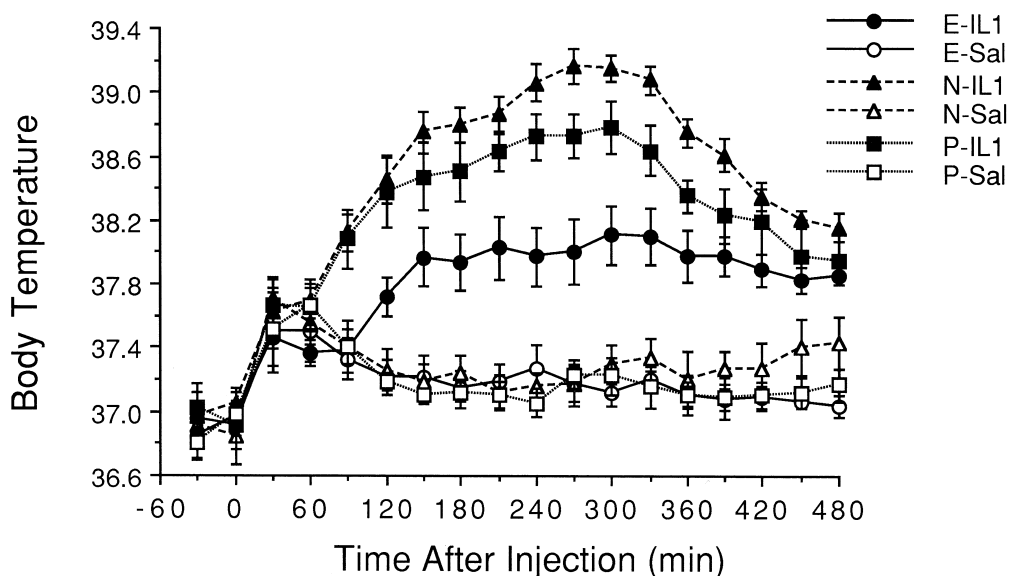


Fig. 2. Effect of fetal alcohol exposure on icv IL-1-induced alteration in body temperature. Mean (\pm SEM) body temperature ($^{\circ}$ C) in rats exposed prenatally to ethanol (E), pair-feeding (P), or normal diets (N). Rats within each of the prenatal treatment groups were injected icv with either IL-1 β (20 ng/rat) or saline (Sal) early in the light period, and body temperature was recorded continuously by a biotelemetric system for 8 h.

Table 2

Mean \pm SEM 24-h food and water consumption and body weight change after icv injection of IL-1 β (20 ng/rat) or saline. Food, water and body weight change (all prenatal groups combined): IL-1 < Saline, $p < 0.01$

Prenatal group	Injection	Food (g)	Water (ml)	Body weight change (g)
Ethanol	IL-1 (3) ^a	20.53 \pm 2.03	45.37 \pm 1.69	-6.93 \pm 2.60
	Saline (3)	30.23 \pm 0.80	56.03 \pm 3.84	6.60 \pm 3.21
Pair-fed	IL-1 (3)	19.10 \pm 2.50	39.07 \pm 7.30	-7.13 \pm 1.79
	Saline (2)	26.70 \pm 0.60	46.90 \pm 1.10	3.25 \pm 1.05
Normal	IL-1 (5)	18.38 \pm 1.29	41.98 \pm 1.75	-6.58 \pm 2.42
	Saline (2)	24.65 \pm 1.75	49.95 \pm 6.15	6.00 \pm 2.20

^aNumber of animals is in parentheses.

Activity levels increased transiently in all groups, peaking at 30 min after injection of either saline or IL-1 (data not shown). By 90 min after the injection, activity returned to the low levels which characterize this period of the diurnal cycle, and there were no significant effects of either prenatal treatment or injection. Nor did the morning injection interfere with the occurrence of the subsequent night-time increase in motor activity in all groups.

Food and water consumption and body weight were measured at 0800–0900 h for 24 h pre- and postinjection. There were no differences between prenatal treatment groups in preinjection food or water consumption and body weights (Table 2). However, compared to saline injection, IL-1 produced its well-established anorectic effect, i.e., significant reduction in food [$F(1,12) = 28.55$, $p < 0.001$] and water consumption [$F(1,12) = 6.78$, $p < 0.05$] and body weight change [$F(1,12) = 28.95$, $p < 0.001$] across all groups.

3.3. Experiment 3: effect of icv administration of PGE₂ on the febrile response

Fig. 3 shows the effect of icv injection of PGE₂ or saline at 0800–0900 h on body temperature. Both injections produced immediate hyperthermic responses in all animals, however, PGE₂ caused a markedly greater response than saline in all three prenatal treatment groups. Repeated measures ANOVA of the temperature data recorded at 5-min intervals during the period between 0 and 75 min postinjection indicated a significant effect of the injection [$F(1,33) = 33.34$, $p < 0.0001$] without any effect of prenatal treatment.

4. Discussion

In the present study, we investigated mechanisms for the attenuated febrile response of fetal alcohol-exposed rats to ip administration of LPS or IL-1 that we reported previously (Yirmiya et al., 1993, 1996). Two lines of evidence presented here, i.e., reduced levels of hypothalamic IL-1 following ip LPS and an attenuated febrile response to icv IL-1, indicate that the responsiveness of central mechanisms which mediate fever is impaired by FAE. Nevertheless, given that E rats respond with a normal body temperature increase to PGE₂, the hypothalamic thermoregulatory system, per se, does not appear to be affected by FAE.

The results of Experiment 1 are in agreement with previous reports establishing that peripheral administration

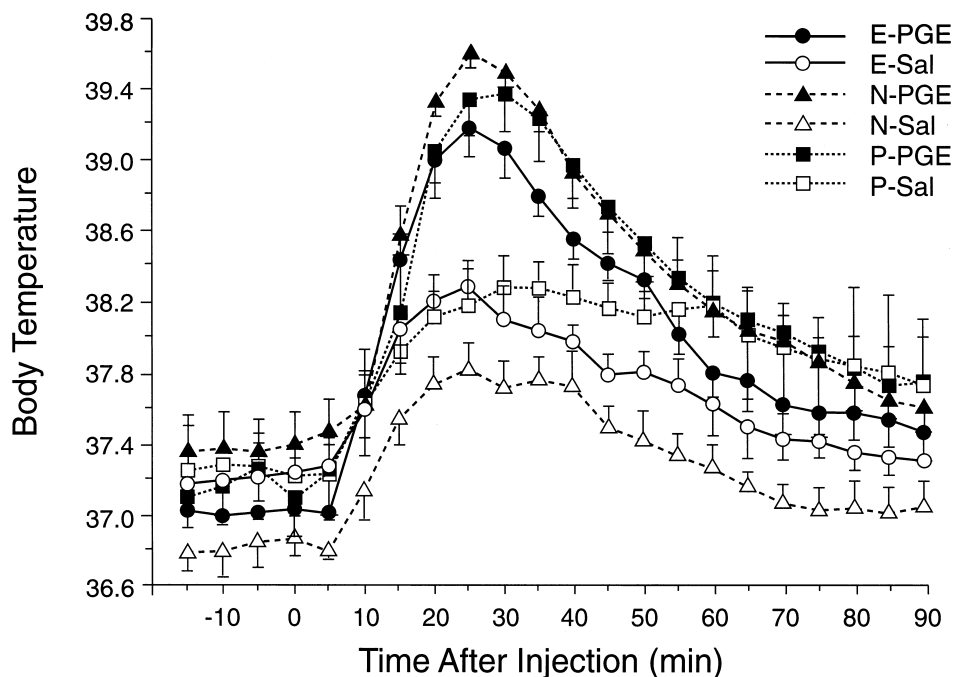


Fig. 3. Effect of fetal alcohol exposure on icv PGE₂-induced alteration in body temperature. Mean (\pm SEM) body temperature ($^{\circ}$ C) in rats exposed prenatally to ethanol (E), pair-feeding (P), or normal diets (N). Rats within each of the prenatal treatment groups were injected icv with either PGE₂ (200 ng/rat) or saline (Sal) early in the light period, and body temperature was recorded continuously by a biotelemetric system for 90 min.

of LPS induces IL-1 production in the brain, as reflected by increased brain IL-1 immunoreactivity (Hagan et al., 1993; Hillhouse and Mosley, 1993; Van Dam et al., 1995; Nguyen et al., 1998), bioactivity (Quan et al., 1994; Laye et al., 1995), mRNA and gene expression (Goujon et al., 1995; Laye et al., 1995). These studies indicate that in addition to the hippocampus and brain stem, the hypothalamus is a particularly rich source of IL-1, which is detectable at two or more hours following injection of LPS. In another study (Klir et al., 1993), IL-6- and TNF α -like activity were detected in push–pull perfusates of rat anterior hypothalamus following ip injection of LPS, with peak bioactivity occurring at 3 h postinjection. IL-10 has been reported to show only a modest increase in gene expression in brain in response to LPS (Wong et al., 1997). As we report here, hypothalamic IL-1 increased in all animals from 2 to 4 h post LPS while hypothalamic TNF α was maximal at 2 h postinjection and declined thereafter. Our assays did not permit us to detect any stimulatory effects of LPS on hypothalamic IL-6 or IL-10 (data not shown). In comparison to LPS-induced increases in hypothalamic IL-1 and TNF α in normal rats, IL-1 was significantly reduced by prenatal ethanol exposure while TNF α was significantly reduced by prenatal pair-feeding. These results indicate that the altered kinetics of IL-1 appearance in E rats may be a contributory factor to their blunted LPS- or IL-1-induced febrile responses. Interestingly, whereas P rats do not generally show a blunted febrile response, they do, in fact, show augmented febrile responses when IL-1 is administered ip prior to the dark phase of the diurnal cycle (Yirmiya et al., 1996). This augmented response may well reflect reduced hypothalamic TNF α levels, given that TNF α also has cryogenic properties (Kluger, 1991).

The temporal patterns of serum IL-1 and TNF α responses to LPS were similar to those observed for hypothalamic IL-1 and TNF α , with IL-1 showing sustained levels for 6 h postinjection while the 2-h-increased TNF α levels subsided by 4 h postinjection. LPS-stimulated serum IL-6 levels also persisted for 6 h. We (Yirmiya et al., 1996) and others (Givalois et al., 1994) have reported similar temporal profiles of LPS-stimulated blood concentrations of TNF α , IL-1 and IL-6. IL-10, an inhibitor of IL-1 expression (Fiorentino et al., 1991), showed only a moderate response at 2 h which did not persist beyond 4 h. In contrast to the differential effects of FAE on hypothalamic IL-1 and of pair-feeding on hypothalamic TNF α at 2 h postinjection, there were no differential effects of the prenatal procedures on blood levels of any of the cytokines at any time point post LPS. The lack of a correlation between blood and brain cytokine levels at the 2-h time point indicates an effect of the prenatal treatments on processes affecting hypothalamic cytokine levels, e.g., synthesis, transport from the circulation to the brain, binding to cell-bound or soluble receptors, and degradation. The effects of FAE on one or more of these processes requires further clarification.

In order to circumvent any potential effects of lower hypothalamic IL-1 levels on IL-1-mediated actions on febrile, anorectic, and locomotor responses, in Experiment 2 we administered IL-1 directly into the brain. The results indicate that the febrile response to icv IL-1 was significantly attenuated in E rats in comparison to the response of N and P rats, as we had found previously with ip administration of IL-1 (Yirmiya et al., 1996). However, in contrast to our previous report (Yirmiya et al., 1996) that the anorexia following ip administration of IL-1 prior to the dark phase of the diurnal cycle was attenuated in E rats, FAE did not attenuate the anorectic response to icv IL-1 administered at the onset of the light phase (Table 1). Nor was the IL-1-induced reduction in locomotor activity affected by FAE (data not shown). It remains to be seen whether FAE would attenuate the anorectic response to a lower dose of icv IL-1 or to administration prior to the dark phase. Interestingly, the specific blunting of the febrile response to icv IL-1 in FAE rats is in contrast to normal febrile responses to icv IL-1 in aged (Plata-Salaman et al., 1998) and chronic alcohol consuming rats (Taylor et al., 1997) who, like FAE rats, show attenuated febrile responses to peripherally administered IL-1. The effects of IL-1 in the present study were most likely mediated centrally rather than by leakage into the periphery, since (a) the small dose of IL-1 which was used in this study cannot cross the blood–brain barrier to produce measurable levels in the blood (Rothwell and Hopkins, 1995; Maier and Watkins, 1998), and (b) even if some IL-1 did reach the periphery, doses at least 40-fold higher are required for comparable effects following peripheral administration of IL-1 (Yirmiya et al., 1996).

Our results with icv IL-1 suggest that FAE impairs transmission of the brain IL-1 signal to the hypothalamic thermoregulatory center. It was therefore of interest to determine whether FAE attenuates the IL-1-induced febrile response because thermoregulatory systems in the hypothalamus are impaired. PGE₂, a known pyrogen, produces fever by acting on temperature sensitive neurons in the anterior hypothalamus (Klir et al., 1993; Scammell et al., 1996; Blatteis and Sehic, 1998). The results of Experiment 3 showed that E rats have a normal febrile response to icv injection of PGE₂ (Fig. 3, cf. Scammell et al., 1996; Milligan et al., 1997). Thus, with an intact effector mechanism for the febrile response, it appears that attenuation of the response in E rats may be due to a defect in IL-1-effector signalling, perhaps in IL-1-PGE₂ signalling, itself. One possibility that is currently being investigated in our laboratory is that FAE impairs IL-1-induced PGE₂ production within the brain.

There are various other mechanisms, in addition to prostaglandins, which mediate the febrile effects of IL-1 and which are known to be affected by exposure to alcohol in utero, and thus may be involved in the effects of FAE on fever production. One such mechanism is corticotropin-releasing hormone (CRH)-induced activation

of sympathetic nervous system innervation of brown adipose tissue (Rothwell, 1989; Rothwell et al., 1996). CRH content in the median eminence has been shown to be decreased following FAE, while levels of hypothalamic CRH mRNA are either increased or unaffected by FAE (Lee and Rivier, 1993; Lee et al., 1990; Redei et al., 1993). Furthermore, as reviewed above, adult E rats show an exaggerated HPA response to certain stressors (Taylor et al., 1982; Lee et al., 1990; Weinberg, 1992) and glucocorticoids are potent inhibitors of many actions of LPS and IL-1, including their effects on thermogenesis, body temperature and behavior (Strijbos et al., 1993; Johnson et al., 1996). Despite the lack of any prenatal treatment effect on ACTH and corticosterone responses to LPS in the present study (Table 1) and in our previous studies (Yirmiya et al., 1996, 1998), the contribution of augmented HPA activity cannot be ruled out given the reported (Lee and Rivier, 1996) differential effects of IL-1 and LPS on ACTH and corticosterone secretion at an earlier postinjection time point. Clarification of the contribution of these, as well as other mechanisms, to the attenuated febrile response to infection in fetal alcohol-exposed offspring awaits further investigation.

In conclusion, we report here that FAE altered the kinetics of brain IL-1 production/appearance following peripheral immune activation, i.e., at 2 h, but not at 4 and 6 h following LPS administration, hypothalamic levels of IL-1 were lower in E than in N rats in the presence of comparably increased serum levels in both groups. Furthermore, FAE produced a decrease in the responsiveness of the brain thermoregulatory centers to icv administration of IL-1. This effect is probably mediated by altered signalling of IL-1 within the brain, because the effector thermoregulatory pathways, activated by brain PGE₂, were not affected by FAE. The reduced production/presence of and responsiveness to brain IL-1 may account for our previous findings of a blunted febrile response to peripheral immune challenges in rats exposed to alcohol in utero.

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