Psychopharmacology (1985) 85:92–96

Psychopharmacology

Opioid but not nonopioid stress-induced analgesia is enhanced following prenatal exposure to ethanol

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Abstract. Two neurochemically distinct forms of stress-induced analgesia were examined in adult rats following prenatal ethanol exposure. Rats were exposed to ethanol during the last 2 weeks of gestation through a liquid diet presented to the dams. Analgesia testing was conducted when the offspring were 150–210 days of age. Two forms of footshock stress were administered; one that resulted in a naloxone-sensitive (opioid-mediated) analgesia and one that resulted in a naloxone-insensitive (nonopioid) form of analgesia. Rats prenatally exposed to ethanol demonstrated significantly enhanced opioid-mediated analgesia, but unaltered nonopioid analgesia compared to controls. These results confirm previous findings that prenatal exposure to ethanol leads to long-term alterations in responding to some, but not all forms of stress. The possibility that prenatal exposure to ethanol leads to perturbations in the endogenous opioid systems is discussed.

Key words: Endogenous opioid peptides – Ethanol – Fetal alcohol syndrome – Prenatal ethanol exposure – Stress-induced analgesia

Fetal exposure to ethanol has been shown to produce a spectrum of anatomical, physiological, and behavioral abnormalities in both humans and animals (Abel 1980; Rosett 1980; Streissguth et al. 1980). Controlled studies utilizing laboratory animals have demonstrated that, whereas some of the effects of prenatal exposure to alcohol are evident for only a conscribed period of time after birth, others persist throughout adulthood (Bond 1981; Druse 1981). We have been studying the long-lasting effects of fetal exposure to ethanol on rat responses to pharmacological agents and environmental stressors. For example, it has been shown that adult rats exposed prenatally to ethanol show an enhanced endocrine response, namely activation of the hypothalamo-pituitary adrenal axis, following administration of ethanol (Taylor et al. 1981). In addition, fetal ethanol-exposed rats respond to certain stressors, but not others, with an enhanced release of adrenal corticosteroids (Taylor et al. 1982; Weinberg and Gallo 1982). In light of this evidence suggesting an altered endocrinological response to pharmacological and environmental stressors in rats prenatally exposed to ethanol, we chose to investigate a behavioral response to stress; namely, stress-induced analgesia.

Exposure to a variety of inescapable stressors has been shown to produce decreased sensitivity to pain as measured by several standard analgesiometric tests (Lewis et al. 1983 for review). Lewis et al. (1980) showed that, depending on the temporal parameters of its administration, inescapable footshock can elicit qualitatively different forms of stress analgesia. In male Sprague-Dawley rats, intermittent footshock (20 min) causes an analgesia that, by several criteria (i.e., reversal by naloxone, development of tolerance, cross-tolerance with morphine analgesia) appears to be mediated by opioid peptides and relies both on neural and hormonal systems. Continuous footshock (3 min), on the other hand, causes an analgesia that by these same criteria appears to be nonopioid in nature and independent of hormonal systems (Lewis et al. 1982). The present study compared opioid and nonopioid analgesic responses to footshock stress in rats prenatally exposed to ethanol and in controls. A preliminary report of these data has been published (Nelson et al. 1982).

Materials and methods

The subjects were adult female offspring, 150–210 days of age, derived from 73 timed-pregnant, nulliparous Sprague-Dawley rats received from Charles River Laboratories (Wilmington, MA) when 6 days pregnant. On day 8 of gestation, the dams were divided into weight-matched groups for subsequent feeding of the following three different diets: (1) ad libitum casein-supplemented liquid diet (Bioserv, Frenchtown, NJ) containing 5.0% w/v ethanol; (2) an isocaloric liquid diet (Bioserv) with maltose dextrin added to replace ethanol, pairfed to the amount consumed by the ethanol dams; (3) standard lab chow and water ad libitum. Fresh diet was presented daily at 9 AM from day 8 of gestation until parturition. Diet consumption was recorded daily and dams were weighed biweekly. On the day of birth, all pups in each prenatal treatment group (E = ethanol, PF = pairfed, N = none or normal) were culled, randomly placed into groups of 10, and fostered to dams which were fed the standard lab chow diet. The pups and dams were weighed at weekly intervals until weaning on postnatal day 21 when pups were group housed by sex. Female offspring (N = 36–44), randomly selected from a total of approximately 105 in each prenatal treatment group, were used for these experiments. At 150–210 days of age, these offspring were placed on a 12-h reverse

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light-dark cycle (lights on 8 PM). After a 2-week acclimation period, behavioral testing was conducted (9AM–1PM).

Experiment 1. Pain responsiveness was assessed using a modified version of the tail-flick test (D'Amour and Smith 1941). A maximum response latency of 7 s was employed on all trials to prevent tissue damage in analgesic animals. Prior to footshock exposure, five tail-flick trials were conducted at 1-min intervals and the average of the last three trials defined baseline pain responsiveness. Footshock stress consisted of 2.5 mA 60-Hz sine waves scrambled and delivered to the grid floor of a 23-× 23-× 23-cm Plexiglas chamber according to one of the following two procedures: intermittent footshock consisting of 1-s pulses delivered every 5 s for 10 min; continuous footshock for 2 min. Tail-flick testing resumed 1 min after footshock termination and continued at 1-min intervals for the first 9 min, and at 2-min intervals from 9–15 min poststress. Rats from each prenatal treatment group received either 10-min intermittent (n = 20, 15, and 12 for groups E, PF, and N, respectively) or 2-min continuous (n = 8 for each group) footshock stress. Data for the 10-min intermittent footshock condition were collected in two independent experiments and the results were pooled.

Experiment 2. The purpose of this experiment was to assess the involvement of opioid peptides in the analgesic response to both 2-min continuous and 10-min intermittent footshock. Rats from each prenatal treatment group (n = 16 for each group) were injected with either naloxone (2 mg/kg SC) or saline 5 min prior to baseline testing. To maintain an equal interval between drug injection and poststress analgesia testing (20 min), continuous footshock stress onset was delayed 8 min following baseline testing. Each rat was exposed to one form of footshock (2-min continuous or 10-min intermittent) on two occasions; once after saline and once after naloxone. The order of drug administration was counterbalanced and the experimenter was blind to the drug condition.

Data analysis. All tail-flick latency data were analyzed in two ways. Raw scores were subjected to analysis of variance for repeated measures and Tukey's tests for specific comparisons (Keppel 1973). Also, raw scores were transformed to total analgesia scores, calculated as the area between the poststress tail-flick latency curve and the baseline for each subject. These areas were also subjected to analysis of variance and Tukey's tests. Both methods of data analysis, raw scores and total analgesia scores, yielded the same statistical results in all cases.

Results

Experiment 1. As shown in Fig. 1, 10-min intermittent footshock stress caused marked analgesia, indicated by elevations in poststress tail-flick latencies in all groups. Analysis of variance of the raw scores or the total analgesia scores indicates that this analgesia was significantly greater in rats prenatally exposed to ethanol (F = 14.7, df 2,44; $P < 0.01$ compared to N or PF). In fact, as the total analgesia scores indicate, the analgesic response of the E rats was approximately twice that shown by either N or PF rats (Fig. 1). Tail-flick latencies following 2-min continuous footshock are shown in Fig. 2. Whereas the continuous footshock procedure was equipotent to the intermittent procedure in eliciting analgesia (compare total analgesia scores of normal offspring in Figs. 1 and 2), there were no differences in the analgesic response among the prenatal treatment groups following 2-min continuous footshock (Fig. 2). In no case did the groups differ significantly in baseline pain sensitivity (Figs. 1 and 2).

Experiment 2. Figure 3 shows the analgesic response to 10-min intermittent footshock in saline and naloxone

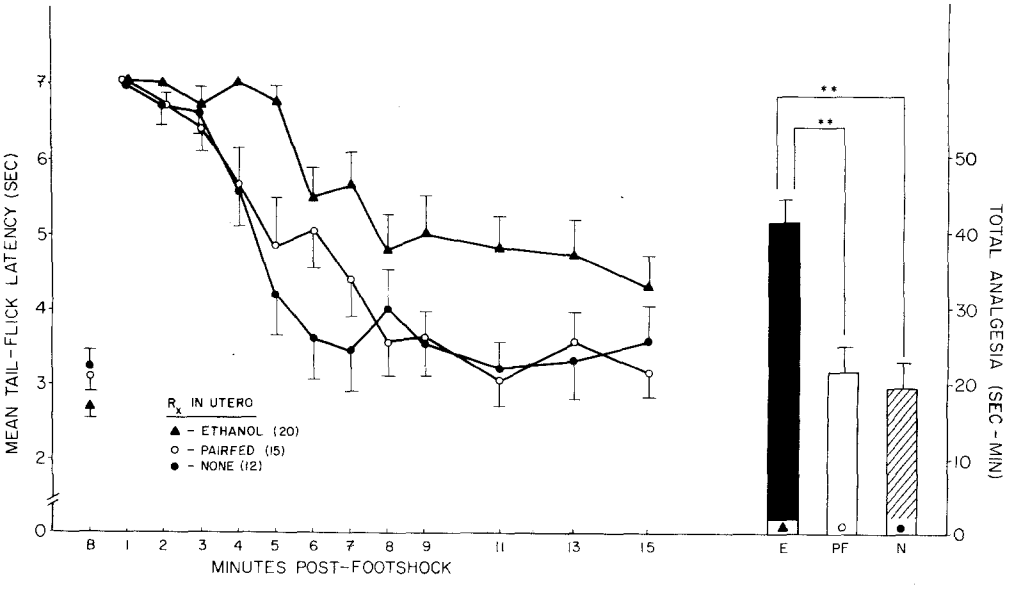

Fig. 1. The left half of the figure portrays the mean (± SEM) tail-flick latencies at baseline testing (B) and following 10-min intermittent footshock. The number in parentheses indicates the number of animals from each prenatal treatment group. The right half of the figure depicts the mean (± SEM) total analgesia scores for the same three groups (** indicates a significant difference between the groups at $P < 0.01$, using Tukey's test)

treated rats. In Fig. 3, the data from the N and PF groups have been combined into a single control group for clarity of presentation, although they were separately analyzed statistically. At no time were the tail-flick latencies of the N and PF groups significantly different from each other. In all three prenatal treatment groups, naloxone significantly antagonized the analgesic response to 10-min intermittent footshock stress ($F = 16.16$, df 5,42; $P < 0.05$, compared to saline in each case), suggesting the involvement of opioid peptides. Similar to the findings reported in experiment 1, the total analgesia scores indicate that prenatal ethanol-exposed rats were significantly more analgesic than control rats following 10-min intermittent footshock when pretreated with saline ($F = 27.58$, df 3,44, $P < 0.01$). However, after pretreatment with naloxone and exposure to 10-min intermittent footshock, there were no differences in tail-flick latencies among the prenatal treatment groups.

In contrast to its effect on analgesia following 10-min intermittent footshock, naloxone pretreatment had no effect on analgesia following 2-min continuous footshock. Table 1 shows the mean total analgesia scores for each prenatal treatment group after saline or naloxone and 2-min continuous footshock. Naloxone pretreatment did not reduce the total analgesia score of any of the prenatal treatment groups as compared to the analgesia that occurred following saline pretreatment. Additionally, there were no significant differences among the prenatal groups in either the saline or naloxone condition, which is in accord with the findings of experiment 1. Statistical analysis of the raw scores also yielded no significant differences between the groups.

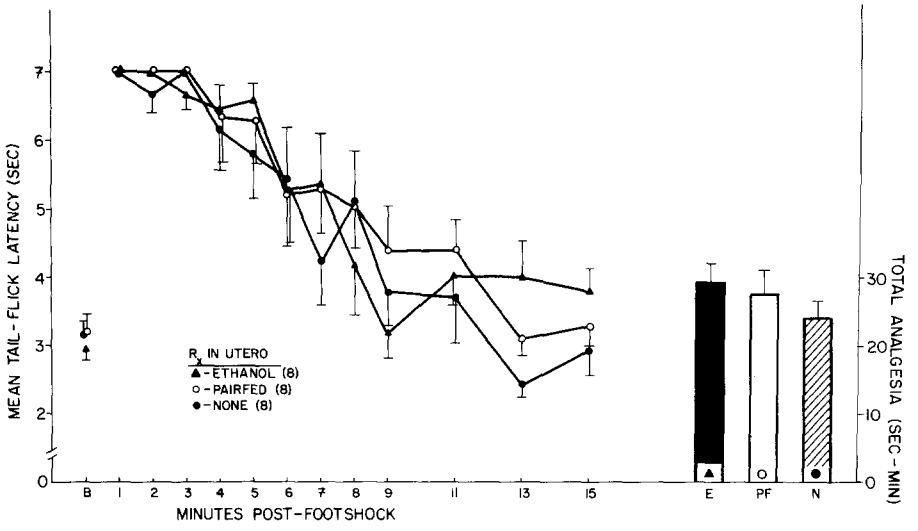

Fig. 2. The left half of the figure portrays the mean (± SEM) tail-flick latencies at baseline testing (B) and following 2-min continuous footshock. The number in parentheses indicates the number of animals from each prenatal treatment group. The right half of the figure depicts the mean (± SEM) total analgesia scores of these same three groups

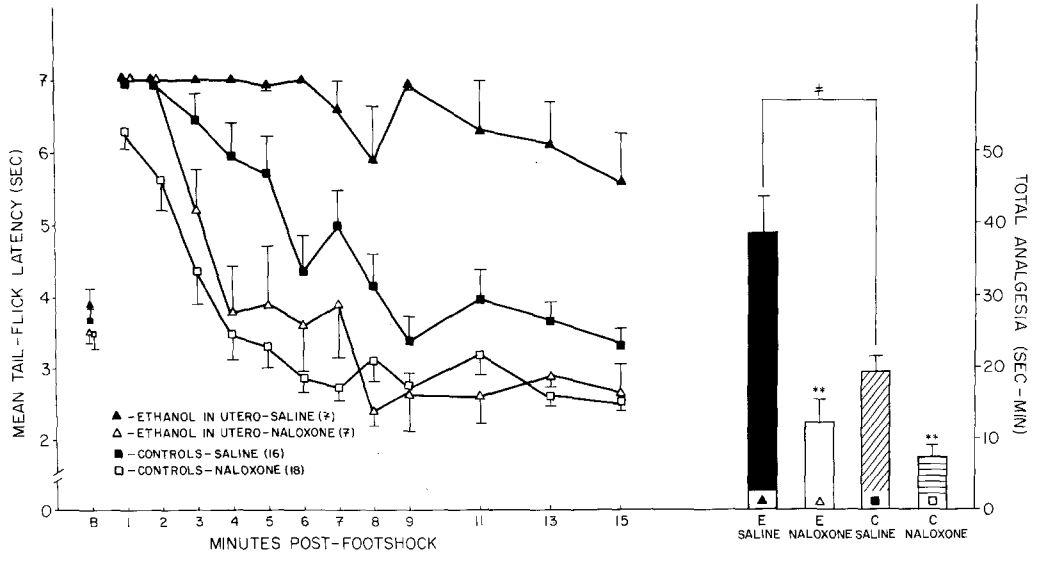

Fig. 3. The left half of the figure portrays the mean (± SEM) tail-flick latencies at baseline (B) testing and following 10-min intermittent footshock preceded by either saline or naloxone (2mg/kg). Data from the N and PF animals were not significantly different and were combined into a single control group (C) for clarity of presentation. The number in parentheses indicates the number of animals from each prenatal treatment group. The right half of the figure depicts the mean (± SEM) total analgesia scores of these same four groups

Table 1. Effect of naloxone (2 mg/kg) on analgesia following 2-min continuous footshock

Group[a]	Total analgesia (s-min)[b]
Ethanol – saline	26.21 ± 2.41
Ethanol – naloxone	23.44 ± 3.28
Pairfed – saline	27.18 ± 5.05
Pairfed – naloxone	29.00 ± 2.27
Normal – saline	30.37 ± 4.16
Normal – naloxone	24.41 ± 3.10

[a] $n = 8$ for each group
[b] Values are means ± SEM

Discussion

In female Sprague-Dawley rats, two paradigms of footshock presentation were found to elicit equipotent but pharmacologically different forms of stress-induced analgesia. Intermittent footshock stress produced a naloxone-blockable analgesia, suggesting that this form of stress-induced analgesia is at least partially mediated by endogenous opioids. Continuous footshock stress produced a naloxone-insensitive analgesia, which is thus nonopioid in nature. Interestingly, only the opioid form of stress-induced analgesia was enhanced by fetal ethanol exposure, while the nonopioid form was completely unaffected. Moreover, this potentiation of stress-induced opioid analgesia in prenatal ethanol-exposed rats is probably not due to impaired performance in the tail-flick test, since these rats did not differ from controls when tested before stress or following continuous footshock stress. Thus, the potentiation of stress-induced analgesia, as indexed by increased tail-flick latencies, is specific to the type of stress administered.

It is possible that the potentiated analgesia seen in the prenatal ethanol-exposed rats could represent the summation of a nonopioid analgesic response with the opioid analgesia normally seen following 10-min intermittent footshock stress. To examine this possibility, we looked at the analgesic response following the administration of naloxone, which would not be expected to reduce analgesia resulting from nonopioid mechanisms. As shown in Fig. 3, the stress-induced analgesia was attenuated to a similar degree in all groups of rats, indicating that the potentiation seen in the prenatal ethanol-exposed rats was indeed opioid in nature. These results suggest that fetal exposure to ethanol produces long-lasting perturbations in the endogenous opioid systems which subserve this analgesic response. Alternatively, the critical alterations may not be in the opioid systems themselves, but in another neurochemical system which modulates their expression.

While it is suggested that prenatal exposure to ethanol alters endogenous opioid systems, precisely which of these systems are affected and to what degree is not known. Accumulating evidence indicates that there may be widespread alterations in opioid systems following fetal ethanol exposure. There have been reports of elevations in brain levels of two opioid peptides; β-endorphin in the midbrain and hindbrain (Shoemaker et al. 1983a), and *met-* and *leu*-enkephalin in the basal ganglia (McGivern et al. 1983). In addition, Govoni et al. (1983) reported that children 4–12 years of age, who were born to chronic alcoholic mothers, had decreased levels of *met*-enkephalin-like immunoreactivity in plasma compared to age-matched controls. Alterations in opiate receptors have also been documented: Shoemaker et al. (1983b) found a decreased number of both high- and low-affinity sites for dihydromorphine in newborn rat pups prenatally exposed to ethanol and Shah and West (1983) reported decreased ^{3}H-naloxone binding in the striatum of prenatal ethanol-exposed rats during postnatal days 4–25.

In addition, we have been using morphine as a pharmacological probe to assess the functioning of the opioid systems: Three responses to morphine (hypothermia, corticosteroid release, and analgesia) were all augmented in adult rats prenatally exposed to ethanol (Nelson et al. 1983). These findings add further support to the contention that there are widespread and long-lasting alterations in the endogenous opioid systems following fetal ethanol exposure.

Acknowledgements. This research was supported by the VA Medical Research Service, NIH grant NS 07628, and a gift from the Brotman Foundation. L. Nelson was supported by MHTP grant MH 15345.

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Received January, 16, 1984; Final version July, 25, 1984