Prenatal Antagonism of Stress by Naltrexone Administration: Early and Long-lasting Effects on Emotional Behaviors in Mice

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The effects of prenatal exposure to stress and to naltrexone on emotional behaviors were studied in CD1 mice during ontogeny and in the adulthood. During ontogeny (a) lower body weights were initially found in pups born by mothers injected with naltrexone; (b) treatments did not affect sensory motor development except in the case of the cliff aversion reflex which occurred earlier in pups prenatally exposed to stress; (c) measures of ultrasonic vocalizations in stressful context showed that the amount of vocalizations emitted by pups born by stressed mothers was significantly higher than that emitted by pups born by naltrexone injected and control mothers (d) an examination of mother–offspring interactions on the very first day of observation indicated a consistent trend in stressed mothers to be more responsive to their pups. In adulthood, ultrasonic calls in courtship after short and long periods of isolation showed a time-dependent decrease of vocalizations in males prenatally exposed to naltrexone. These results indicate that the modifications of emotionality evident during early development are directly related to the reactivity of the mothers to the experimental treatments.

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Moreover, the long-term reduction of ultrasonic vocalizations in adult males prenatally exposed to naltrexone shows in these animals a lowered reactivity to stressful contexts which probably results from specific physiological alterations due to the prenatal administration of the opiate antagonist.

A number of studies suggest a coordinate role for pituitary β-endorphins and ACTH in the behavioral response to stress (Amir, Brown, & Amit, 1981; Axelrod & Reisine, 1984; Jacquet, 1979). When applied to pregnant females, many stressors have been shown to produce both developmental and long-lasting physiological and behavioral modifications in the offspring which outline the importance of the interactive endocrine and opioid systems in the early CNS organization and its subsequent functioning.

Prenatal stress was demonstrated to affect gross physiological parameters such as litter size (Smith, Joffe, & Heseltine, 1975) body weights at birth and weaning (Beckhardt & Ward, 1983) or sensorimotor development (Fride & Weinstock, 1984). More specific neurobiological and hormonal alterations were also detected in rats or mice prenatally exposed to various stressors such as change in cortical biogenic amines (Peters, 1982; Plaut, Graham, & Leiner, 1972) and in plasmatic corticosterone levels (Peters, 1982; Politch, Herrenkhol, & Gala, 1978). Finally, from a behavioral point of view, modifications of activity rates (Sobrian, 1977), early emotionality (Ader & Plaut, 1968; Chapman & Stern, 1979; Hockman, 1961; Thompson, 1957), active avoidance scores (Smith et al., 1975) together with disruptions of sexual and reproductive behaviors (Beckhardt & Ward, 1983; Politch & Herrenkhol, 1984; Ward, 1972) were reported in prenatally stressed animals, some of these effects being antagonized by blocking completely (adrenalectomy) or partially (dexamethasone injections) the adrenocortical response of the pregnant females to stress (Smith et al., 1975).

However, in spite of numerous data indicating that acute administration of opiate antagonists can block stress-induced corticosterone release (Gibson, Ginsberg, Hall, & Hart, 1979), no experiments have until now been concerned with prenatal blocking of the effects of stress by opiate antagonists. The present experiment was designed to investigate this possibility.

Since many authors have reported that stressing pregnant females modify emotional reactivity in the offspring (see Archer & Blackman, 1971 for a review) the effect of prenatal exposure to stress and of concomitant administration of naltrexone on emotionality was examined in developing and adult CD1 mice. For this purpose, ultrasound emission in pups placed in stressful context, i.e., submitted to a short period of isolation, was chosen as a criterion of emotionality and recorded in prenatally stressed, prenatally naltrexone-injected and control litters. In addition, some parameters of sensorimotor development and maternal behavior were also examined. Finally, in order to detect eventual long-lasting effects of prenatal treatments on adult ultrasonic vocalizations, ultrasound emission in presence of a mature female after two different period of isolation housing was recorded in males born by mothers exposed to each treatment during gestation.

Method

Subjects

Nulliparous CD1 adult female mice (Charles River, Como, Italy) were individually housed with food and water available ad libitum and maintained on a 12

hr light/dark cycle (7:00 AM–7:00 PM) with constant temperature (21 ± 2°C) for a free period treatment of 6 days. On the 7th day, the males were introduced in the cages (ratio 1 : 1) for a 10-day period. On the 17th day, corresponding to a maximum of 11 and a minimum of 9 days of pregnancy, the males were removed from the cages and the females randomly divided into 3 groups. From the following day the females were weighed daily and treatments began according to the following procedure. The first group was stressed according to a previously described method (Peters, 1982), i.e., by receiving subcutaneous injections of saline twice a day. The second group received increasing doses of naltrexone: day 10–11: .5 mg/kg; days 12–13: 1 mg/kg; 14–15 days: 2 mg/kg; days 16–17: 3 mg/kg; days 18–19: 4 mg/kg (Oliverio, Castellano, Puglisi-Allegra, 1984). This schedule was selected on the basis of preliminary experiments showing high abortion rates in mice injected with naltrexone (3–4 mg/kg) during pregnancy. The third group was left undisturbed during gestation, except for daily handling during weight check. Injections were given twice a day between 9:00–10:00 AM and 4:00–5:00 PM. Since no control of the first day of pregnancy was carried out in order to limit the manipulations of the females, the litters retained for this study were those in which females received the entire injection schedule and delivered on the last day of the treatment or on the day after. The cages were daily inspected for live births and the number of pups per female was culled to 8. Each group involved at least 5 females and their offspring.

Developmental and Behavioral Measures

Litter Sizes

The number of pups born by mothers exposed to each treatment was recorded in the whole litters.

Body Weights and Reflexes

Considering the day of delivery as postnatal day 1, pups were weighed and tested for sensorimotor development every other day from postnatal day 2 until postnatal day 18 according to a previously described method (Castellano & Oliverio, 1976). The following developmental parameters were recorded: rooting, cliff aversion, righting, forelimb placing, forelimb grasping, bar holding, vibrissae placing. The age at which a given response was present in the adult form indicated the score for each mouse.

Maternal Behaviors

Observations of maternal behaviors were daily conducted from postnatal day 2 to postnatal day 10 between 8:30 and 11:30 AM. The home cages were transferred in a soundproof chamber, maintained at a constant temperature, 30 min before the beginning of the observation period, in order to restaure the mothers' basal arousal level. The mothers were observed for 30 min and 30 sampling points per female—one per minute—were taken for the following behavioral parameters: presence in the nest, nursing posture, grooming and licking pups, self-grooming and scratching, nest-building, and carrying pups.

Distress Vocalizations of Pups

Each day from postnatal day 3 to postnatal day 10, 2 pups per litter were separately taken out from their home cage and checked for the amount of ultrasonic vocalizations in another cage. Checking was carried out in isolation and at the room temperature (21°C) according to a previously described method (Noirot, 1966). Ultrasounds were detected with a bat detector (QMC Instruments). The detected ultrasounds were transformed into whistles audible for human ear which were recorded by the experimenter. The detector was tuned to a center frequency of 70 kHz which is within the range of ultrasonic calls of young mice (Noirot, 1966). In order to rule out habituation effects, each pup was marked after the test and retested four days later.

Ultrasonic Calls in Courtship

At 1 month of age, the males were weaned and caged according to the treatment with 6 animals per cage. At 4 months of age, 12 males for each treatment were tested for ultrasonic vocalizations in presence of a sexually mature female. Each animal was isolated and tested first after 20 hr and then again after 10 days of isolation housing. The number of ultrasonic calls emitted during the first 3 min after the female had been introduced in the cage was detected. The bat detector was tuned to a center frequency of 70 kHz since male mice emit 70 kHz ultrasonic vocalizations when paired with a conspecific female (see Pomerantz, Nunez, & Bean, 1983).

Data Analysis

Due to the small but constant differences among samples, the data concerning litter sizes, body weights and maternal behaviors were compared by a Kruskall Wallis nonparametric analysis of variance. Subsequent pair-comparisons were made by a Mann Whitney *U*-test. Ultrasonic vocalizations recorded in the 3 groups during ontogeny were analyzed by a random blocks 2-factor (treatment × days) analysis of variance, since calls were recorded in different pups on consecutive days (see Method). Finally, ultrasonic vocalizations recorded in adult animals after a short and a long period of isolation housing were analyzed by a 2-factor (treatment × repeated measures) analysis of variance since in this case each animal was tested twice.

Results

Litter Sizes

No difference was found in the number of pups per litter according to the treatment ($H = 1.69$, $df = 2$, NS).

Body Weights and Reflexes

Pups born by mothers exposed to naltrexone initially showed the lowest body weights (day 2: $H = 6.98$, $df = 2$, $p < .05$), this deficit being rapidly recovered (Table 1). Mothers' weight gain during pregnancy was not affected by treatments ($H = 1.10$, $df = 2$, NS). Treatments did not affect sensorimotor development

TABLE 1. Mean Body Weight (±SE) in Prenatally Stressed, Prenatally Naltrexone-Injected and Control Pups During Ontogeny.

Age of Pups (days)	Pups (g)		
	Pre-Str.	Pre-Ntrx.	Control
2	1.98 (0.05)	1.86 (0.11)*	2.07 (0.06)
4	2.81 (0.04)	2.60 (0.19)	2.73 (0.15)
6	3.96 (0.05)	3.70 (0.24)	3.69 (0.21)
8	4.90 (0.09)	4.81 (0.23)	4.57 (0.24)
10	5.87 (0.11)	5.78 (0.26)	5.56 (0.25)
12	6.56 (0.28)	6.67 (0.26)	6.46 (0.21)
14	7.16 (0.39)	7.60 (0.27)	7.19 (0.24)

* $p < 0.05$.

except in the case of the cliff aversion reflex which occurred earlier in pups prenatally exposed to stress.

Maternal Behavior

No significant treatment effect was found in maternal behaviors by comparing each parameter over the whole observation period. However, an examination of the data recorded on the very first day of observation revealed in stressed mothers a consistent trend to be more responsive toward their pups. In fact, as can be seen in Figure 1, plotting together the scores obtained in testing active maternal behaviors (grooming pups, carrying pups and nest building) stressed mothers scored significantly higher than naltrexone injected and control mothers ($H = 7.59$, $df = 2, p < .05$; Pre-Str./Pre-Ntrx.: $U = 3, p < .008$; Pre-Str./Control: $U = 2, p < .004$; Pre-Ntrx./Control: $U = 25$, NS).

Distress Vocalization of Pups

The amount of ultrasonic vocalizations emitted by pups of each group during ontogeny is shown in Figure 2. The results show that pups prenatally exposed to

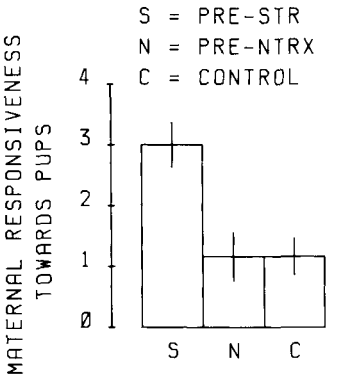

Fig. 1. Responsiveness towards pups in stressed, naltrexone-injected and control mothers on day 2 after delivery. Values reported in ordinate correspond to the mean number (±SE) of sampling points in which responsiveness towards pups was observed.

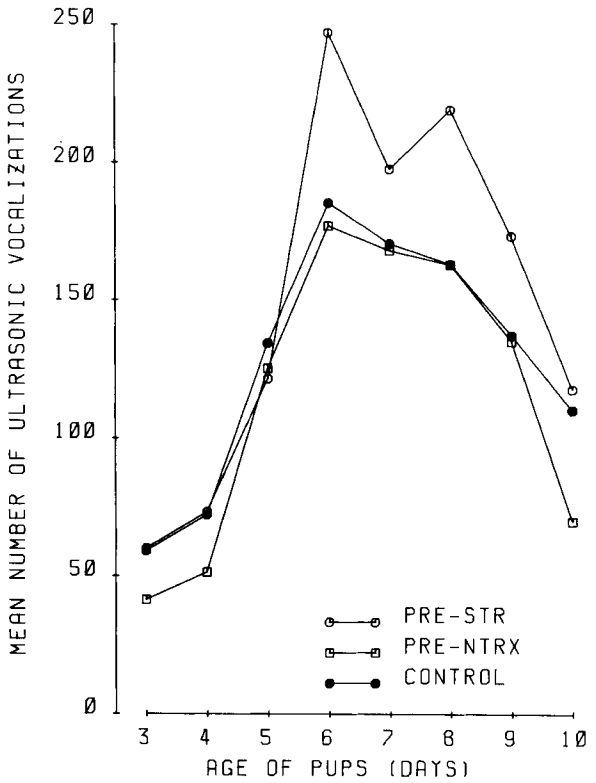

Fig. 2. Mean number of ultrasonic vocalizations emitted by prenatally stressed, prenatally naltrexone-injected, and control pups during ontogeny.

stress emitted significantly more ultrasonic vocalizations than pups from the other groups (significant treatment effect: $F(2,216) = 4.58, p < .01$). The rate of emission varied across the days (significant days effect: $F(7,216) = 16.55$, $p < .01$), but followed the same trend in the three groups (no significant interaction effect).

Ultrasonic Calls in Courtship

The amount of ultrasonic vocalizations emitted by males of each group in presence of a sexually mature female after a short and a long period of isolation housing is shown in Figure 3. The results show that males prenatally exposed to naltrexone emitted significantly less ultrasonic vocalizations than males from the other groups (significant treatment effect: $F(2,33) = 4.37, p < .001$). The increase in time spent in isolation housing enhanced the amount of vocalization (significant repeated measures effect: $F(1,71) = 61.69$, $p < .01$) but this enhancement was significantly less pronounced in males prenatally exposed to naltrexone (significant treatment $\times$ repeated measures interaction: $F(2,33) = 3.99$, $p < .012$).

Discussion

This study was undertaken to analyze the effect of prenatal stress (saline injections) and its possible antagonism by prenatal administration of an opiate

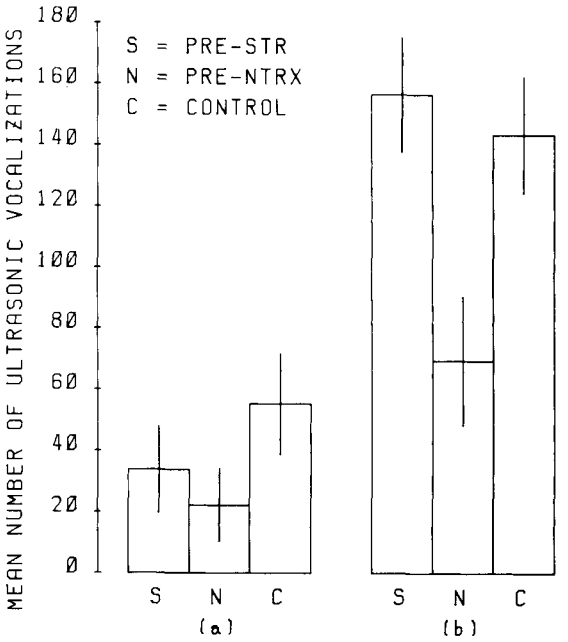

Fig. 3. Mean number (±SE) of ultrasonic vocalizations emitted by prenatally stressed, prenatally naltrexone-injected, and control males in adulthood after (a) 20 hr and (b) 10 days of isolation-housing in presence of a sexually mature female.

antagonist (naltrexone)—on the amount of ultrasounds emitted by the progeny in stressful contexts during development and in the adulthood.

The present results first demonstrate that until the first 2 weeks of postnatal life, the amount of ultrasound emitted by pups during 5 min of isolation did not differ between animals prenatally exposed to naltrexone and control mice but was significantly increased in prenatally stressed animals.

Measures of maternal behaviors during the same period also revealed that females stressed during gestation were more responsive toward their pups 48 hr after delivery than naltrexone-injected and control females. These observations indicate a consistent effect of prenatal stress on ultrasound emission in reaction to stress together with early modifications of maternal behaviors which is antagonized by prenatal naltrexone administration.

The increased reactivity to stressful situations observed in the progeny prenatally exposed to stress is consistent with previous data from the literature: intense defecation and reduced ambulation in the open field situation has been in fact reported in juvenile rats born by mothers exposed to conditioned anxiety during gestation (Hockman, 1961) while enhanced levels of plasmatic corticosterone in reaction-to-stress tests have been found (1) in prenatally handled animals (Ader & Plaut, 1968) and (2) in 23-day-old rats born by saline-injected mothers, i.e., in animals exposed to the same kind of prenatal stress we used in the present experiment (Peters, 1982). It can therefore be assumed that pups born by stressed mothers experienced a high concentration of plasmatic corticosterone together with a high endorphin level during their fetal life which might have lowered their postnatal threshold of reactivity to stressful situations while the main effect of prenatal naltrexone injections may have consisted in restoring the maternal re-

lease of corticosterone and endorphin during gestation at the level of control mothers. It must be noted that, according to the findings reported above, the effect of prenatal stress on offspring emotionality seems limited to the development period and disappears when the animals reach the adulthood (Archer & Blackman, 1971).

In the same way, the initial increase in responsiveness toward pups observed in stressed mothers might also be interpreted in terms of sharp modifications of ACTH and endorphin levels before and after parturition, these levels being high during the chronic stress period—gestation—and dropping abruptly after the cessation of the treatment—parturition. This drop should be responsible for the high responsiveness of these mothers toward their pups close to delivery since according to Panksepp, Herman, Vilberg, Bishop, and DeEskinazi (1980) the diminution of endorphin level enhance social cohesion.

Therefore, the only early effect which can be attributed to naltrexone during development is the initial deficit in body weight. This result can not be ascribed to the anorexigen effect of naltrexone, since no difference was discovered in females' weight gain during pregnancy.

However, while clear effects of prenatal stress were found on the amount of ultrasound emitted in stressful context during ontogeny, no difference was then observed in the adulthood between the number of ultrasonic vocalizations emitted by prenatally stressed and control male animals in presence of a sexually mature female, even after 10 days of isolation-housing. Conversely, male animals born by naltrexone-injected mothers emitted fewer vocalizations than the other animals when placed in the same situations, this difference being particularly significant after the long period of isolation-housing.

It must be noted that ultrasound vocalizations in courtship, in particular after isolation-housing, can be considered an index of both reactivity to a sexual partner and emotionality. As we previously mentioned, prenatal stress does not generally induce modifications of emotionality in adult offspring (Hockman, 1961; Ader & Plaut, 1968; Peters, 1982; Archer & Blackman, 1971; Chapman & Stern, 1979) while some abnormalities of sexual behaviors in prenatally stressed animals have been previously described (Ward, 1972; Herrenkohl & Whitney, 1976). In fact, feminization has been reported in males whose mothers were stressed during pregnancy, this effect being reversed by concomitant administration of naltrexone (Ward, Monaghan, & Ward, 1986).

However, since no difference in vocalization was found between prenatally stressed and control adult males in the presence of the female after a short or long period of isolation-housing, it seems possible to conclude that not only emotionality but also reactivity to a sexual partner was unaffected by the prenatal stress applied in this experiment. Moreover, these observations could suggest that no sexual differentiation was induced by the stress procedure applied in this experiment. Conversely, the fact that long-lasting effects of naltrexone injections were observed on the number of ultrasonic vocalizations, which were more markedly reduced after the long period of isolation-housing, mainly suggests a lowered reactivity to stressful experience in adult animals prenatally exposed to an opiate antagonist. This possibility is supported by recent evidence indicating a reduced development of regional [3]Met binding sites (Tsang & Ng, 1980) in animals born by mothers exposed to naloxone during pregnancy. Concerning this point, it must be also underlined that an enhanced responsiveness to stress has been observed in adult mice prenatally exposed to morphine (Castellano & Ammassari-Teule, 1984).

Taken together these findings outline the relevance of longitudinal studies to identify the behavioral effects of prenatal opiate manipulations. Moreover they indicate that the modifications of emotionality recorded in the offspring during development seem directly related to the reactivity of the mothers to the experimental treatments and might be viewed as a consequence of the induced variations in maternal release of corticosterone and endorphin during gestation. However, the early modifications of emotionality detected in the developing offspring appear unrelated to those observed in the adulthood which seemed to result from specific physiological alterations due to the administration of the opiate antagonist.

Notes

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