Analgesic Response to Stress Is Reduced in Perinatally Undernourished Rats1,2

A. C. Gutiérrez and E. A. Keller

Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Agencia Postal 4, C.C. 61, 5000 Córdoba, Argentina

ABSTRACT Stress-induced analgesia was evaluated in adult rats submitted early in life to a protein deprivation schedule. Rats were undernourished with a hypoproteic diet containing 80 g casein/kg diet from d 14 of gestation until 50 days of age. Rats were thereafter fed a balanced nonpurified diet until 140 days of age, when they were exposed to two stressors: forced swimming and acute restraint, after which the analgesic response was evaluated. In addition, the analgesic response induced by different morphine doses was determined in another group of rats. Basal latency was not different in deprived and control rats. Undernourished rats presented a significantly lower analgesic response in both stress situations. However, when the analgesic response induced by different morphine doses (1, 2, 4 and 8 mg/kg, s.c.) was assessed, a significantly higher response occurred in undernourished rats compared to control rats. This lower stress-induced analgesia in undernourished rats may account for the behavioral alterations attributed to early undernutrition. J. Nutr. 127: 765–769, 1997.

KEY WORDS: • perinatal undernutrition • opiate agent • rats • stress • analgesia
• protein undernutrition

Early-age undernutrition induces, even after a nutritional recovery period, alterations in different behavioral paradigms, such as exaggerated behavioral responses to stress (Barnes et al. 1968, Levitsky and Barnes 1978, Smart 1971). We previously suggested that this insult during early life probably affects the ability of undernourished rats to cope with environmental demands in adulthood (Keller et al. 1994). This point of view is based on much evidence suggesting that the adaptive changes in monoaminergic receptors may play a role in the maintenance of normal behaviors after exposure to stressful or aversive events (Cancela and Molina 1987, Cancela et al. 1988, Kennett et al. 1985, Stone 1987) and on the fact that rats undernourished during early life are unable to develop these adaptive changes in monoaminergic receptors following successive stress exposures (Keller et al. 1990 and 1994).

On the other hand, a large number of findings show that exposure to a variety of stressors leads to a pain suppression effect, termed stress-induced analgesia (SIA),3 which may be mediated by either opiate or nonopiate mechanisms (Akil et al. 1978, Amir et al. 1980, Grisel et al. 1993, Lewis et al. 1980, Olson et al. 1994). For example, SIA induced by restraint and long forced swims has been reported to have an opiate origin, since this analgesic response is blocked by naloxone and potentiated when enzymatic degradation is prevented (Amir and Amit 1978, Bodnar et al. 1980, Christie et al. 1981, Greenberg and O’Keefe, 1982, Kelly and Franklin 1987) and, conversely, short swims induced nonopiate analgesia (Galea et al. 1993, Tierney et al. 1991). Several pieces of evidence suggest that, apart from inducing a pain-suppressing effect, SIA would play an adaptive role in aversive situations, necessary for coping with stressful conditions (Amit and Galina 1986, Sumová and Jakoubek 1989).

In accordance with our hypothesis that early undernutrition probably affects the adult’s ability to cope with aversive situations and that SIA could be regarded as an adaptation of the organism to aversive situations, we have evaluated the pain-suppression effects induced by two aversive situations, namely forced swimming (FS, short and long) and acute restraint, in rats undernourished in early life. In addition, the analgesic responses induced by different morphine (MOR) doses were evaluated in rats given the same dietary treatment.

MATERIAL AND METHODS

Animals and diets. A previously described protein deprivation regimen (Marichich et al. 1979) was used. Briefly, Wistar strain female rats from our colony were divided into two groups at d 14 of pregnancy and fed isocaloric diets containing 240 and 80 g casein/kg, respectively, for control and undernourished rats. Diets also contained 4 g D,L-methionine/kg. After weaning (30 d), pups continued to receive the same diet as their dams until 50 d of age. Both groups were thereafter fed a balanced nonpurified diet (g/kg diet: protein, 180; fiber, 55; fat, 40; energy, 11.6 MJ/kg diet; Cargill, Buenos Aires, Argentina) for at least 90 d before the experiment (i.e., rats were at least 140 d old). Undernourished and control male rats used in these experiments came from different litters, so that sibling replication was consistently avoided. When the experiments were performed,
body weights for control and undernourished rats were $312 \pm 8.7$ g and $239 \pm 6.1$ g, respectively. Rats were kept at $22 \pm 2^\circ C$ with a 12-h light:dark cycle, lights on at 0700 h, with free access to food and water.

These conditions meet the standards for the care of laboratory animals as outlined in the Guide for the Care and Use of Laboratory Animals (NRC 1985).

**Drugs.** Morphine-HCl (Lab Verardo, Buenos Aires, Argentina) was dissolved in saline, 9 g NaCl/L (SAL). Injections were administered at 1 mL/kg body weight for all doses (1, 2, 4, and 8 mg/kg body weight).

**Analgesic response to acute restraint.** Male adult rats from control and undernourished groups were habituated to manipulation by the experimenter, to the tail-flick apparatus (handling) and to the plexiglas restraining device daily, starting 5 d before immobilization. This handling procedure was carried out so that the novelty of the experimental situation would not influence the effect of restraint. On the sixth day, each rat was placed for 2 h in a plexiglas device capped with a wooden plug, the whole device fitting the rat’s body for complete immobilization. The analgesic response was evaluated 30, 60 and 120 min after the restraint session started.

**Measurement of analgesic response.** Analgesia was measured by the tail-flick procedure (D’Amour and Smith 1941), with modifications. Each rat was held loosely wrapped in a towel or placed in its restraint device and then taken to the tail-flick apparatus. The ventral side of the tail (about 4 cm from the tip) was exposed to a beam of radiant heat. An automated counter recorded the latency in seconds between the onset of the beam and the movement of the tail (tail-flick), which cut the beam off. The stimulus intensity was adjusted so that baseline tail-flick latency for the non-stressed control rats was in the 1.40-3.70 s range. To prevent tissue damage, a 12-s cut-off time was used.

Tail-flick latency data are expressed as the mean percent maximum possible effect (%MPE) according to the formula (Dewey and Harris 1975): 

$$\%MPE = \frac{\text{TL} - \text{BL}}{12 - \text{BL}} \times 100$$

where TL is observed latency after stressor application, 12 is cut-off time in seconds and BL is baseline latency determined for unstrained rats (control and undernourished) in three trials conducted on different days at 5-s intervals. The average of the last two trials was taken as the baseline measure for each rat.

**Analgesic response to forced swimming.** Male adult rats, both control and undernourished, were habituated to manipulation by the experimenter, to the tail-flick apparatus and to the experimental conditions of forced swimming (FS). This habituation process lasted for 5 d and was conducted between 0900 and 1200 h. It consisted of carrying the rats in individual cages from the animal room to the stressor room, placing them in a drying cage and then taking them to the analgesia-recording room. On the sixth day, the basal analgesic response (baseline latency) was determined for each rat as described above. Five minutes later, rats were segregated into two groups, each comprising both control and undernourished rats, and groups were subjected to the FS task for 5 or 15 min. Swimming was forced by placing the rats individually in acrylic cylinders (40 cm high, 18 cm wide) filled with 25°C water to 17 cm deep. Thereafter each rat was placed in a cage in front of a fan delivering hot air for 10 min, put back in the home cage and then taken to the analgesia-recording room. Tail-flick response was recorded 15, 30, 45, 60, 120 and 180 min after stressor.

**Analgesic response induced by different morphine doses.** Male adult rats from control and undernourished groups were subjected to the handling process for 5 d and also were habituated to being placed back in the home cage and taken to the analgesia-recording room. On the sixth day, each rat was placed for 2 h in a plexiglas device. The analgesic response was evaluated 30, 60 and 120 min after the restraint session started.

**Statistical analysis.** Stress-response data was analyzed by a two-way ANOVA for repeated measures followed by Fisher’s least significant difference test. In order to draw a morphine dose-response curve for each group, the areas below the %MPE curves were computed in terms of time using a statistical and pharmacokinetic data analysis program (PKCALC, Shumaker 1986). Morphine-dose response data was analyzed by three-way ANOVA for repeated measures and by Fisher’s least significant differences test (Steel and Torrie, 1980) for post-hoc comparisons of groups. A difference of $P < 0.05$ was considered significant. A software package (STATISTICA Version 4.3, Statsoft, Inc. 1993) was used to calculate statistics.

**RESULTS**

**Effect of acute restraint on the analgesic response.** Acute immobilization induced a significant increase in tail-flick latency by control rats (Fig. 1). However, acute stress-induced analgesia was significantly lower in undernourished rats. Diet ($P < 0.04$) and time ($P < 0.00001$), but not their interaction, affected %MPE. The increase in tail-flick latency in control rats was significantly greater than that observed in undernourished rats at 30 min ($P < 0.05$).
Effect of forced swimming on the analgesic response. Control rats subjected to the 15 min FS test had an increase in tail-flick latency, which reached its peak between 15 and 30 min after test (Fig. 2). This increase was significantly lower in undernourished rats. Diet \( (P < 0.05) \), time \( (P < 0.0001) \) and their interaction \( (P < 0.01) \) affected %MPE. The increase in the tail-flick latency in control rats was significantly lower than that observed in undernourished rats at 15, 30 and 45 min \( (P < 0.05) \).

When the FS test lasted for 5 min, the analgesic response of undernourished rats did not differ from controls (data not shown).

Effect of different MOR doses on the analgesic response. The subcutaneous administration of different MOR doses was effective in inducing analgesia, as evidenced by the increased tail-flick latency for control and undernourished rats (Fig. 3). However, this analgesic effect was significantly stronger in undernourished rats than in controls. On the other hand, as expected, SAL administration (vehicle) produced no significant difference in analgesic response between the two groups (Fig. 3). Diet \( (P < 0.0007) \), MOR dose \( (P < 0.00001) \), time \( (P < 0.00001) \) and the three-way interaction \( (P < 0.02) \) affected %MPE. The increase in tail-flick latency in undernourished rats administered the 1 and 2 mg/kg MOR injection, expressed as % MPE, was significantly higher than that observed in control rats at 30 and 60 min \( (P < 0.05) \). The 4 mg/kg MOR injection produced an increase in post-drug latency in undernourished rats that was significantly greater than that of control rats at 90, 120 and 180 min \( (P < 0.02) \).

To compare the effects of different MOR doses in the control and undernourished groups, tail-flick latency expressed as %MPE was transformed to the area under the dose-effect curve for the 30-180 min period, for each group and for each dose (Fig. 4). In undernourished rats different MOR doses (1, 2, 4 and 8 mg/kg) provoked a left-hand displacement of the dose-response curve compared to that for similarly treated control rats (Fig. 4). The dose-response curve for the different MOR doses in undernourished rats was significantly displaced to the left compared to that of the controls \( (P < 0.001) \). In addition, intracerebroventricular administration of MOR also produced a significantly greater tail-flick latency in undernourished rats compared to controls. Drug \( (P < 0.007) \), time \( (P < 0.0001) \) and the interactions diet \( \times \) time \( (P < 0.003) \), drug \( \times \) time \( (P < 0.00001) \) and diet \( \times \) drug \( \times \) time \( (P < 0.03) \) affected %MPE. The Fisher’s test for the posterior individual comparisons revealed that a 5 μg per rat MOR injection provoked an increase in tail-flick latency in undernourished rats expressed as %MPE, significantly higher than that of control rats at 15 and 30 min. Percent MPE at 15 min was 23.53 ± 4.56 for control rats and 36.56 ± 4.41 for undernourished rats; at 30 min, values were 27.91 ± 4.98 for control rats and 48.49 ± 10.44 for undernourished rats \( (P < 0.001) \).

**DISCUSSION**

As previously reported, after acute immobilization the analgesic response of control animals increases over time (Amir and Amit 1978, Bodnar et al. 1980). However, in the stressed undernourished rats, this analgesic response was lower than that of controls. Similar results were found after a 15 min FS session; that is, stressed controls displayed a higher response than stressed undernourished rats. It should be noted that no differences were observed in the latency of analgesic response between nonstressed controls and nonstressed undernourished rats. The stress-induced analgesic response is considered to be an adaptive response necessary for coping with stressful situations (Amir and Galina 1986, Sumová and Jakoubek 1989). Because our present results show that undernourished rats have a lower analgesic response after enduring the stress of restraint and 15 min of forced swimming, these data confirm our hypothesis that early-life undernutrition affects the ability to cope with aversive situations.

It has been shown that opioid mechanisms mediate analgesic responses induced by restraint and long-duration swims (Amir and Amit 1978, Bodnar et al. 1980, Christie et al. 1981, Greenberg and O’Keefe 1982, Kelly and Franklin 1987); therefore, the lower analgesic response observed in undernourished rats may be mediated by opioid mechanisms. In support of this, the same analgesic response was observed in control and undernourished rats after a short swim that induces non- opiate analgesia (Galea et al. 1993, Tierney et al. 1991). On the other hand, when the analgesic response elicited by s.c. administration of different morphine doses was assessed in both groups, the response was significantly higher in undernourished rats than in control rats. Also, since both intracerebroventricular and s.c. MOR administration in undernourished rats induced the same analgesic response, the possibility of a pharmacokinetic alteration may be discarded. Thus, the analgesic response observed after the administration of the opiate agonist may indicate supersensitivity of the opioid brain receptors in undernourished rats. Since MOR induces its analgesic effect by selectively interacting with the µ-type opioid receptor (Olson et al. 1994), it follows that undernourished rats present supersensitive µ-type opiate brain receptors. In turn, this higher reactivity to MOR indicates that the lower analgesic response observed in the undernourished rats subjected to stressful situations may be the consequence of reduced release of opiates caused by such situations, and that the development of supersensitivity of the opiate receptors was not sufficient to compensate for the deficit in opiate release. In support of this, a reduced release of endorphin following stressful situations has been demonstrated in undernourished rats (Perry and Izquierdo 1989, Vendite et al. 1985).
our laboratory has demonstrated that the combined treatment with MOR or β-endorphin and stress in undernourished rats causes adaptive changes in their brain monoaminergic receptors (Keller et al. 1994, and in press), suggesting that the absence of this adaptive change in response to stress following early undernutrition was due, at least in part, to a functional deficiency in the activation of an endogenous opiate mechanism, triggered by repeated aversive experiences (Keller et al. 1994, and in press). Since several reports demonstrated alterations in different brain receptors as a consequence of early life undernutrition (Keller et al. 1982, Wiggins et al. 1984), the supersensitivity observed in undernourished rats may be due to a higher density of μ-type brain receptors; however, signal transduction alterations (G protein and/or effectors) cannot be discarded. Even though further investigation is necessary to clarify this topic, it should be kept in mind that present as well as previous evidence from our laboratory strongly suggest that perinatal undernourishment may result in a permanent functional deficit in the opiate process involved in behavioral responses to stress.

Recent evidence has shown that the modification taking place in the endogenous opiate system during development induces in adult rats behavioral alterations similar to those reported for undernourished rats (De Cabo et al. 1995). Furthermore, several reports indicate that the endogenous opiate system participates directly in the elaboration of behavioral adaptations to stressful stimuli, and that the analgesia induced by opiate release when animals are confronted with a certain stressful situation would allow the animal to adequately interact with the stressful stimulus, and therefore reach an appropriate balance with the environment (Amir et al. 1980, Amit and Galina 1986). That is to say, opiates also participate in the elaboration and expression of the emotional response to stress (Amir et al. 1980). The preceding results strongly indicate that further research on the functioning of the endogenous opiate system in undernourished animals may elucidate the neural basis of behavioral alterations observed in these animals.

ACKNOWLEDGMENTS

We are grateful to Gabriela Bazán for her English technical assistance and to Elsa R. Pereyra for her laboratory technical assistance.

LITERATURE CITED

