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A Developmental Study on Stress-Induced Antinociception Measured by the Tail Electric Stimulation Test

ANDRÉS PUJOL,* CARLOS DE CABO,*
MARIA ISABEL MARTÍN† AND MARIA PAZ VIVEROS*[1]

**Departamento de Biología Animal II, Facultad de Biología, and †Departamento de Farmacología, Facultad de Medicina, Universidad Complutense, E-28040 Madrid, Spain*

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PUJOL, A., C. DE CABO, M. I. MARTÍN AND M. P. VIVEROS. *A developmental study on stress-induced antinociception measured by the tail electric stimulation test.* PHARMACOL BIOCHEM BEHAV 46(2) 373–376, 1993.—The possible influence of weaning on the development of different neural mechanisms involved in stress-induced antinociception (SIA) was studied. Male Wistar albino rats were used for studies on adult and pre- and postweanling rats of 20 and 25 days of age, respectively. Animals were stressed by warm-water (20C) swimming for 3-min periods. Antinociception wasd assessed by the tail electric stimulation test. The thresholds for the motor response (tail withdrawal) (TW), vocalization during stimulus (V), and vocalization afterdischarge (VAD) were recorded. These responses are considered to be integrated at spinal, medulla oblongata, and diencephalon-rhinencephalon levels, respectively. In 20-day-old neonates, swimming stress only induced significant increases in the VAD thresholds that were not significantly reversed by naloxone (NAL) (1 mg/kg). Twenty-five-day-old rats showed increased thresholds for the three nociceptive responses after stress, the effects on TW and V being antagonized by NAL. Adult rats subjected to stress showed increased thresholds for the three responses, an effect that was antagonized by NAL in all cases. These results suggest that the weaning period might be critical for the development of the mechanisms mediating SIA. Besides, a different involvement of opioid systems throughout development, particularly in relation to the affective/emotional component of pain, is also suggested.

Pre- and postweanling rats Stress-induced antinociception Opioid mediation Tail electric stimulation test

THERE is a substantial amount of literature indicating that diverse forms of stress activate intrinsic pain-inhibitory systems. This is the well-known phenomenon of stress-induced antinociception (SIA), which has been widely studied in adult rodents. There are both opioid and nonopioid systems involved in SIA that can be differentially activated depending not only upon the type of stressor but also upon different parametric variables including severity, duration, and/or pattern of exposure to stress [for review, see (1, 2)].

There are relatively few reports on SIA during development, but several studies using maternal deprivation (8), electric shock (4), cold-water swimming (5), cold stress (6), and warm-water swimming (7,10) demonstrated that all those stressors induce antinociception in the neonate. Some of the mentioned ontogenetic studies indicate that either opioid or nonopioid forms of SIA can be activated depending upon the developmental period studied (4,7). Moreover, an interesting difference between preweanling and postweanling rats has been recently reported, showing that swim stress-induced antinociception appears to involve μ-receptor activation at day 20 and δ-receptor mediation at day 25 and in adulthood (7,10). A major question that remains to be elucidated is the possibility of a differential implication of neural structures at different levels within the CNS in the phenomenon of SIA in pre- and postweanling rats. In fact, to evaluate antinociception the above-mentioned developmental studies used nociceptive responses that are essentially spinally coordinated reflexes (tail immersion, tail-flick). The aim of this work was to investigate the hypothesis indicated by using a warm swimming protocol to induce antinociception and evaluating this SIA by the tail electric stimulation test. This procedure, which allows the study of different pain reactions integrated at different levels in the CNS (3), has been recently employed by our group to study the development of basal nociceptive responses in pre- and postweanling rats (16).

[1] To whom requests for reprints should be addressed.

METHOD

Animals and Experimental Conditions

Male Wistar albino rats were used for studies on adult (355 g) and 20- and 25-day-old rats. All animals were maintained at a constant temperature (20 ± 1 °C) and a reverse 12 D : 12 L cycle (light on at 2000 h). Food and water were available ad lib. Litters were sex balanced, culled to 10 pups per dam, and, when possible, cross fostered. Twenty-five-day-old rats were weaned at day 21, defining the day of birth as day 0. Twenty-day-old pups (of both genders) remained with the mother except during drug administration, nociceptive testing, and swimming to minimize stress due to maternal deprivation. Adult and postweanling rats were housed in groups of five males per cage.

Animals were equilibrated in a quiet, windowless room during approximately 1 h before experimentation. Experimental procedures were carried out between 0930 and 1430 h under similar temperature, humidity, and illumination conditions as those in the animal house. Each experimental group was formed by at least two litters or home cages (in the case of adult rats) that were tested on separate days to avoid interlitter and interday variation. The testing and data recording were performed by an observer who was unaware of the diverse treatments in each experiment.

Swim Stress Procedure, Nociceptive Testing, and Drug Administration

Ten minutes before swim stress, animals received either 0.9% saline or naloxone HCl (Sigma Chemical Co., St. Louis, MO) (1 mg/kg) IP in dose volumes no greater than 0.4 ml.

The swim stress protocol consisted of placing animals individually in 20 ± 1 °C water for a period of 3 min as previously described for the three ages studied in this work (7). For adult rats, the water was contained in a methacrylate tank (40 × 60 × 44 cm deep) and for neonates a smaller plastic tank (30 × 50 × 30 cm deep) was employed. At the end of the swimming period, rats were removed from the water, gently dried, and returned to the home cage before nociceptive testing was performed. Nociception was assessed using the tail electric stimulation test. Animals were placed in horizontal aerated plastic cylinders (Cibertec) of adequate dimensions and their tails carefully cleaned before spreading conductive gel at the base of the tail, where two electrodes were attached 1–2 cm separated from each other. The electrodes were connected to a stimulator (SH-92 Model, Cibertec) delivering the current (pulse frequency 60 Hz, train duration 100 ms, train interval 5 s, 64 steps). The initial intensity was 0.05 mA, increasing in steps of 0.06 mA until the responses were observed. The thresholds for the motor response (tail withdrawal) (TW), vocalization during stimulus (V), and vocalization after cessation of the stimulus [vocalization after discharge (VAD)] were assessed for each rat. These responses are considered to be integrated at spinal, medulla oblongata, and diencephalon-rhinencephalon levels, respectively (3,12,14,15). All animals were tested for nociceptive responses immediately before drug or saline administration and 5 min following swimming stress. Control, unstressed rats were tested at equivalent time points. Thus, experimental groups for each age were: saline-injected unstressed (S), saline-injected swim-stressed (SSW), and naloxone-injected swim-stressed (NSW). The number of animals per condition was as follows: for 20-day-old rats, S = 9, SSW = 14, and NSW = 14; for 25-day-old rats, S = 8, SSW = 17, and NSW = 17; and for adult animals, S = 10, SSW = 10, and NSW = 8. To compare the different treatments, antinociception was quantified using the following formula:

$$\frac{\text{Intensity after treatment}}{\text{Intensity before treatment}} = \frac{\text{TI}}{\text{CI}}$$

Additional comparisons between pretreatment and posttreatment responses were performed to assess possible sensitization phenomena. In all animals, the intensity (mA) was recorded for: tail withdrawal, vocalization, and vocalization afterdischarge before (CI) as well as after (TI) each treatment.

Statistical Analysis

Data analysis was carried out using the Wilcoxon signed rank test for comparisons between nociceptive responses be-

TABLE 1

NOCICEPTIVE RESPONSES TO THE TAIL ELECTRIC STIMULATION TEST BEFORE AND AFTER EACH TREATMENT

		S		SSW		NSW	
Response	Age	CI	TI	CI	TI	CI	TI
TW	20 d	0.39 ± 0.05	0.40 ± 0.04	0.36 ± 0.03	0.40 ± 0.03	0.36 ± 0.02	0.31 ± 0.02*
	25 d	0.39 ± 0.04	0.40 ± 0.03	0.29 ± 0.02	0.38 ± 0.03†	0.32 ± 0.02	0.25 ± 0.01*
	Adults	0.49 ± 0.05	0.47 ± 0.04	0.36 ± 0.06	0.52 ± 0.06*	0.62 ± 0.09	0.47 ± 0.07
V	20 d	0.92 ± 0.06	0.82 ± 0.06*	0.84 ± 0.03	0.89 ± 0.04	0.80 ± 0.04	0.76 ± 0.07
	25 d	1.19 ± 0.09	0.97 ± 0.09*	0.94 ± 0.08	0.94 ± 0.08	0.97 ± 0.07	0.80 ± 0.05*
	Adults	2.47 ± 0.28	1.99 ± 0.23*	1.54 ± 0.16	1.84 ± 0.21	1.85 ± 0.32	1.35 ± 0.26
VAD	20 d	1.15 ± 0.07	1.06 ± 0.07	1.04 ± 0.04	1.42 ± 0.06‡	0.96 ± 0.04	1.20 ± 0.10*
	25 d	1.59 ± 0.10	1.35 ± 0.13*	1.29 ± 0.10	1.82 ± 0.16‡	1.27 ± 0.08	1.56 ± 0.11*
	Adults	3.20 ± 0.30	2.73 ± 0.29	2.44 ± 0.39	3.11 ± 0.36	2.74 ± 0.49	1.88 ± 0.25

*$p < 0.05$, †$p < 0.001$, ‡$p < 0.01$, (Wilcoxon signed rank test).

S = saline injection, SSW = saline injection plus swimming, NSW = naloxone injection plus swimming, TW = tail withdrawal, V = vocalization, VAD = vocalization after discharge. Values are means (intensity in mA ± SE. Number of animals: 9–14 for 20-day-old rats, 8–17 for 25-day-old rats, and 8–10 for adults. Comparisons between responses before (CI) vs. after (TI) each treatment.

fore and after each treatment and Kruskal-Wallis one-way analysis of variance (ANOVA) followed by Mann-Whitney *U*-test for comparisons between treatments.

RESULTS

The analysis of the data from control unstressed groups revealed that saline injection did not significantly increase the nociceptive thresholds in any of the cases studied. Further, vocalization responses appeared at significantly lower intensities during the second exposure to the nociceptive test in 20- and 25-day-old rats and in adults (Table 1).

Statistical comparisons among the different experimental treatments rendered the following results. In 20-day-old rats, swimming stress did not significantly increase the thresholds for the motor response or vocalization. However, a significant increase was found for vocalization afterdischarge following swim stress ($p < 0.001$), an effect that was reduced but not antagonized by naloxone (Fig. 1a). Twenty-five-day-old rats showed increased thresholds for the three nociceptive responses after swimming stress ($p < 0.05 - p < 0.001$); this effect was antagonized by naloxone for both motor response and vocalization. In contrast, and similarly to 20-day-old neonates, the stress-induced effect on vocalization afterdischarge was only reduced but not significantly reversed by the opioid antagonist (Fig. 1b). Thus, at 20 and 25 days of age naloxone did not antagonize but appeared to attenuate the increases in thresholds for VAD. In fact, at both ages the differences between NSW and S treatments were of lower significance than the differences between SSW and S treatments. Adult rats subjected to swimming stress showed significantly increased thresholds for the three responses ($p < 0.05$), an effect that was antagonized by naloxone in all the cases (Fig. 1c).

Discussion

The relatively few works on SIA during development indicate that this phenomenon can be observed in the neonate (4-8,10). All these studies used nociceptive tests that measure responses that are essentially spinally coordinated reflexes, that is, tail immersion, tail-flick. Such responses provide little information about the emotional/affective aspects of pain, while measures like vocalization response to electrical stimulation of the tail are more informative in this respect (12).

An interesting neonatal transition in the type of opioid receptor mediating SIA around the weanling period (7,10) has been recently reported. This finding raises the question of a possible differential involvement of neural structures at different levels within the CNS in pre- and postweanling rats. The present work represents the first approach to this hypothesis by using the tail electric stimulation test, which allows the study of different pain reactions integrated at different levels within the CNS (3,14,15).

The results obtained for the control groups suggest the existence of learning processes that might account for the diminished nociceptive thresholds in the second exposure to the nociceptive test. This effect may involve structures, such as the limbic system, associated with learning and memory, particularly in the case of the vocalization afterdischarge, a response that is considered to be integrated at diencephalon-rhinencephalon levels (3,14,15). Besides, the results allow us to rule out any antinociceptive effect induced by handling. In fact, the only significant effect observed following saline injection was actually a decrease in the nociceptive thresholds for vocalization.

According to previous data (7,10), a 3-min swim at 20°C induced antinociception in 20- and 25-day-old rats and in adults. Besides, the use of the tail electric stimulation test provided additional information with respect to the neural structures involved in SIA throughout development. Comparisons between treatments at each age show that there are interesting differences regarding the effects observed at the three ages studied. Thus, at 20 days of age (preweanling rats) swimming stress caused only a significant increase in the threshold

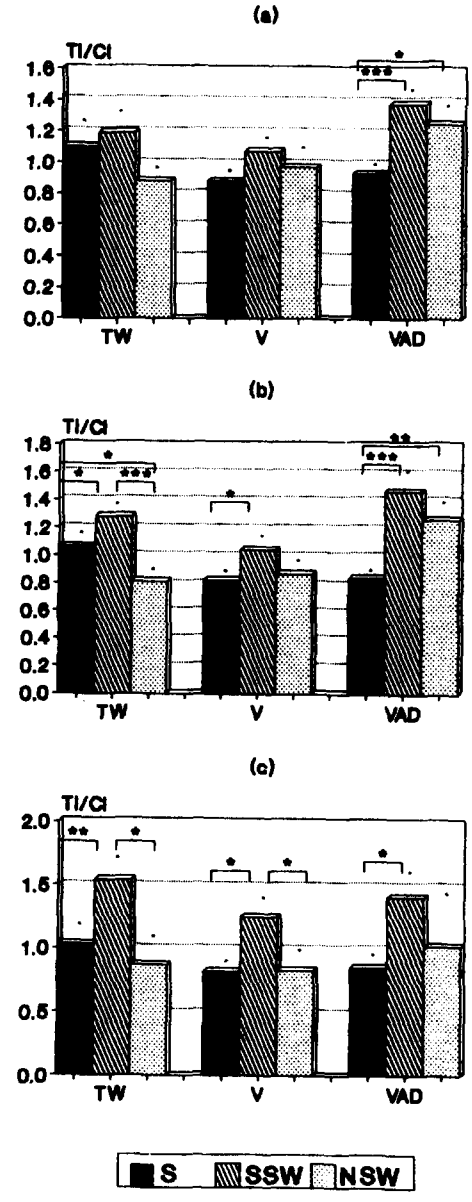

FIG. 1. Comparisons between treatments in (a) 20-day-old rats, (b) 25-day-old rats, and (c) adult rats. S, saline injection; SSW, saline injection plus swimming; NSW, naloxone injection plus swimming; TW, tail withdrawal; V, vocalization; VAD, vocalization afterdischarge. Histograms show means ± SEM of values obtained by using the formula indicated in the text: intensity after treatment/intensity before treatment (TI/CI). Significant differences between each two treatments are indicated by horizontal lines. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ (Kruskal-Wallis and Mann-Whitney *U*-test)

for vocalization afterdischarge, although a similar tendency was observed for the other nociceptive responses. However, in 25-day-old rats (postweanling animals) the stress induced a marked antinociception at the three levels studied. Moreover, the SIA observed in these rats, at spinal and medulla oblongata levels, was antagonized by naloxone and therefore appears to be an opioid-mediated phenomenon. In contrast, the stress-induced effect on vocalization afterdischarge was not significantly reversed by naloxone in 20- or 25-day-old rats, which indicates the involvement of nonopioid components. Mediation of opioid receptors other than μ should not be disregarded because naloxone appears to attenuate the antinociceptive effect of stress at these early ages. However, an exclusively opioid mediation at this level is unlikely given the relatively high dose of naloxone administered.

The relative discrepancy with respect to previous works (7) in which a clearly opioid-mediated SIA was reported in 20-day-old rats—as measured by the tail immersion test—might be due, at least partially, to the different nociceptive stimulus employed. However, our observations indicate that the motor response was the most difficult to evaluate due to the slight but continuous trembling of the tail after swim stress.

With respect to adult animals, our results indicate that swim stress induced a marked antinociception that was shown in the three responses recorded. Besides, the effect was antagonized by naloxone at spinal, medulla oblongata, and subcortical levels, indicating an opioid mediation in all cases.

It has been recently reported that κ-agonists are inactive against electrical stimulation of the tail (11). Therefore, it might be that non-κ-opioid receptors are involved in the antinociceptive effect integrated at subcortical levels in the adult rats. Moreover, the stimulus used in this work can be considered of relatively high intensity, and it has been previously reported that the effects of κ-agonists tend to disappear as the intensity of the stimulus increases (12,13).

In a previous study using the tail electric stimulation test, we found that the weaning process might influence the basal nociceptive responses integrated at subcortical levels (16). As a whole, the present results suggest that there is a progressive maturing of the mechanisms mediating SIA throughout development. Although the effect of age per se cannot be ruled out as accounting for the observed effects, there is strong indirect evidence that indicates that the weaning process is a major factor involved. As mentioned above, it has been reported that SIA is mediated by μ-opioid receptors in 20-day-old pups and by μ- and δ-receptors in 25-day-old rats and adults when animals are weaned at day 21 (7,10). More recently, the same research group reported that if weaning is delayed the change in opioid receptor control of SIA is concomitantly delayed. In fact, when weaned and nonweaned 25-day-old rats were compared only weaned animals showed a δ-mediated SIA (9). Taking into account these findings, it is likely that the process of weaning, more than age per se, can account at least for the differences between 20- and 25-day-old animals observed in this work.

In conclusion, the special characteristics of the nociceptive test employed allowed us to provide new data concerning diverse aspects of pain perception and tolerance, which were beyond previous works using other methodology. The present work supports the view that weaning period may be critical for the establishment of the neuroendocrine mechanisms mediating SIA.

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