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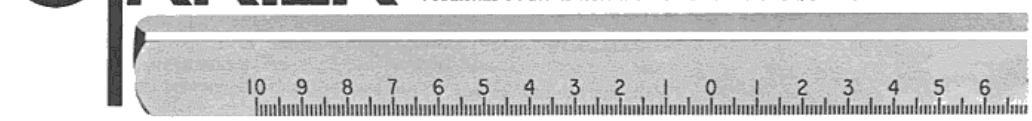

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LONG-TERM EFFECTS OF PRENATAL STRESS ON ACUTE AND TONIC PAIN IN MALE RATS

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Introduction

Published data indicate an alteration of pain sensitivity to acute nociceptive irritants in offspring born to females subjected to stress during pregnancy (8, 18-20). Changes in characteristics of acute pain produced by prenatal stress were demonstrated both in early postnatal ontogenesis and in adulthood. Tonic nociceptive system activity after prenatal stress during "critical periods" of embryogeny (7) was investigated at the time of early ontogenesis only (1). Data on anatomical, neurophysiological and neurochemical differences between acute and tonic nociceptive systems (2, 11) let us suggest that there is a distinct mechanism in influences of prenatal stress on these systems. Effects of prenatal stress on pain sensitivity can be mediated by changes in the hormonal, monoaminergic and opioid systems in the developing brain (6, 8, 12, 13, 15-17). However, the basic neurobiological mechanisms responsible for these effects remain unclear. The formalin test, a widely accepted model for the study of nociception, permits an evaluation of acute and tonic pain by characteristics of two different phases of the specific behavioral response to subcutaneous injection of formalin (5). The first acute phase is presumed to result from the activation of small diameter primary afferent nociceptive fibers. The second tonic phase is characterized by patterns of prolonged shaking, flexing and licking of the paw injured with formalin. This second or tonic phase develops as tissue inflammation and is considered to result from sensitization of

primary afferents and spinal cord dorsal horn neurons. There is the interphase between them during which there are no pain behaviors. The first and the second phases are believed to represent acute and tonic nociception, respectively (3). The aim of the present study was to investigate effects of prenatal stress on acute and tonic phases in the formalin test in 90-day-old male rats, the age generally used for the evaluation of long-term consequences of prenatal stress on an organism.

Methods

The guidelines published by the Committee for Research and Ethical Issues of the IASP on ethical standards for investigations of experimental pain in animals were followed (4). Ninty to 100-day-old Wistar rats (n=17) were used for breeding. Male-female pairs were housed together overnight and the next morning a vaginal smear to detect sperm was used to determine pregnancy. Pregnant females were individually maintained in standard conditions with access to food and water. Eight females were subjected to daily immobilization for 30 min twice a day (at 10 and 16 hr) during days 16-21 of pregnancy. Nine females remained undisturbed throughout pregnancy. On postnatal day 3 all litters were reduced to eight pups. Sixteen males born to stressed females, and 18 males born to non-stressed (intact) females were maintained in the laboratory vivarium in groups of 4-5 animals until 90 days of age. Two males only were used from each litter. Before the experiment each animal was placed in a Plexiglas box (25x25x25 cm) for 10-15 min of adaptation. Each male was injected subcutaneously with formalin (2,5%, 50 µl), into the plantar pad of the left hind paw and observed in the experimental box. Five males born to intact and five to stressed females were used as controls. Controls were injected with saline and observed like experimental rats. The number of flexes and shakes and duration of licking were recorded each min during 90 min. The intensity of formalin-induced behaviors (the number of flexes+shakes and duration of licking) was analyzed during the first and the second phases of the specific behavioral response. In addition, the time course of formalin-induced pain was evaluated by analyzing separately the duration of the first phase, the interphase and the second phase. Pain-related behavior is a reliable measure of the central transmission of nociceptive signals in the formalin test.

Statistical analysis was performed by Dr. E.A. Vershinina, in the Department of Applied Mathematics of the I. P. Pavlov Institute of Physiology headed by Dr. R.A. Bedrov. Data were analyzed with the use of the Wilcoxon rank pair test, along with Student's t-test for dependent variables and the Mann-Whitney test for independent variables. For all tests $P < 0.05$ was considered as statistically significant.

Results

In prenatally non-stressed (intact) males injection of formalin evoked the specific behaviors of lifting, flexing, shaking and licking the injected paw. There were two phases of responses: the first short phase (4.6 ± 0.7 min) and the second prolonged phase (47.6 ± 3.3 min). The interphase lasting 12.7 ± 2.0 min occurred between two phases during which the specific behaviors were not shown. Similar injection of saline to controls did not produced any nociceptive behaviors. The average intensity of behavioral responses did not differ during the first phase of the formalin test between intact and prenatally stressed male rats both in flexing+shaking and in licking behaviors and was 19.9 ± 4.5 vs 17.5 ± 4.6 and 14.1 ± 4.0 min vs 15.9 ± 6.9 min, respectively.

During the second phase of the formalin test the average intensity of flexing+shaking in intact males reached 190.4 ± 29.9 while in stressed males 112.3 ±

28.8 ($p<0.05$) (Fig. 1, a). The duration of licking in intact rats averaged 251.3 ± 25.9 min, while that in prenatally stressed rats significantly increased ($p<0.05$) and averaged 404.1 ± 56.9 (Fig. 1, b).

The average duration of both phases measured both in flexing+shaking and in licking behaviors did not differ between intact and prenatally stressed males (Fig. 2). The duration of the first phase in flexing+shaking and licking behaviors was 4.6 ± 0.7 min and 3.7 ± 0.8 min, in intact and 1.3 ± 0.4 min and 1.4 ± 0.4 min, in prenatally stressed males. The duration of the interphase in flexing+shaking behavior in intact males was 12.7 ± 2.0 min, in stressed males it significantly decreased to 6.7 ± 1.5 ($p<0.05$). The duration of the interphase in licking behavior did not differ between non-stressed (7.7 ± 2.2 min) and stressed (7.9 ± 2.7 min) males.

In controls, injection of saline both in intact and prenatally stressed rats did not produce any pain behaviors.

Discussion

The important results of the data presented here are, first, establishment of the influence of prenatal stress on tonic pain, caused by inflammation in adult rats, and second, the discovery of differences in effects of prenatal stress on characteristics of two distinct phases of the specific behavioral response in the formalin test, that are considered to represent acute and tonic pain. Prenatal stress resulted in a decrease in intensity of pain responses organized at the spinal level (flexing+shaking behavior) and an increase in intensity of pain responses organized at the supraspinal level (licking behavior). Such reorganization of patterns of behavioral responses in the formalin test could result from impairment of the mechanisms of inner inhibition in CNS caused by imbalance of monoamines that could be created by prenatal stress (9, 17). Females were subjected to immobilization during the last week of pregnancy, critical for the development of hormonal and monoaminergic systems of a fetus (12, 13, 15-17). Prenatal stress is known to evoke irreversible long-term alterations of neuroendocrine regulation of behavior and changes of adrenocortical reaction on acute stress (17). Significant alterations in brain noradrenergic and serotonergic systems were shown in adult rats after prenatal stress during particular "critical" periods of embryogeny (16, 21). The appearance of responses during the interphase and decrease of its duration that we observed in prenatally stressed males may be accounted for by the impairment of the brain monoaminergic descending inhibitory system, that controls nociceptive signals at the level of the dorsal horn of the spinal cord. Such an assumption can be made on the basis of results obtained with the use of antagonists for noradrenaline and serotonin in the formalin test (14). The behavioral response of adult rats observed by the authors in that study was similar to that after prenatal stress in our experiments.

In prenatally stressed males characteristics of behavioral responses during the first, acute phase, that has a protective function, showed no changes. Significant alteration of the second phase associated with the process of inflammation that is under the influence of sympathetic and hormonal systems, indicates that the effect of prenatal stress on tonic pain can be mediated through these systems. Our results find confirmation in studies showing differences between acute and tonic pain in the neurochemical nature and in the modulating inhibitory descending NA influences (2, 10).

Thus, effects of prenatal stress on the acute and tonic phase in the formalin test were firstly studied in adult 90-day-old rats. Long-term consequences of prenatal stress on pain sensitivity in the formalin test manifested themselves in impairments of

mechanisms of tonic pain evoked by inflammation while characteristics of acute phase were not changed. Results obtained suggest that the effects of prenatal stress on acute and tonic pain in the formalin test are mediated by different mechanisms and support data on distinct mechanisms of two types of pain.

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Figure legends

Fig. 1. The effect of prenatal stress on the intensity of the biphasic specific nociceptive behavior response in the formalin test in 90-day-old males.

a - the number of flexes+shaking, b – the duration of licking (sec). 1 – the first phase, 2 – the second phase of responses. The data are represented as mean ± S.E.M., * P <0.05, prenatally stressed, vs intact.

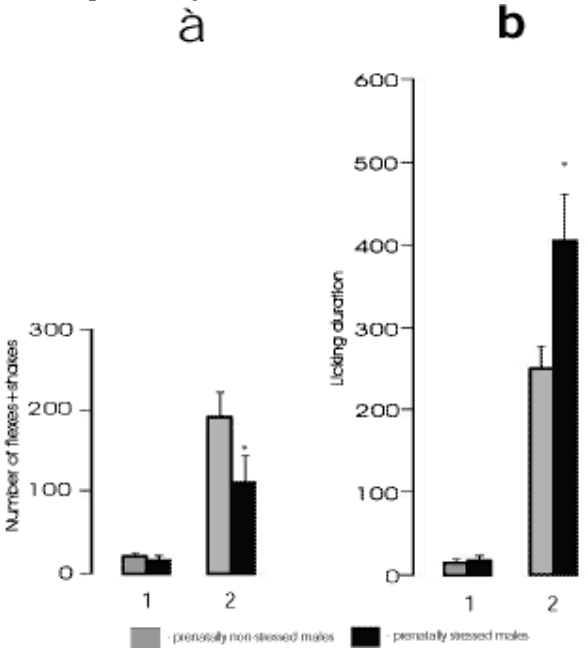

Fig. 2. The effect of prenatal stress on the duration of phases and the interphase in the formalin test in 90-day-old males.

a, b – the duration of the first (1), second (3) phases and the interphase (2) in flexing+shaking and licking behaviors, respectively.