Effect of Prenatal Stress on Tonic Pain in Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 131, No. 6, pp. 608-611, June, 2001
Original article submitted February 6, 2001

The effect of prenatal stress on specific biphasic behavioral response in 25-day rat pups was studied using the experimental model of formalin-induced tonic pain. Prenatally stressed rats showed hypersensitivity to tonic nociceptive stimulation manifested in increased amplitude and duration of the response. Behavioral responses observed during the interphase period attest to impairment of inhibitory processes (more pronounced in females). These findings suggest that the interphase interval is the period of active inhibitory process rather than rest period.

Key Words: *prenatal stress; tonic pain; formalin test; ontogenesis; rats*

Stress factors acting during critical periods of embryogenesis can disturb structure and function of cerebral structures and lead to various behavioral abnormalities in the offspring [1,2,9,13]. Published data on functional changes in phasic nociceptive and antinociceptive systems induced by prenatal stress in rats of both sexes are scanty and contradictory [8,14,15]. The effects of early postnatal stress on parameters of tonic pain were reported [4]. However, we found no reports on the effect of prenatal stress on these parameters. Clinical studies demonstrated changes in nociception after interventions made during the prenatal period. It is well known that the effects of early postnatal and prenatal stress exposures on developing organism are different.

Formalin test is widely used as a model of tonic pain [6]. Injection of formalin into hindpaw of mature rats induces a biphasic pattern of nociceptive behavior: short phase I and more prolonged phase II separated by a rest period (interphase interval, IPI). Phase I is a response to acute pain, phase II reflects the development of inflammatory process resulting from sensitization of primary afferents and neurons in the dorsal horns of the spinal cord. The mechanisms underlying these phases are now intensively studied. Phase I can be induced in fetuses and neonatal rats, while phase II appears only on postnatal week 3. There are published data that phase II can be induced in 25-day-old pups [5]. We assume that ontogenic approach can help to study the peculiarities of the development of tonic pain system after early prenatal damages.

Taking into consideration the importance of the effect of prenatal stress on adaptive behavior and growing attention to the problem of tonic pain (less studied compared to phasic pain [10]), our aim was to evaluate the effect of prenatal stress during critical ontogenic periods on tonic pain in 25-day-old rats.

MATERIALS AND METHODS

Experiments were carried out on random-bred albino male Wistar rats. Five males were caged with 8 females. The day when the spermatozoa were detected in vaginal smear was considered as pregnancy day 1. Pregnant rats were caged individually and maintained under standard conditions. Control rats (n=5) were not stressed, while experimental rats (n=5) were immobilized for 30 min in the morning and evening every day during pregnancy days 16 to 21. Starting from day 3 after delivery, each dam was allowed to rear 8 pups (four male and four female pups, when possible). In 25-day-old stressed and non-stressed males and females (13 rats in each group) the biphasic behavioral response (BPBR) induced by plantar subcutaneous injection of formalin (2.5%, 20 μl) [6] was studied. The controls received the same volume of physiologi-

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0007-4888/01/1316-0515$25.00

cal saline. Before the experiment, the rats were adapted during 30 min in a glass chamber (25×25×25 cm). The temperature was maintained at 20°C. In each rat, the numbers of flexions and shakes and the duration of licking of injected hindpaw per 1 min (indices of pain in the formalin test [6]) were recorded for 60 min. The total number of flexions and shakes and the duration of licking in phases I and II of specific BPBR of nonstressed and prenatally stressed males and females were analyzed. We also analyzed the duration of IPI, phase I, and phase II.

The data were statistically analyzed using Wilcoxon's test for linked and Mann—Whitney's test for independent variables, respectively. The differences were significant at $p<0.05$ (for two-way alternative).

RESULTS

In 25-day-old pups born by nonstressed females, subcutaneous injection of formalin induced a specific BPBR (flexion, shaking, and licking of the damaged paw). Duration of phases I and II were 1-4 and 12-24 min, respectively, IPI lasted for 6-9 min (during this period no specific behavioral acts were observed). Subcutaneous injection of physiological saline produced no such response [5].

The 25-day-old pups, which were stressed prenatally, demonstrated significant changes in the intensity of BPBR induced by formalin and in the duration of IPI and both phases of BPBR. Significant increase in the number of flexions+shakes of the injected paw was observed in females during phase II ($p<0.05$), while in males it was manifested during both phase I ($p<0.02$) and phase II ($p<0.001$). These differences were more pronounced in males (Fig. 1, *a*). Licking was insignificantly prolonged in prenatally stressed pups in both phases (Fig. 1, *b*), although females demonstrated significant difference at 5% level within one-way alternative, while in males there was a decrease in data scattering during phase II ($p<0.01$).

Analysis of the total number of flexions+shakes showed that stress markedly decreased the duration of IPI in females ($p<0.001$) compared to males ($p=0.07$, Fig. 2, *a*). Scattering this index in prenatally stressed rats drasticly decreased and the differences between males and females became significant ($p<0.001$, Fig. 2, *a*). Mean duration of phase II evaluated by the total number of flexions and shakes increased both in females and males ($p=0.006$ and $p<0.001$, correspondingly). Analysis of duration of licking of the injected paw in stressed pups revealed the difference between females and males in IPI duration ($p=0.014$). The scattering of this index decreased pronouncedly, while its mean value dropped insignificantly. A significant increase of phase II duration was observed in stressed females and males ($p=0.034$ and $p=0.002$, correspondingly), while the duration of phase I significantly increased in females ($p=0.004$), but did not change in males (Fig. 2, *b*).

Therefore, significant changes in the parameters of tonic pain in prenatally stressed 25-day-old rat pups were demonstrated using the formalin test. An increase in specific BPBR, prolongation of its phases, and shortening of IPI attest to enhancement of nociceptive sensitivity to tonic nociceptive stimulation. The observed changes in BPBR can result from disturbances in monoaminergic mechanisms responsible for the control of long-term nociceptive traffic to suprasegmentary structures and the spinal cord caused by prenatal stress. This hypothesis is based on the data attesting to activation by tonic pain of the monoaminergic (noradrenergic and serotoninergic) descending

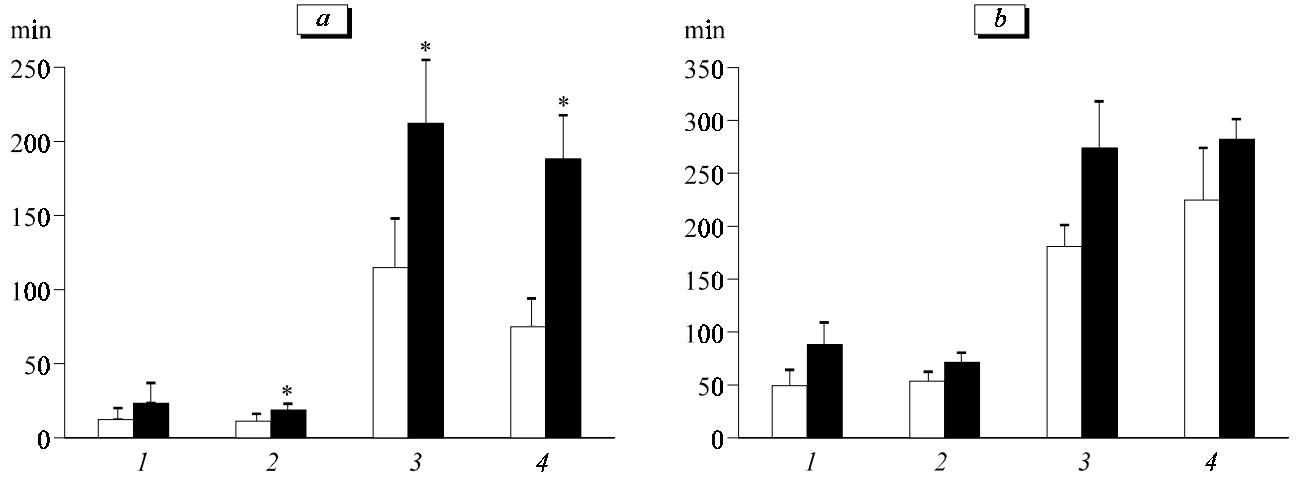

Fig. 1. Effect of prenatal stress on intensity of specific biphasic behavioral response induced by formalin injection in 25-day-old rat pups: *a*) the number of flexions and shakes and *b*) duration of licking. Phase I (*1*, *2*) and phase II (*3*, *4*) of the response in female (*1*, *3*) and male (*2*, *4*) rat pups. Here and in Fig. 2: open and solid bars correspond to nonstressed and stressed rats. **p*<0.05 compared to nonstressed rats.

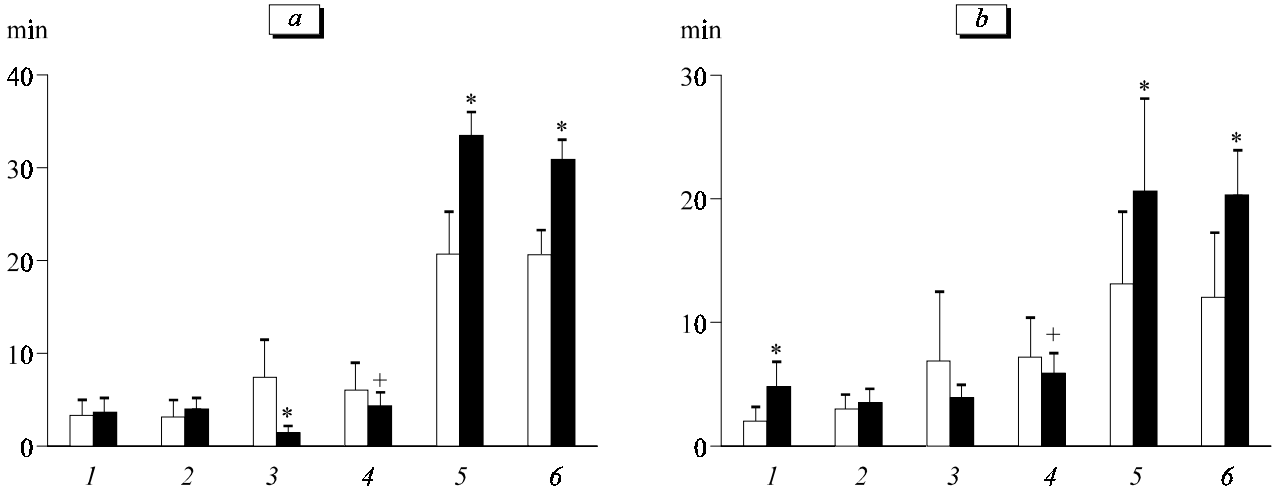

Fig. 2. Effect of prenatal stress on duration of phases I (*1*, *2*) and II (*5*, *6*) of specific biphasic behavioral response induced by formalin injection in 25-day-old rat pups. *a*) Duration of flexions and shakes and duration of licking (*b*) in females (*1*, *3*, *5*) and males (*2*, *4*, *6*), respectively. $^{+}p<0.05$ difference between stressed males and females are significant.

inhibitory system of medulla oblongata, which controls nociceptive traffic via the feedback mechanism at the level of dorsal horns of the spinal cord [11]. Subcutaneous injection of formalin pronouncedly increases the level of norepinephrine and serotonin in the dorsal horns of the spinal cord and activates the nociceptive supraspinal structures, which in its turn activate the monoaminergic descending inhibitory system in medulla oblongata. The data showed that intrathecal injection of epinephrine and serotonin antagonists to rats, which were not stressed during the prenatal period, evoked specific response during IPI and prolonged BPBR [11]. It is noteworthy that similar changes in specific BPBR were also observed in prenatally stressed rat pups.

In our experiments pregnant rats were immobilized during the last week of embryogenesis, which is critical period for the development of monoaminergic neurons [12]. Stress-induced elevation of blood glucocorticoids content in pregnant rats is a powerful factor, which can disturb the development of monoaminergic system in the fetus, the consequences of which will be manifested in the offspring [13]. Disturbances of monoaminergic neurotransmitter mechanisms in CNS produce an abnormal hyperactive generator, which damages the inhibitory mechanisms [3]. In our experiments this was indicated by facilitation of specific behavioral responses to tonic stimulus during IPI. This fact corroborates the arguments in favor of the hypothesis on inhibitory nature of IPI [7]. Since formalin test evokes no behavioral response to nociceptive stimulation during IPI, this period was considered as a rest period [6].

The criterion used for evaluation of BPBR evoked by formalin in this study is wider than the conventional criteria not only by the magnitude, but also by the duration of its phases and IPI. This criterion made it possible to reveal sex related differences in the effects of prenatal stress on tonic pain even in prepubertal rats: facilitation of the response during IPI was more pronounced in female rats.

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