Effects of Prenatal Stress on Formalin-Induced Acute and Persistent Pain in Adult Male Rats

I. P. Butkevich

Translated from *Byulleten' Eksperimental'noi Biologii I Meditsiny*, Vol. 133, No. 2, pp. 154-157, February 2002
Original article submitted October 25, 2001

Behavioral responses of 90-day-old male offspring from female Wistar rats exposed to restraint stress during the last week of pregnancy were studied in the formalin test. Specific biphasic behavioral response characterized acute (phase 1) and persistent tonic pain (phase 2). The intensity of nociceptive responses (evaluated by the number of flexions+shakings and by the duration of paw licking) in prenatally stressed rats changed only during phase 2. During interphase, facilitation of the flexion+shakings pattern (but not the licking pattern) in response to nociceptive stimulation was seen. The response intensity during phase 1 and the duration of both phases remained unchanged. Our findings suggest that prenatal stress modulates nociceptive sensitivity in 90-day-old offspring: it affects the duration of tonic (inflammatory), but not of acute pain. It is concluded that different mechanisms are responsible for the effects of prenatal stress on acute and persistent pain in the formalin test.

Key Words: *prenatal stress*; *formalin test*; *acute and tonic pain*

Prenatal stress modulates sensitivity to short-term nociceptive stimuli not only during early ontogeny, but also in adult animals [9,12,14,15]. We previously demonstrated that immobilization of pregnant females modulated specific biphasic response to nociceptive stimulation in their 25-day-old offspring [2]. Taking into account recent evidence on different neurophysiological and neurochemical characteristics of acute and persistent pain we assumed that the effects of prenatal stress on these two types of pain are realized via different mechanisms [6,10]. Formalin test is a classic model for evaluation of nociceptive sensitivity allowing evaluation of the two types of nociceptive systems [7]. Formalin injected subcutaneously into the paw induces a specific behavioral response (flexion, shaking, licking of the paw) consisting of two phases of different origin. The first (acute) phase is a result of chemical stimulation of nociceptive C and Aδ afferent fibers. The second phase (caused by inflammation) reflects sensitization of primary afferent fibers and neurons of the dorsal horns [13].

The aim of this work was to study the effects of restraint stress applied to female rats during critical period of fetal development on parameters of acute and tonic phases of specific behavioral response in the formalin test in their 90-day-old male offspring, *i. e.,* delayed effects of prenatal stress.

MATERIALS AND METHODS

Experiments were carried out on 90-day-old male Wistar rats (n=44). Their parents, 17 female and 10 male Wistar rats, were maintained in a vivarium. Pregnancy was determined by the presence of spermatozoa in vaginal smears. Pregnant rats were kept under standard vivarium conditions with free access to food and water. On 16 to 21 days of gestation, rats of the test group (n=8) were subjected to restraint stress consisting of two daily 30-min sessions in the morning and in the evening. To this end, the rats were placed into a narrow adjustable

Laboratory of Ontogeny of the Nervous System, I. P. Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg. ***Address for correspondence:*** but@kolt.infran.ru. Butkevich I. P.

0007-4888/02/1332-0130$27.00

cylinder. Control pregnant rats (n=9) were not subjected to immobilization. Prenatally stressed (n=21) and not stressed (n=23) male offsprings were kept in a vivarium before tests.

After 30-min adaptation in a individual chamber (25×25×25 cm glass aquarium), male rat was injected with formalin (2.5%, 50 μl, subcutaneously) into the hindpaw. The number of flexions and shakings per minute and the duration of paw licking (parameters of pain in the formalin test) were visually evaluated [7]. In the control group, prenatally stressed (n=5) and nonstressed male rats (n=5) received the same volume of saline. The number of flexions+shakings and duration of paw licking were analyzed during phase 1 and 2. The duration of phase 1 and 2, and the interphase were also measured.

The data were statistically analyzed by non-parametric Wilcoxon test for dependent variables and Mann-Whitney test for independent variables at p<0.05.

RESULTS

Formalin injected into the hindpaw induced a nociceptive biphasic response with specific flexing+shaking and paw licking patterns. Phases 1 and 2 of the response were separated by the interphase, when the animal did not demonstrated nociceptive behavior. Injection of physiological saline produced no nociceptive responses.

The intensity of acute response to formalin (phase 1) was similar in prenatally stressed and intact male rats, as evidenced from similar numbers of flexions+ shakings and duration of paw licking (Table 1). During the second phase, the mean number of flexion+ shakings in intact rats was significantly higher than in prenatally stressed rats (p<0.05; Table 1). During phase 2, the mean duration of licking in intact rats was significantly shorter than in prenatally stressed rats (p<0.05; Table 1).

The duration of phase 1 and 2 measured by the number of flexions+shakings and duration paw licking in male offspring of intact females and females subjected to restraint stress was similar (Table 2). The duration of interphase determined by flexion+shakings pattern differed significantly in intact and prenatally stressed male rats, but this difference was insignificant if licking duration was used as a measure of pain (Table 2).

Our findings indicate that stress during the last week of gestation affects nociception in 90-day-old male offspring. Our findings suggest that stress stimuli applied during critical periods of ontogeny [3] can produce delayed effects on various forms of behavior [1] and on pain sensitivity to not only transient, but also long-term painful stimulation [9,14,15]. We demonstrated different effect of prenatal stress on phase 1 and 2 behavioral response in formalin test (acute and persistent pain, respectively). Parameters of acute, or nocifensive reactions in prenatally stressed rats remained unchanged, while parameters of persistent pain changed significantly, compared to the control. The second phase, corresponding to inflammation-induced tonic pain is controlled by humoral and sym-

TABLE 1. Intensity of Behavioral Response in Formalin Test in Prenatally Stressed (n=21) and Nonstressed (n=23) Male Rats (M±m)

Parameters	Nonstressed		Stressed	
	phase 1	phase 2	phase 1	phase 2
Number of lexions+shakings	19.9±4.5	190.4±29.9	17.5±4.6	112.3±28.8*
Duration of raw licking, sec	14.1±4.0	251.3±25.9	15.9±6.9	404.1±56.9*

Note. Here and in Table 2: *p<0.05 compared to intact rats

TABLE 2. Duration (min) of Various Phases of Behavioral Response in Formalin Test in Prenatally Stressed (n=21) and Nonstressed (n=23) Male Rats (M±m)

Phases	Nonstressed		Stressed	
	flexions+shakings	paw licking	flexions+shakings	paw licking
1st	4.6±0.7	3.7±0.8	1.3±0.4	1.4±0.4
Interphase	12.7±2.0	7.7±2.2	6.7±1.5*	7.9±2.7
2nd	47.6±3.3	41.6±2.7	24.8±2.4	22.7±2.1

pathoadrenal systems. Functional activities of these systems in prenatally stressed animals are considerably changed [5]. Therefore, prenatal stress produced different effects on different parameters of pain. During phase 2, the paw-licking behavior realized at the supraspinal level in prenatally stressed rats dominated over spinal flexions+shakings pattern. This redistribution of behavioral patterns caused by prenatal stress probably reflects structural and functional rearrangements in CNS associated with impairment of inhibitory mechanisms. Shortening of the interphase in prenatally stressed rats, determined by the number of flexinons+shakings (but not licking duration), also attested to impairment of inhibitory mechanisms. In our experiments, pregnant rats were exposed to stress during the last week of gestation, which was critical for the development of monoaminergic and hormonal regulatory systems [5]. Neurotransmitter imbalance induced by prenatal stress can impair central inhibitory mechanisms in CNS [4] and monoaminergic descending inhibitory system modulating nociceptive signals in the spinal cord [11]. We hypothesize that different effects of prenatal stress on acute and tonic phases in the formalin test are due to different modulatory effects of the monoaminergic descending inhibitory pathways on acute and persistent pain.

We revealed long-term effects of prenatal stress on nociceptive sensitivity in the formalin test in male rats. Ninety-day-old male offspring from females subjected to restraint stress during the last third of gestation demonstrated considerable shifts in persistent but not acute pain. This study provided an additional evidence on inhibitory nature of the interphase [8] and different mechanisms responsible for acute and persistent pain [6,10].

Authors are grateful to E. A. Vershinina (Department of Statistics, I. P. Pavlov Institute of Physiology) for her help in statistical analysis of the results.

REFERENCES

1. A. S. Batuev, E. P. Vinogradova, and O. N. Polyakova, *Zh. Vyssh. Nervn. Deiat*., **46**, No. 3, 558-563 (1996).
2. I. P. Butkevich and E. A. Vershinina, *Byull. Eksp. Biol. Med*., **131**, No. 6, 608-611 (2001).
3. V. G. Kassil', V. A. Otellin, L. I. Khozhai, and V. B. Kostkin, *Ros. Fiziol. Zh.*, **86**, No. 11, 1418-1425 (2000).
4. G. N. Kryzhanovskii, *Byull. Eksp. Biol. Med*., **129**, No. 2, 124-128 (2000).
5. E. V. Naumenko, M. Vigash, A. L. Polenov, *et al.*, *Ontogenic and Genetic and Evolutionary Aspects of Neuroendocrine Regulation During Stress*, Novosibirsk (1990).
6. A. I. Basbaum, *Reg. Anesth.*, **24**, No. 1, 59-67 (1999).
7. D. Dubuisson and S. G. Dennis, *Pain*, **4**, No. 1, 161-174 (1977).
8. J. L. Henry, K. Yashpal, G. M. Pitcher, and T. J. Coderre, *Ibid.*, **89**, No. 1, 57-63 (1999).
9. C. H. Kinsley, P. E. Mann, and R. S. Bridges, *Pharmacol. Biochem. Behav.*, **30**, No. 1, 123-128 (1988).
10. W. J. Martin, N. K. Gupta, C. M. Loo, *et al.*, *Pain*, **80**, Nos. 1-2, 57-65 (1999).
11. K. Omote, T. Kawamata, M. Kawamata, A. Namiki, *Brain Res*., **814**, Nos. 1-2, 194-198 (1998).
12. D. A. V. Peters, *Pharmacol. Biochem. Behav.*, **17**, No. 6, 721-725 (1982).
13. S. Puig and L. S. Sorkin, *Pain*, **64**, No. 2, 345-355 (1994).
14. J. W. Smythe, C. M. McCornick, J. Rochford, M. J. Meaney, *Physiol. Behav.*, **55**, No. 5, 971-974 (1994).
15. W. F. Sternberg, *Ibid.*, **68**, Nos. 1-2, 63-72 (1999).