Genotype-Dependent Changes in Pain Thresholds in Adult Mice after Neonatal Treatment

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We studied the effect of neonatal treatment with pharmacological preparations (Semax and buspiron) and solvents (distilled water and physiological saline) on the pain threshold in 3-4-month-old mice of 6 genotypes. Neonatal administration of the solvent (nociceptive stimulation) decreased pain thresholds in DBA/2, 101/HY, and RSB males, but not in female mice and animals of other strains. Neonatal administration of Semax significantly increased pain thresholds in adult DBA/2 and 101/HY males compared to those in animals neonatally treated with the solvent. Injection of buspiron in the neonatal period decreased pain thresholds in RLB males.

Key Words: *pain sensitivity; neonatal treatment; linear mice; behavioral genetics*

Administration of pharmacological preparations and bioactive substances to rat pups during the first days of life modulates behavioral activity and affects general physiological characteristics of adult animals [1,3-5,11]. The study of the side effects produced by this treatment should involve control animals exposed only to nociceptive stimulation. Published data show that injection of physiological saline (nociceptive stimulation) to rat pups of three strains over the first week of life decreases pain thresholds in adult animals in the tail-flick test [1,2]. Here we measured the pain threshold in adult mice neonatally injected with the solvent, Semax, or buspiron. The effect of neonatal nociceptive stimulation on pain thresholds was studied on animals of various genotypes.

MATERIALS AND METHODS

Experiments were performed on 512 male and female mice of various genotypes. Inbred DBA/2 (n=128), CBA (n=42), 101/HY (n=92), and C3H mice (n=81) and RSB (n=85) and RLB animals (n=84) were obtained from the Laboratory for Physiology and Genetics of Behavior (Biological Faculty, M. V. Lomonosov Moscow State University). The mice were bred from female animals of two strains selected for low and high weight of the brain; the embryos were transplanted into the uterus of a recipient mouse.

Semax, buspiron, distilled water, and physiological saline (5 μl) were injected subcutaneously in the withers of treated and control mice. The preparations were administered daily for 5 days (days 2-7 of life). Some intact animals were subjected to handling, while others remained in home cages. The doses of Semax and buspiron were 5 and 25 μg, respectively.

Pain thresholds in adult 3-4-month-old animals were measured in the tail-flick test. The latency of tail-flick response to thermal stimulation of the skin with light beam was estimated semiautomatically. The test was performed in 3 repetitions at 10-15-min intervals.

The results were analyzed by ANOVA (Statistica software). The significance of differences was estimated by LSD test.

RESULTS

Three-factor ANOVA (genotype, sex, and treatment) showed that pain thresholds depend on the effect of 2

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TABLE 1. Effect of Mouse Genotype (Factor 1), Sex (Factor 2), Type of Neonatal Treatment (Factor 3), and Interaction of These Factors (1, 2; 2, 3; 1, 3; 1, 2, 3) on Mean Tail-Flick Latency (3 Tests, 3-Factor ANOVA)

Factor	df Effect	df Error	F	p
1	5	478	4.686471	0.000345
2	1	478	1.185515	0.276785
3	2	478	3.990273	0.019114
1, 2	5	478	4.305410	0.000768
1, 3	10	478	0.423776	0.935147
2, 3	2	478	0.027218	0.973151
1, 2, 3	10	478	1.933688	0.038841

factors: genotype (factor 1, 5 gradations) and type of neonatal treatment (factor 3, 3 gradations, Table 1). However, pain thresholds did not depend on sex of animals (factor 2). This is probably related to various signs of sex differences in neonatally treated animals of studied strains. Two-factor ANOVA (sex and treatment) revealed a significant effect of sex on delayed consequences of neonatal treatment ($F_{df\ 1.508}$=4.1955, p<0.01559).

Post-hoc-LSD test showed that the mean pain threshold in mice exposed to neonatal nociceptive stimulation was lower than in intact and neonatally injected animals. It should be emphasized that the observed effects depended on sex and strain of mice. The decrease in pain thresholds after neonatal nociceptive stimulation was most pronounced in adult males of strains DBA, 101/HY, and RSB (p=0.0351, p=0.0318, p=0.0424), but insignificant in adult females. It is important that pain thresholds decreased in male and female CBA mice (statistically insignificant). Pain thresholds remained unchanged in C3H and RSB mice, but slightly increased in RLB males.

In 2 groups of adult mice with the maximum effect of neonatal nociceptive stimulation (DBA and 101/HY males), neonatal administration of Semax was accompanied by an increase in pain thresholds (1 or 2 of 3 tests) compared to control animals (solvent administration or nociceptive stimulation). These data suggest that administration of Semax to newborn mice normalizes changes in the nociceptive system produced by strong nociceptive stimulation. Neonatal treatment with buspiron significantly decreased pain thresholds in RLB males, but had no effect on RLB females and RSB mice.

Our experiments revealed genotype-dependent changes of pain thresholds (tail-flick test) in adult mice receiving pharmacological preparations (Semax or buspiron) or exposed to nociceptive stimulation (injection of the solvent) over the first week of life. Neonatal nociceptive stimulation decreased pain sensitivity in adult animals of various genotypes. These data confirm the results of our previous experiments [2].

Newborn and especially preterm infants are characterized by increased pain sensitivity [6,8]. Clinical observations indicate that painful sensations in the neonatal period affect the development of CNS and some psychophysiological characteristics [10]. Therefore, it is important to study delayed consequences of nociceptive stimulation in the early period of life [8]. Attempts were made to determine the morphophysiological basis for the increase in pain sensitivity [13]. Experiments with nociceptive stimulation (needle prick) over the first 7 days of life showed that pain sensitivity increases in 16-day-old rats, but not in adult animals [8]. The results of our study contradict published data, which is probably related to differences in the strength of nociceptive stimulation. Stress exposure over the first 2 weeks of life (repeated isolation of newborn NMRI albino mice from mothers) increases pain thresholds in adult animals in the tail-flick test. These data are consistent with our results. Therefore, changes in the pain thresholds produced by treatment in the early period of life are a general phenomenon [7]. We revealed interstrain differences in the delayed effect of neonatal nociceptive stimulation.

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