PHARMACOLOGY AND TOXICOLOGY

Role of Inherent and Individual Acquired Factors in the Development of Morphine Sensitivity in WAG/G and Fischer-344 Rats

Yu. V. Lyupina, O. F. Medvedeva, I. V. Tyurina, and S. K. Sudakov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 5, pp. 535-538, May, 1998
Original article submitted June 10, 1997

Individual differences in sensitivity to morphine-induced suppression of vertical motor activity and analgesia in WAG/G and Fischer-344 rats are determined mainly by genetic factors, while sensitivity to morphine-induced suppression of total motor activity depends primarily on environmental factors. The severity of morphine dependence is determined predominantly by individually acquired factors.

Key Words: *motor activity; pain sensitivity; addiction; morphine; heritability; rat strains*

Phenotypic signs are determined by genetic and individually acquired factors. We have shown that inbred WAG/G rats have low sensitivity to morphine-induced locomotor depression and pain sensitivity in comparison with inbred Fischer-344 rats (F-344) [1]. Effects of environmental challenge were evaluated in subsequent experiments when WAG/G offspring was reared by F-344 dams and vise versa. We found that sensitivity to morphine-induced analgesia was reduced only in F-344 offspring, while sensitivity to its suppressive effect was altered in both strains [3]. Thus, individual morphine sensitivity is determined by both genetic and environmental factors. However, the contributions of these factors to the formation of animal sensitivity to different morphine effects remained unknown.

The aim of the present study was to evaluate the contribution of heredity and environment to the formation of individual sensitivity to morphine-induced locomotor depression and analgesia, and formation of morphine dependence using genetic analysis of F_2 hybrids WAG/G and F-344 rats. It is known that parent strains as well as F_1 hybrids are genetically identical, hence phenotypic differences (general phenotypic variance) in their sensitivity to various morphine-induced effects are determined by environmental factors. In F_2 offspring, phenotypic variance is determined by both genetic and environmental factors due to segregation of genes inherited from parent strains. Comparison of individual deviations in F_1 and F_2 hybrids allows one to determine broad-sense heritability and thereby evaluate the contribution of genetic factors to general phenotypic variance [2].

MATERIALS AND METHODS

Experiments were carried out on 2.5-3-month-old male rats (180-200 g), F_1 and F_2 hybrids of WAG/G and F-344 strains. The groups of F_1 and F_2 hybrids comprised 11 and 20 rats, respectively. Rat pups were raised by mothers for 21 days and then received standard chow and water *ad libitum*. The animals were maintained at 22°C and 12:12 illumination regimen.

On the first day of the animals of both groups were injected with 1 ml/kg isotonic NaCl and 7 min

Laboratory of Neurobiology of Craving, Research Center of Addiction, Ministry of Health, Moscow

0007-4888/98/0005-0474$20.00

later tested in an open field (41× 41×50 cm) for 3 min. Horizontal motor activity, i.e., the number of crossed squares (2.5×2.5 cm) outlined with infrared beams, and vertical activity (rearing) were assessed. Immediately after that the animals were placed into individual Plexiglass boxes, and the latency of tail withdrawal from hot water (56°C) was measured 15, 30, 45, and 60 min postinjection. Seven days later, the same tests were performed after injection of 5 mg/kg morphine hydrochloride. For modeling morphine dependence the rats were treated for 8 days with morphine in doses ascending from 5 to 60 mg/kg (2 times per day with 10-h intervals). Six hours after the last injection, the rats were intraperitoneally injected with 2 mg/kg naloxone, and specific signs of the withdrawal syndrome (wet dog shakes, head and forelimb tremor, posture disorders, piloerection, rhinorrhea, ptosis, writhing, teeth chattering, and diarrhea) were evaluated. The total number of withdrawal signs and the integral index (the sum of these signs in each animal) were determined.

Classical analysis of F_2 population was used to evaluate broad-sense heritability by determining phenotypic and genetic variances [2]. Broad-sense heritability (H) was calculated from the formula:

$$H=\frac{V_{F_2}-V_E}{V_{F_2}}, \text{ where}$$

V_{F_2} the total phenotypic variance for this sign in F_2 population, V_E is the component of phenotypic variance determined by the environmental differences of F_1 population, while $V_{F_2}-V_E$ are its components determined by environmental and genetic differences.

RESULTS

Analysis of heritability of motor activity in the open field test showed that phenotypic differences between F_2 hybrids of F-344 and WAG/G rats in both horizontal (V_{F_2}=530, V_E=504) and vertical (V_{F_2}=11.99, V_E=30.7) activity are determined mainly by environmental factors (Fig. 1). Sensitivity to morphine-induced suppression of horizontal motor activity in the open field was predominantly determined by environmental factors (V_{F_2}=253, V_E=381), while for vertical motor activity the contribution of genetic variance to expression of morphine sensitivity constituted 51% of total phenotypic variance (V_{F_2}=3.99, V_E=1.94, Fig. 1).

The contribution of genetic variance to the total phenotypic variance of pain sensitivity in F_2 hybrids constituted 62% (V_{F_2}=5.13, V_E=1.96). The formation of sensitivity to repeated painful stimulation may be related to environmental factors: repeated testing (45 and 60 min postinjection) yielded negative values of

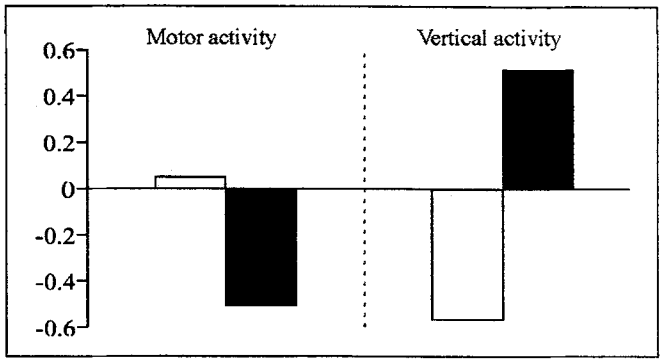

Fig. 1. Heritability of expression of motor activity in the open field test in response to injection of isotonic NaCl (open bars) and morphine hydrochloride (shaded bars). Here and in Figs. 2 and 3: ordinate is the calculated value of broad-sense heritability, rel. units

broad-sense heritability (V_{F_2}=3.1, V_E=5.06, V_{F_2}=3.15, V_E=6.1, respectively, Fig. 2). The contribution of genetic variance to the expression of rat sensitivity to morphine-induced analgesia constituted 51% of phenotypic variance (V_{F_2}=13.36, V_E=6.63) on the 30th minute and 65 and 78% in successive tests (V_{F_2}=12.99, V_E=4.59 and V_{F_2}=13.07, V_E=2.99 on the 45th and 60th min, respectively, Fig. 2). Sensitivity to morphine-induced analgesia is apparently determined by different gene systems and these systems have different phenotypic expression at different stages of the experiment.

Analysis of broad-sense heritability for the expression of morphine withdrawal syndrome in morphine-dependent F2 hybrids showed that only 30% of its phenotypic variance is determined by genetic differences (V_{F_2}=1.69, V_E=1.19), hence the major contribution to the phenotypic variance is made by environmental factors. Genetic variance of expression of some specific morphine withdrawal symptoms exceeded 50%: head tremor (V_{F_2}=3.28, V_E=0.19), forelimb tremor (V_{F_2}=6.75, V_E=0.53), writhings (V_{F_2}=1.78, V_E=0.11), ptosis (V_{F_2}=5.5, V_E=1.94), rhinorrhea

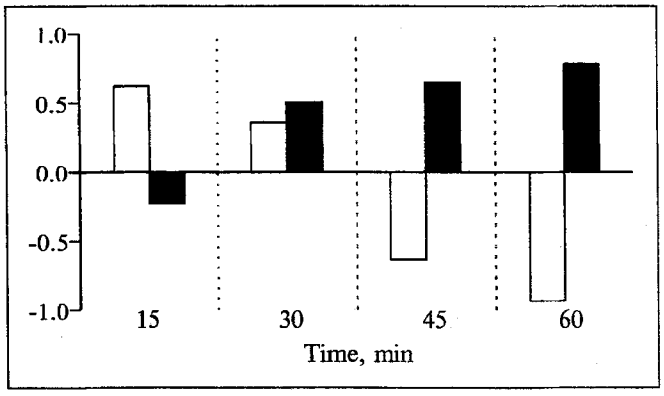

Fig. 2. Heritability of pain sensitivity and sensitivity to morphine-induced analgesia after injection of isotonic NaCl (open bars) and morphine (shaded bars).

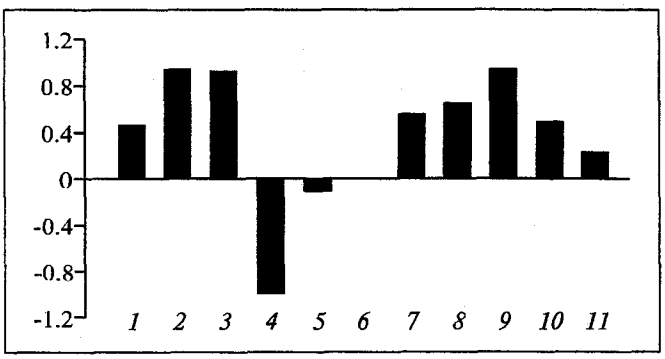

Fig. 3. Heritability of expression of specific morphine-withdrawal symptoms. 1) wet dog shakes; 2) head tremor; 3) forelimb tremor; 4) posture disorders; 5) respiratory disturbances; 6) piloerection; 7) rhinorrhea; 8) ptosis; 9) writhing 10) teeth chattering; 11) diarrhea.

(V_{F_2}=0.25, V_E=0.11, Fig. 3), while for other signs the contribution of environmental variance was predominant and increased in the following order: teeth chattering, wet dog shakes, diarrhea, piloerection, respiratory disturbances, posture disorders (Fig. 3).

These and our previous data suggest the existence of a gene system determining individual sensitivity to morphine-induced suppression of motor activity, analgesia, and morphine dependence. The contribution of genetic variance varied for sensitivity to different morphine-induced effects. It should be noted that classical analysis of broad-sense heritability considers the contribution of both additive and nonadditive genetic factors in F_2 population. These conclusions can be verified by evaluation of narrow-sense heritability.

The study was supported by Russian Foundation for Basic Research (grant 97-04-48063).

REFERENCES

1. R. Plomin, J. C. DeFries, and G. E. McClearn, in: *Behavioral Genetics*. New York (1990), pp. 273-275.
2. S. K. Sudakov, E. V. Borisova, and Yu. V. Lyupina, *Neuropharmacology*, **35**, No. 8, 1131-1134 (1996).
3. S. K. Sudakov, R. S. Goldberg, E. V. Borisova, *et al.*, *Psychopharmacology* (Berlin), **112**, 183-188 (1993).