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Strain Differences in the Analgesic and Reinforcing Action of Morphine in Mice

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SEMENOVA, S., A. KUZMIN AND E. ZVARTAU. *Strain differences in the analgesic and reinforcing action of morphine in mice.* PHARMACOL BIOCHEM BEHAV **50**(1) 17–21, 1995.—The analgesic and reinforcing effects of morphine were compared in four strains of mice (C57BL/6, BALB/c, DBA, CBA). The analgesic action of morphine was measured in tail immersion (49°C), hot plate (60°C), and tail clip (four-point scale of nociceptive response) tests. The reinforcing action of morphine was studied in IV self-administration and conditioned place preference techniques. The results demonstrate strain differences in the analgesic and reinforcing action of morphine in mice. The relative rank order of the strains varied for the several tests as well as for the morphine effects. The lack of correlation between analgesic and reinforcing action of morphine in inbred strains supports the conclusion that analgesia and reinforcement are separate processes with different genetic control.

Morphine Inbred strains Mice Analgesia Reinforcement

THE ROLE of genetic predisposition in sensitivity to the central effects of morphine as a substance of abuse has been received increasing attention in recent years [for current review, see (4)]. A naive individual's initial sensitivity to the drug effect is known to be substantially influenced by genotype. The proliferation of increasingly sophisticated pharmacogenetic animal models has led to a better understanding of the mechanisms of action of such substance of as morphine. Numerous studies have demonstrated that genetically defined stocks of mice differ in sensitivity to the acute analgesic and locomotor effects of opiates (2,14). Also, it has been shown that mice of different inbred strains can vary in the predisposition to prefer or avoid morphine solution in the drinking water (9). The data currently available clearly indicate that genetic factors regulate the behavioral responses, both acute and chronic, that accompany morphine administration. However, little is known about the differences in the sensitivity of inbred strains of mice to the reinforcing effects of morphine in the classical tests like conditioned place preference and IV self-administration. The study presented here comprises an attempt to establish whether inbred mouse strains differ in their sensitivity to the reinforcing and analgesic action of morphine using the analgesic tests with the different modalities of the nociceptive stimuli and tests measuring primary and secondary reinforcing actions of morphine.

METHOD

Animals

The experiments were carried out in male C57BL/6, BALB/c, DBA, and CBA inbred strains of mice weighing approximately 25–30 g. All the animals were obtained from the state breeding laboratory in Rappolovo (Russia) and kept under the standard laboratory conditions with an unlimited access to gronulated food and water. The animals were housed 12 per cage in a light-controlled room (12 L : 12 D cycle, 1000 h lights on) at 22°C and 60% humidity.

Analgesic Tests

The analgesic effect of morphine were measured using tail immersion (49°C), hot plate (60°C), and tail clip (four points scale of nociceptive response: 0—no response, 1—turning, 2—turning + vocalization, 3—turning + vocalization + biting the clip) tests. In all the tests the data after morphine administration were recalculated as the percent of analgesia to the initial level of nociceptive reaction. The percents of analgesia were calculated using formulas: $[E-C]/[15-C] \times 100$ for tail immersion test $[E-C]/[30-C] \times 100$ for hot plate test, and $[C-E]/C \times 100$ for tail clip test, where E—experimental and C—control scores for the latency of paw licking or jumping in

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the hot plate test or intensity of pain response to clip, 15−cut off time (s) in the tail immersion test, 30−cut off time (s) in hot plate test. The ED_{50} of morphine in each analgesic test were calculated for all the strains with the aid of the Lietchfied and Wilcoxon procedure.

Place Preference in Mice

Each experiment started at 0900 h. All strains were tested simultaneously. The experiments were performed in plastic shuttle boxes separated by guillotine doors into two equal size (30 × 30 × 30 cm) compartments. These two compartments were distinguished by degree of illumination color (white and black), and floor texture (metal grid floor and plastic solid floor, respectively). The whole experiment consists of preconditioning, conditioning, and postconditioning periods. During pre- and postconditioning tests animals were placed in the shuttle box for 30 min. Time spent in the white compartment was calculated. Place conditioning procedure in mice was biased. Animals showed initial preference of black compartment spending 24.3 + 9.6% (mean + SEM) of time during preconditioning test in white part of the box. Conditioning period consisted of two daily 30-min sessions. First, animals received saline SC before placing in a black part and then saline or morphine (SC) before placing in the white compartment. This procedure was replicated in the 3 following days. The postconditioning test was held on day 6 when mice were allowed to investigate shuttle boxes freely in the drug-free state for 30 min. During this session, the time spent in the white compartment has been recorded in the same manner as during preconditioning test using automatic system with infrared sensors. The difference in time spent in white compartment during post- vs. preconditioningtests has been calculated as the measure of drug reward. Shuttle boxes were deodorized with 3% H_2O_2 solution after each animal placement. The data, obtained with different strains, were analyzed by two-way ANOVA followed by Dunkan test with respect to the groups conditioned with vehicle. Also the ED_{50} for morphine were calculated for all strains with the aid of Lietchfied and Wilkoxon procedure according to the percent of animals in group with the time (post vs. pre) differences higher than upper confidence limit in control group.

Intravenous Self-Administration in Drug-Naive Mice

Details of the experimental set-up and procedure have been published (10). Briefly, mice were tested in pairs in the identical test cages. Both animals in the pair had been chosen according to the results of preliminary testing without injections and exhibited approximately equal levels of nose poking. Each cage presented a frontal hole for nose poking. Each nose poke of the active mouse resulted in a contingent injection of 1.4 μl of either saline or morphine solution to the lateral tail veins of both active mouse and yoked passive mouse. Nose pokes of the yoked control were counted but had no programmed consequences. After the first 10 min of habituation in the test cage, IV injections were made contingent upon each nose poke of the active animal. As a gradual measure of the reinforcing effect of a drug, the ratio, R, between the cumulative number of the nose-poke responses (NPR) of the active and passive mouse during a 30-min period subtracted by one (initial level of ratio without drug injections) was used. As an alternative measure of the reinforcing effect of the drug, the number of pairs of animals in the group with R higher than the upper confidence limit of R in a group with saline self-administration was used. The effect of the drug was considered reinforcing, neutral, and aversive when R was higher, equal and smaller than 0, respectively. The significance of values with respect to the level of saline self-administration has been calculated with the aid of the Mann-Whitney *U*-test.

RESULTS

Tail Immersion Test

Significant differences in the pain thresholds were found for different strains (Fig. 1). The distribution of strains in order of the increasing of thresholds was: CBA < BALB < DBA < C57BL. However, the repeated testing (4 times) leads to the disappearance of the differences between strains in pain thresholds. Morphine was tested in the range of doses of 2.5–20 mg/kg, and induced significant analgesia in all mice. The significant effect of morphine was noted in BALB strain only because the dose of 5 mg/kg, while in all other strains morphine even at the dose of 2.5 mg/kg, induced significant analgesia. Significant ($p < 0.05$) strain differences in the analgesic action of morphine were founded at the scale of doses between 6 mg/kg and 12 mg/kg. Strain distribution in order of the increasing of the sensitivity to the analgesic action of morphine (according to ED_{50} values) was: DBA < BALB < CBA < C57BL. However, ED_{50} values of morphine did not differ significantly in different strains. The duration of the analgesic action of morphine in this test also differ in strains. The shortest action was in CBA strain and the longest one in C57BL strain. BALB and DBA mice have an intermediate position.

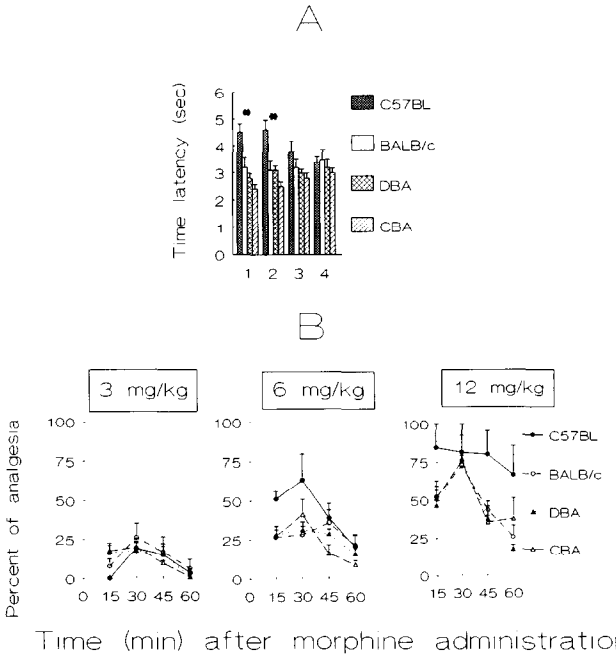

FIG. 1. Strain differences in the sensitivity to the analgesic effect of morphine in the tail immersion test. (Plate A) Initial pain thresholds. OX−four following tests. Time intervals between tests were 15 min. Data as means ± SEM (n = 12–20) *$p < 0.05$−significant differences between strains (Student's t-test). (Plate B) Strain differences in the analgesic action of particular doses of morphine. Data as means ± SEM. See text for details and significance of strain differences.

Hot Plate Test

The distribution of strains in order of the increasing of pain thresholds was: DBA < CBA < C57BL = BALB (Fig. 2). Also, the repeated testing equilibrate the pain thresholds in strains. Strain distribution in order of the increasing of the sensitivity to the analgesic action of morphine was: CBA = C57BL < DBA = BALB. The calculated ED_{50} values (with confidence limits of ED_{50}) of morphine were 6.2 (3.8-10.1), 7.1 (5.5-9.1), 3.4 (2.3-4.6), and 2.3 (0.9-3.9) mg/kg, respectively, and differed significantly in different strains.

Tail Clip Test

The distribution of strains in order of the increasing of pain thresholds was: C57BL < CBA < DBA = BALB (Fig. 3). Pain thresholds were significantly higher in BALB and DBA strains in comparison with CBA and C57BL. Strain distribution in order of increasing sensitivity to the analgesic action of morphine was: C57BL < DBA < CBA = BALB. C57BL exhibited the smallest decreasing of pain thresholds after morphine administration, compared with other strains. The calculated ED_{50} values (with confidence limits of ED_{50}) of morphine were 16.4 (7.4-36.6), 13.1 (9.3-18.5), 4.1 (2.2-7.9), and 3.9 (2.6-5.8) mg/kg, respectively, and differed significantly in different strains.

Conditioned Place Preference

The were no significant strain differences in strains in pretest (Fig. 4). All the strains also exhibited similar reaction to the conditioning with saline. However, after conditioning with morphine, the significant differences between strains occurred. BALB and DBA stains have a highly significant reaction even after conditioning with morphine at the smallest dose of 5 mg/kg. On the contrary, in CBA strain a significant effect of morphine was found only at the dose of 20 mg/kg, and the percent of mice in group with positive conditioning did not exceed 50%. C57BL mice had the intermediate position among strains and exhibited significant effect of morphine at the doses of 10 and 20 mg/kg. The distribution of sensitived strains to the place conditioning effects of morphine in this test was: CBA < C57BL < BALB = DBA.

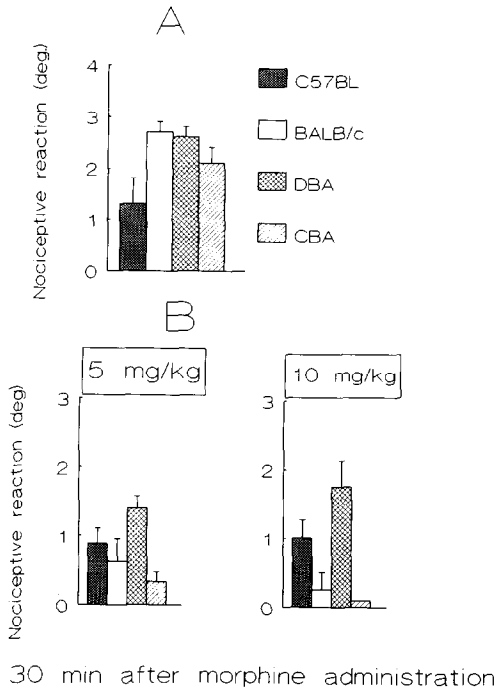

FIG. 3. Strain differences in the sensitivity to the analgesic effect of morphine in the tail clip test. (Plate A) Initial pain thresholds. Data as means ± SEM (n = 12-20). (Plate B) Strain differences in the analgesic action of particular doses of morphine. Data as means ± SEM. See text for details and significance of strain differences.

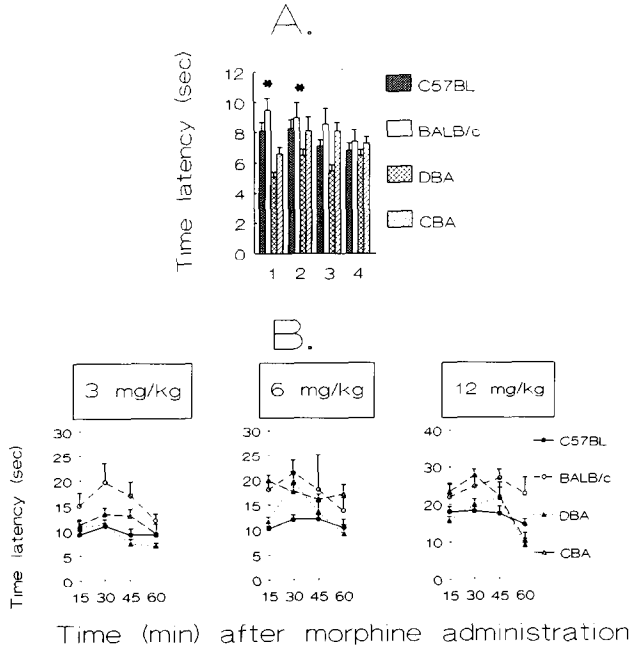

FIG. 2. Strain differences in the sensitivity to the analgesic effect of morphine in the hot plate test. (Plate A) Initial pain thresholds. OX–four following tests. Time intervals between tests were 15 min. Data as means ± SEM (n = 12-20). (Plate B) Strain differences in the analgesic action of particular doses of morphine. Data as means ± SEM. See text for details and significance of strain differences.

Intravenous Self-Administration

Significant differences in strains were found in morphine self-administration on a wide scale of morphine concentrations (0.125-2.0 mg/ml). CBA and DBA mice exhibited typical bell-shaped concentration for response (R criterion) dependence with the existence of the optimal drug-concentration point, 1.2 mg/ml and 0.63 mg/ml, respectively, which was characterized by the highest operant output (Fig. 5). In C57BL mice, the concentration for response dependence was unstable, irrespective the fact that some animals exhibited very high level of self-administration, especially at the concentrations of 0.5 mg/ml and 2 mg/ml. However, according to the quantal score, the optimal concentration for C57BL mice also was calculated (0.5 mg/ml). We failed to find any tendency for self- administration in BALB mice on the hole scale of tested concentrations. Moreover, in BALB mice high level of aversion to morphine self-administration was found.

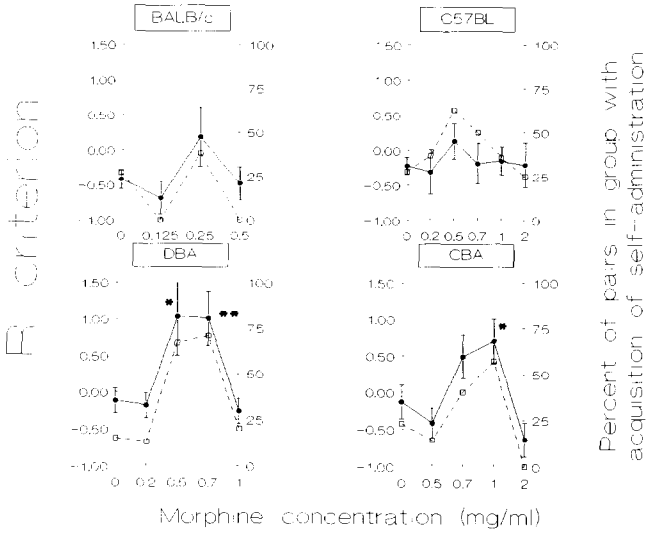

FIG. 4. Strain differences in the sensitivity to the reinforcing effects of morphine in conditioned place preference test. Filled circles—shift of time sent in drug paired side (s) in postconditioning test vs. preconditioning test. Data as means ± SEM (n = 8–16). * and **$p < 0.05$ and 0.01, respectively (Student's t-test). Opened squares—percent of animals in group with place conditioning (for details see the Method section).

DISCUSSION

The results presented demonstrate strain differences in the analgesic and reinforcing action of morphine in mice. The relative rank order of the strains varied for the various tests as well as for the morphine effects. In analgesic tests the strains differed in rank order for consistent relationship between baseline activity and morphine effect. The observed strain divergence cannot be explained by the differences in the brain morphine level after single injection (1). Therefore, the different strain responses to morphine are likely due to differences in tissue sensitivity. Similar results were obtained in ethanol studies (11). The results of different studies suggest that there are genetically controlled differences in the amount of narcotic receptors in the brain. However it was shown that there are no significant differences between BALB/c and C57BL strain in the number and affinity of μ receptors (14). In our experiments, BALB/c mice displayed higher response to morphine than C57BL in hot plate and tail clip tests but not in the tail immersion test which excluded methodologically the possibilities of locomotion (mice were placed in the small plastic boxes with the tail protruded through the hole). It is possible that the hot plate assay is affected by the increased motor activity produced by morphine. C57BL but not CBA strain has been shown to be deficient in κ receptor levels (14). Thus, it seems possible that the κ receptors also might be responsible for the strain differences in analgesic action of morphine. However, genotypic changes in postreceptor mechanisms involved in morphine analgesia cannot be ruled out.

In our studies, DBA mice demonstrated the highest sensitivity to the reinforcing effects of morphine in both CPP and ISA tests among other strains and also in comparison to C57BL strain. BALB/c mice exhibited high sensitivity to morphine reinforcement in CPP test but fail to acquire IV self-administration of morphine. It is likely that such results in BALB strain are not due to the lack of the sensitivity of these mice to the reinforcing effect of morphine but can be explained by the high level of excitability of BALB mice and long-lasting reaction to the partial immobilization stress. These results are in conflict with previous studies that dealt mainly with oral self-administration of morphine or ethanol solutions (4,8,9). However, there is little convincing evidence that animals prefer ethanol because of its postabsorption interoceptive CNS properties rather than other factors such as taste (7). It has been shown that C57BL showed a higher morphine consumption when compared to CBA mice, and C57BL preferred a morphine solution, while DBA mice avoided it (4). It is widely assumed that animals that are less sensitive to ethanol consume greater amounts of this drug and, in fact, the high preferring C57BL show much shorter sleep times in response to ethanol then do their nonpreferring DBA and BALB/c counterparts (3,5). Therefore, it is quite possible that animals that are relatively more sensitive to morphine and, thus, consume relatively less amount of morphine may actually be more reinforced by this substance than their less sensitive counterparts. These findings provide evidence that genetic factors play an important role in determining the persistence of nonalcohol and alcohol drug taking behaviors.

Another explanation of the results, obtained in reinforcing tests might be in the differences of locomotor response to morphine administration. It has been shown that after intraperitoneal injection of morphine, mice of C57BL strain showed the highest increase in locomotor activity, while they were the least sensitive to the analgesic effect of morphine (6). In contrast, DBA mice were more sensitive to the analgesic effect of morphine, but the drug did not enhance their locomotor activity (1). These differences have been attributed to striatal dopamine release, which occurs in C57BL but not in DBA mice after morphine administration (4). Therefore, this

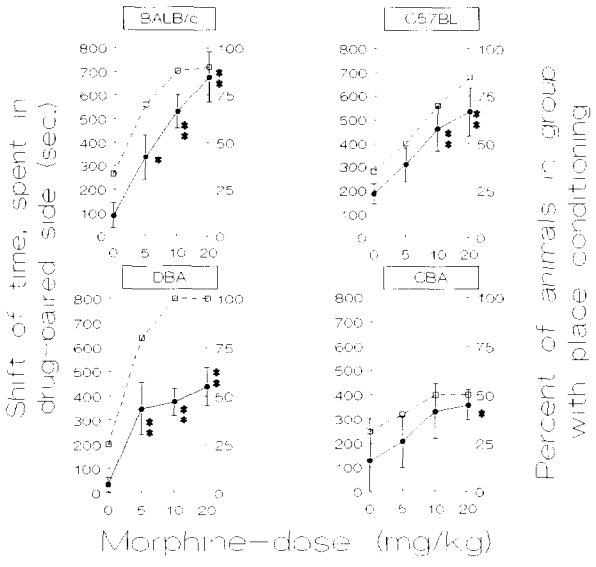

FIG. 5. Strain differences in the sensitivity to the reinforcing effects of morphine in self-administration test. Filled circles—absolute levels of R criterion (for details see the Method section). Data as means ± SEM (n = 10–14). * and **$p < 0.05$ and 0.01, respectively (Student's t-test) as compared to the level of R during saline self-administration (0). Opened squares—percent of pairs in group with acquisition of self-administration in active animals (for details see the Method section).

locomotor activation might be the obstacle for memory processes, which are of importance in these tests.

In conclusion, the results demonstrate that genetic factors regulate the response of the mouse to the analgesic and reinforcing action of morphine. The dissociation between analgesic and reinforcing effects of morphine were found among different inbred strains of mice. This dissociation might be connected with the genetic differences at the functioning level of opiatergic and dopaminergic systems. The lack of correlation between analgesic and reinforcing action of morphine in inbred strains supports the conclusion that analgesia and reinforcement are separate responses with different genetic loci.

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