Differential Sensitivity to Physical Dependence on Morphine and Codeine in Three Inbred Strains of Mice

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ABSTRACT—The purpose of this experiment is to investigate genetic differences in the development of physical dependence on morphine and codeine in inbred strains of mice, C57BL/6, C3H/He and DBA/2. Mice were treated with morphine- or codeine-admixed food (1, 2 and 3 mg/g of food) for 3 to 9 days. After the termination of drug treatment, the mice were given naloxone (5 mg/kg, s.c.). The incidences of jumping and teeth chattering by naloxone challenge in morphine- and codeine-treated C57BL/6 mice were much greater than those in C3H/He and DBA/2 mice. However, the incidences of other naloxone-precipitated withdrawal signs, such as ptosis and diarrhea, were not different among the three inbred strains of mice. These results indicate that genotype is an important determinant of the degree of most naloxone-precipitated withdrawal signs in morphine- and codeine-treated mice.

Several studies have examined the contribution of genetics to the determination of responsiveness to morphine (1–8). These studies examined the initial sensitivity to morphine analgesia as well as the development of analgesic tolerance to and physical dependence on morphine.

In assessing the physical dependence on morphine, most of these studies employed naloxone-precipitated or abrupt withdrawal jumping. The withdrawal jumping in morphine dependent mice was more sensitive in C57BL/6 than in DBA/2 (7, 8). Furthermore, Gwynn and Domino (8) reported that naloxone elicited similar high frequencies of weight loss, shivering and irritability in C57BL/6 and DBA/2 mice chronically treated with morphine. However, other withdrawal signs from morphine, such as body shakes, diarrhea, ptosis and teeth chattering, have scarcely been examined.

Codeine and dihydrocodeine have been

widely used as over-the-counter cough drugs. In recent years, the abuse of over-the-counter cough syrups has been spreading among adolescents (9, 10). As for genetic differences in the development of physical dependence on codeine or dihydrocodeine, however, there have been no studies so far reported.

In the present study, physical dependence on morphine and codeine in three inbred strains of mice, C57BL/6, C3H/He and DBA/2, was systematically investigated using the drug-admixed food (DAF) method.

MATERIALS AND METHODS

Animals

Male C57BL/6N, C3H/HeN and DBA/2N mice, 5 weeks of age, were supplied by Charles River Japan, Inc. (Atsugi, Japan). The mice were housed in individual cages (143 × 293 × 148 mm) under a 12-hr light-dark cycle (light period: 08:00–20:00 hr), and the

room was maintained at a temperature of $22.0 \pm 1^\circ\text{C}$. Each group of mice consisted of 10–12 animals. Food and water were continuously available *ad libitum*.

Induction of morphine and codeine dependence

After several days of acclimation to the laboratory, all animals were fed with a powdered food diet (CA-1, Clea Japan Inc., Tokyo, Japan). Subsequently, the normal powdered food was replaced with the morphine- or codeine-admixed food diet (11). Morphine and codeine were mixed with normal powdered food at a drug/food ratio of 1, 2 and 3 mg/g, respectively. Each strain of mice was divided into 6 groups: 3 groups treated with morphine-admixed food and the other 3 groups treated with codeine-admixed food, using a food dispenser for powdered food (Charles River Japan Inc., Atsugi, Japan). The mice were fed with drug-admixed food for 3 to 9 days by the method of Suzuki et al. (12, 13). The first group was treated with 1 mg/g morphine- or codeine-admixed food. The second group was treated with 1 and 2 mg/g morphine- or codeine-admixed food. The third group was treated with 1, 2 and 3 mg/g morphine- or codeine-admixed food. Each drug concentration was available for 3 days. During the experimental period, body weight, general behaviors and food consumption were routinely assessed at 16:00–17:00 each day. Daily drug intake was calculated from the value of food intake/day.

Naloxone-precipitated withdrawal in morphine and codeine dependent mice

Withdrawal was precipitated by injecting naloxone (5 mg/kg, s.c.) on the fourth, seventh or tenth day of exposure to drug-admixed food. In DBA/2J mice implanted for 3 days with a morphine (75 mg) pellet, the ED_{50} of naloxone for precipitation of withdrawal jumping behavior is 4.38 mg/kg (7). Therefore, 5 mg/kg of naloxone was used in the present study. Body weight was measured at 15, 30, 45, 60 and 90 min after naloxone injection. The number of body shakes was

counted for 15 min after the naloxone challenge. Other signs of withdrawal were observed for 60 min, and the incidence of each withdrawal sign was calculated.

Drugs

The drugs used were morphine hydrochloride (Sankyo Co., Tokyo, Japan), codeine phosphate (Sankyo Co., Tokyo, Japan) and naloxone hydrochloride (Endo Laboratories, Garden City, NJ, U.S.A.). Naloxone was dissolved in sterilized saline and administered s.c. at 0.1 ml per 10 g body weight.

Statistical analysis

Two-way random factorial analysis of variance and Student's *t*-test were used to evaluate the significance of the difference between mean morphine and codeine intakes. Naloxone-precipitated body weight loss was expressed as a percentage of body weight, and then the correlation between body weight loss and drug intake was assessed. Behavioral data were expressed as the number of mice presenting a particular sign over the total number of mice observed.

RESULTS

Morphine intake

Food intakes of normal powdered food before the drug treatment in C57BL/6, C3H/He and DBA/2 were 3.3 ± 0.16 , 3.4 ± 0.18 and 3.4 ± 0.12 g, respectively. There was no difference in food intake among the three inbred strains of mice. During the treatment with morphine-admixed food, the animals did not show daily morphine toxicity and appearance of mortality. The mean daily morphine intakes were 164.4, 341.2 and 570.1 mg/kg for C57BL/6; 125.8, 324.8 and 581.5 mg/kg for C3H/He; and 72.8, 165.2 and 318.1 mg/kg in DBA/2 when the concentrations of morphine-admixed food were 1, 2 and 3 mg/g of food, respectively (Fig. 1). There was a significant difference in morphine intake among the strains ($F(2,81) = 233.14$, $P < 0.01$).

Naloxone-precipitated withdrawal signs in morphine dependent mice

Naloxone-precipitated weight losses in morphine-dependent C57BL/6, C3H/He and DBA/2 mice were positively correlated with morphine daily intakes ($r = 0.967$, 0.990 and 0.997 , respectively; Fig. 2). There was no difference in naloxone-precipitated weight loss among mouse strains. C57BL/6 showed the greatest number of body shakes after naloxone

injection, as compared to C3H/He and DBA/2 (Fig. 2). The incidences of jumping, diarrhea, ptosis, teeth chattering and body shakes related to daily morphine intake are shown in Fig. 3. Naloxone-precipitated jumping, teeth chattering and body shakes showed the greatest incidence in C57BL/6 as compared to C3H/He and DBA/2. There was no difference in naloxone-precipitated diarrhea and ptosis among the strains except at low dai-

Fig. 1. Mean morphine intake during the treatment with the final concentration of morphine-admixed food in the three inbred strains of mice. Each column represents the mean with S.E.M. of 10–12 mice. *** $P < 0.001$ vs. DBA/2N.

Fig. 2. Relationship between the mean morphine intake and naloxone-precipitated body weight loss and body shakes in the three inbred strains of mice. Body weight loss is shown as the value at 90 min after naloxone (5 mg/kg, s.c.). Number of body shakes is the value measured for 15 min after the naloxone injection. Each plot represents the mean with S.E.M. of 10–12 mice. ●—●: C57BL/6N, ○—○: DBA/2N, ▲—▲: C3H/HeN.

ly morphine intake.

Codeine intake

During the treatment with codeine-admixed food, the animals did not show daily codeine toxicity and appearance of mortality. The

mean daily codeine intakes were 169.4, 357.8 and 556.7 mg/kg for C57BL/6; 147.4, 319.7 and 534.3 mg/kg for C3H/He; and 118.5, 270.1 and 438.6 mg/kg in DBA/2 when the concentrations of codeine-admixed food were 1, 2 and 3 mg/g of food, respectively (Fig. 4).

Fig. 3. Relationship between the mean morphine intake and incidence of naloxone-precipitated withdrawal signs, including jumping, diarrhea, ptosis, teeth chattering and body shakes in the three inbred strains of mice. The incidence of withdrawal signs was observed for 60 min after the naloxone injection. ●—●: C57BL/6N, ○—○: DBA/2N, ▲—▲: C3H/HeN.

Fig. 4. Mean codeine intake during the treatment with the final concentration of codeine-admixed food in the three inbred strains of mice. Each column represents the mean with S.E.M. of 10–12 mice. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. DBA/2N.

There was a significant difference in codeine intake among the strains ($F(2,81) = 52.048$, $P < 0.01$).

Naloxone-precipitated withdrawal signs in codeine dependent mice

Naloxone-precipitated weight losses in codeine-dependent C57BL/6, C3H/He and DBA/2 mice were positively correlated with codeine daily intakes ($r = 0.969$, 0.991 and 0.913 , respectively; Fig. 5). There was no difference in naloxone-precipitated weight loss between C57BL/6 and DBA/2, whereas weight loss in C3H/He treated with codeine (3 mg/g of food) was the smallest among the strains. However, all the strains showed a few number of body shakes after naloxone injection, and there was no difference among the strains (Fig. 5). The incidences of jumping, diarrhea, ptosis, teeth chattering and body shakes related to daily codeine intake are shown in Fig. 6. C57BL/6 showed the greatest incidences of naloxone-precipitated jumping and teeth chattering among all the strains (Fig. 6). All the strains showed similar incidences of diarrhea and ptosis after naloxone injection (Fig. 6). In contrast the incidence of body shakes in C57BL/6 and DBA/2 treated with codeine (3 mg/g of food) was higher than

that in C3H/He.

DISCUSSION

Differences between two different inbred strains are known to be significantly greater than individual differences within each strain. The strain differences may be derived from such factors as a lack of homozygosity, micro-environmental differences, and errors of measurement. Strain differences in inbred animals are usually construed to represent a genetic determination for the observed phenotypic difference. The behavioral differences observed between inbred strains may provide some useful insight for possible strategies of behavioral genetic research (14).

Horowitz et al. (15) and Belknap (16) gave various strains of mice a two-bottle choice between water and morphine-saccharin solution. C57BL/6 exhibited a marked preference for morphine-saccharin solution over water. In contrast, mice of the DBA/2 strain avoided morphine-saccharin solution. In the present study, morphine and codeine intakes in C57BL/6 and C3H/He during the treatment were significantly higher than those in DBA/2. It is possible that C57BL/6 and C3H/He prefer opioids, such as morphine and

Fig. 5. Relationship between the mean codeine intake and naloxone-precipitated body weight loss and body shakes in the three inbred strains of mice. Body weight loss is shown as the value at 90 min after naloxone (5 mg/kg, s.c.). Number of body shakes is the value measured for 15 min after the naloxone injection. Each plot represents the mean with S.E.M. of 10–12 mice. ●—●: C57BL/6N, ○—○: DBA/2N, ▲—▲: C3H/HeN.

Fig. 6. Relationship between the mean codeine intake and incidence of naloxone-precipitated withdrawal signs, including jumping, diarrhea, ptosis, teeth chattering and body shakes in the three inbred strains of mice. The incidence of withdrawal signs was observed for 60 min after the naloxone injection. ●—●: C57BL/6N, ○—○: DBA/2N, ▲—▲: C3H/HeN.

codeine, while DBA/2 avoids opioids.

In the present study, the relationship between naloxone-precipitated withdrawal signs and drug intake was shown in Figs. 2, 3, 5 and 6 to consider the differences in drug intake among the three inbred strains of mice. Most of the withdrawal signs in the three strains intensified on a drug intake-dependent manner. However, the degree of withdrawal signs differed from strain to strain.

Almost all of the studies on physical dependence development have used the jumping response as an index of physical dependence following serial injections or morphine pellet implantation. Very large strain differences have been observed in such studies (3, 7, 17, 18). Among the more commonly used inbred strains, the C57BL/6 strain seems to develop a greater degree of physical dependence (sensitivity to naloxone-induced jumping) than the DBA/2 strain. In the present study, after the treatment with morphine-admixed food, the incidence of naloxone-precipitated jumping

was high in C57BL/6, intermediate in C3H/He and very low in DBA/2. We found that the incidence of naloxone-precipitated jumping after the treatment with codeine-admixed food was similar to that in the group treated with morphine. These findings confirmed that the susceptibility to naloxone-precipitated jumping after treatment with opioids, such as morphine and codeine, is: C57BL/6 > C3H/He > DBA/2.

On the other hand, the severity of withdrawal signs from opioids except jumping have scarcely been examined among different inbred strains of mice. Thus diarrhea, ptosis, teeth chattering, body shakes and body weight loss induced by naloxone were also investigated after the treatment with morphine- or codeine-admixed food in the three inbred strains of mice. Naloxone-precipitated teeth chattering and body shakes showed the greatest incidence in morphine-treated C57BL/6 as compared to C3H/He and DBA/2. However, there was no difference in

naloxone-precipitated diarrhea, body weight loss and ptosis among the strains. In codeine-treated mice, the incidence of naloxone-precipitated teeth chattering, diarrhea and ptosis was similar to those in morphine-treated mice. These results suggest that strain difference differs in every opioid withdrawal sign. The intensity of physical dependence on opioid is evaluated by the severity of withdrawal signs (19). Therefore, the link between genetically-determined differences in the opioid systems and every opioid withdrawal sign must be examined.

Recently, we reported using CXBK (μ_1 opioid receptor deficient) mice that withdrawal jumping and body shakes may be mediated by μ_1 receptors, but weight loss and diarrhea may be mediated by μ_2 and/or other receptors including peripheral opioid receptors (20). The incidence of withdrawal jumping (Figs. 3 and 6) and number of withdrawal body shakes (Figs. 2 and 5) in DBA/2 and C3H/He were lower than those in C57BL/6. The possibility that μ_1 opioid receptor density in DBA/2 and C3H/He mice may be lower than that in C57BL/6 mice is suggested.

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