Genetic Differences in Preferences for Morphine and Codeine in Lewis and Fischer 344 Inbred Rat Strains

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Abstract—Preferences for morphine and codeine in two inbred strains of rats, Lewis and Fischer 344 (F344), were systematically investigated using the drug-admixed food (DAF) procedure. Rats were allowed access to food only between 10:00 a.m.-4:00 p.m., but allowed free access to water. The rats were allowed to choose either DAF (0.5 mg/g) or normal food on the first day and then were allowed access only to DAF on the second and third days. After this schedule was repeated 10 times, they were again allowed to choose either DAF or normal food for a successive 8 days. In both strains, preferences for morphine and codeine rapidly increased; the preferences in Lewis rats were significantly higher than those in F344 rats during the daily choice trials. In the range of 0.25 to 1 mg/g for the DAF concentration, there was a negative correlation between the preference and concentration in Lewis and F344 rats, except in the codeine group of F344 rats. When a test dose (60 mg/kg and 30 mg/kg, s.c., in Lewis and F344 rats, respectively) of morphine or codeine was given at 30 min before the beginning of the choice trial, Lewis rats, but not F344 rats, showed a significantly lower preference for the respective drug. The above results indicate that genotype is an important determinant of the degree of preferences for morphine and codeine.

The drug self-administration method has been extensively used for evaluating the psychic dependence liability of drugs (1-3). Procedures for generating self-administration of opioid compounds in laboratory animals have taken one of two forms: either the animal performs some response to receive an injection of the drug or ingests the drug with food or drink (4-10).

Although the genetic bases of the morphine effect have attracted the attention of researchers, pharmacogenetical research with morphine employing the self-administration procedure has been minimal. The only such studies known to the authors are the one in which selective breeding was used to produce two strains of rats that differed in their susceptibility to morphine intake (11) and the studies in which the oral ingestion of morphine-containing water was made in Wistar and hooded rats (12), in selectively bred strains (MNR/Har/Lu, MR/Har/Lu, RCA/Lu, RHA/Lu, and RLA/Lu) of rats (13), and C57BL/6J and DBA/2N inbred mice (14). As for genetic differences in the preference and self-administration of codeine, there has been no report. It is great interest to study the role of genetically selected behaviors in morphine and codeine intakes.

The major source of experimental animals used in behavioral pharmacogenetic research has been inbred strains of mice and, to a much lesser extent, rats. Recently, Suzuki et al. (15) reported that ethanol serves as a strong positive reinforcer for Lewis inbred rats, but a weak positive reinforcer for Fischer 344 (F344) inbred rats. Hence, the main purpose of the present study was to compare the morphine and codeine preferences in Lewis and F344 rats in order study the genetic aspects of the phenomena.

Materials and Methods

Animals: Twenty-four naive male rats, 12
each from Lewis/Crj and F344/DuCrj strains (Charles River Japan, Inc., Atsugi, Japan), were used in this experiment. Each experimental group consisted of Lewis or F344 rats, 5 weeks of age, which were housed individually in cages (21 x 25 x 15 cm) with two food cups placed equi-distantly from the water bottle. Rats were allowed access to food (CA-1, Clea Japan, Inc., Tokyo, Japan) only between 10:00 a.m. -4:00 p.m., but allowed free access to water all day. At the start of the experiment, body weights of the Lewis rats ranged from 125 to 142 g, and those of the F344 rats ranged from 100 to 116 g (4:00 p.m.). The rats were housed in a temperature (22±1 °C) and humidity (55±5%) controlled room under a 12 hr light/dark cycle with the lights on between 8:30 a.m. and 8:30 p.m.

Procedure: At the beginning and end of each trial (10:00 a.m. and 4:00 p.m.) throughout the experiment, food cups were weighed and the amounts of food consumed were recorded. The body weight of each rat was also measured at the corresponding times. Morphine hydrochloride and codeine phosphate (Sankyo, Inc., Tokyo, Japan) were admixed with powder food (CA-1) at drug/food ratios of 0.25, 0.5 and 1.0 mg/g.

The feeding regime was as follows: on the first day and every third day afterwards, both a drug-admixed food and normal food were made available in separate food cups for each rat throughout the six hour feeding periods. This procedure constituted a "choice" trial. On the two days between the successive choice trials, only a single food cup containing a drug-admixed food was provided ("forced" trial). The three day cycle, consisting of a choice trial followed by two forced trials, was imposed repeatedly until the rats had completed 10 choice trials. After the 10 cycles, the choice trial was performed daily for 8 days. During these choice trials, the preference rate for morphine (0.5 mg/g of food) or codeine (0.5 mg/g of food) was compared between the Lewis and the F344 rats. Preference rate was calculated as follows:

Preference rate (%) = drug-admixed food intake (g) \times 100/total food intake (g)

After the initial test, the preference rate for and intake of morphine and codeine concentrations of 0.25, 0.5 and 1.0 mg/g of food, in that order, was studied. Five to eight trials were given of the choice trials in each concentration. Data for each concentration were calculated as the mean preference rate during these trials.

After completion of the concentration-preference investigation, the choice trial was performed again at a morphine or codeine concentration of 0.5 mg/g of food for 9 trials. The choice trial was started 30 min after giving saline s.c. in the first 8 trials, and morphine/codeine at 60 mg/kg, s.c., for Lewis and 30 mg/kg, s.c., for F344 rats in the last trial. These dosages corresponded to approximately 3 times larger than daily drug intake under the 0.5 mg/g of food condition.

Results

Lewis and F344 rats showed essentially the same growth curve and gross behavior throughout the experiment.

The preference rate for morphine (0.5 mg/g of food) in Lewis and F344 rats rapidly increased until the third to fifth choice trials, after which these preference rates became stable at a level of 60-70% in Lewis rats and at a level of 40-50% in F344 rats (Fig. 1). During daily choice trials, mean preference rates for morphine (0.5 mg/g of food) in Lewis and F344 rats were 61.8±6.4% and 45.9±2.4%, respectively (Fig. 3). There was a significant difference (P<0.05) in the preference rates for morphine between Lewis and F344 rats. Preference rates for codeine (0.5 mg/g of food) in Lewis and F344 rats were 61.8±6.4% and 45.9±2.4%, respectively (Fig. 3). There was a significant difference (P<0.05) in the preference rates for codeine between strains.

When the concentration of morphine-admixed food was increased, the preference rate for morphine in both strains decreased in a concentration-dependent manner (Fig. 4).
Fig. 1. Preferences for morphine in each choice trial in Lewis (closed circle) and F344 (open circle) rats. The concentration of drug-admixed food is 0.5 mg/g of food. Each plot represents the mean with S.E. of 6 animals.

Fig. 2. Preferences for codeine in each choice trial in Lewis (closed circle) and F344 (open circle) rats. The concentration of drug-admixed food is 0.5 mg/g of food.
The preference rates for morphine in the 0.25 and 0.5 mg/g of food concentrations were significantly lower in F344 rats than in Lewis rats (P<0.001). On the contrary, mean morphine intake in both strains increased in a concentration-dependent manner (Fig. 4). The mean morphine intake at each concentration was significantly lower in F344 rats than in Lewis rats (P<0.001 at 0.25 and 0.5 mg/g of food, P<0.05 at 1.0 mg/g of food). When the concentration of codeine-admixed food was increased, the preference rate for codeine in Lewis rats also decreased in a concentration-dependent manner (Fig. 5). The preference rate for codeine in each concentration was significantly lower in F344 rats than in Lewis rats (P<0.001 at 0.25 and 0.5 mg/g of food, P<0.01 at 1.0 mg/g of food). Mean codeine intake in both strains increased in a concentration-dependent manner (Fig. 5). There was a significant difference in codeine intake between the strains (P<0.001).

Preference rate for morphine after morphine injection in Lewis rats decreased significantly in comparison with the saline treatment (P<0.01), but the preference rate in F344 rats...
Fig. 5. Effects of concentration of codeine-admixed food on preferences for codeine (left panel) and codeine intake (right panel) in Lewis (closed column) and F344 (open column) rats. Each column represents the mean with S.E. of 6 animals. **P<0.01 and ***P<0.001 vs. Lewis rats.

Fig. 6. Effects of morphine (left panel) and codeine (right panel) injection (s.c.) 30 min before the choice trial on preference rate for each drug. Each column represents the mean with S.E. of 6 animals. **P<0.01 vs. saline (0).
rats did not differ in spite of morphine injection (Fig. 6). The preference rate for codeine after codeine injection in Lewis rats also decreased significantly in comparison with the saline treatment (P<0.01), but the preference rate in F344 rats was not changed (Fig. 6).

Discussion

Differences between two different inbred strains are known to be significantly greater than individual differences within each strain. The 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 (16).

In the present study, we compared the preference for morphine or codeine between the two inbred strains of rats, Lewis and F344. These rat strains were selected because 1) they have had no common ancestors for at least seventy-five years, 2) both strains have a history of good fertility and are readily available from commercial vendors, thereby increasing the likelihood of their use and the possibility of genetic manipulation, and 3) the Lewis strain is derived from Sprague-Dawley stock, thus providing an animal genetically similar to rats which have been used in much related research. Furthermore, it had previously been found that ethanol serves as a strong positive reinforcer for Lewis rats, but as a weak positive reinforcer for F344 rats (15). In our present experiments, Lewis rats exhibited a marked preference for the morphine-admixed food (0.25, 0.5 and 1.0 mg/g of food) over drug-free food (normal food); in contrast, F344 rats exhibited a weak preference for the morphine-admixed food. The strain differences in preference rate for codeine and in codeine intake between Lewis and F344 rats were similar to the results obtained in morphine groups. These results suggest that the order of degree of preference for opioids in the two inbred strains of rats is: Lewis ≫ F344.

Preference for morphine in rats has been established with the two-bottle method (4, 17) and the two-food cup method (9). The schedule for preference test used in the present study was the same as that used in an earlier study (9). The preferences for morphine and codeine (9, 10) in Sprague-Dawley rats were comparable to those in Lewis rats, but higher than those in F344 rats.

The degree of preference for codeine was similar to that for morphine. Codeine is similar to morphine in all its actions, but is less potent. The activities of codeine as compared with morphine are about one-fifth to one-tenth as potent as an analgesic, about one-tenth as potent as a respiratory depressant and emetic, but about one-half as potent as cough suppressant. However, codeine is absorbed well when administered orally, relative to morphine (18). The good absorption of codeine may be the reason why the degree of preference for codeine is similar to the preference for morphine.

When morphine or codeine was given s.c. to both strains at 30 min before the beginning of the choice trial, Lewis rats showed a significantly lower preference for the respective drug, but F344 rats did not. These results in Lewis rats are consistent with those of Stolerman and Kumar (6) and Yanaura and Suzuki (9). The Lewis rats seem to be satisfied with the drug injection and thus preferred ordinary food to drug-admixed food in the choice trial. On the other hand, Suzuki et al. (19) reported that ED50s for the analgesic activity of morphine evaluated by the hot plate method were 3.86 (2.32-5.69, 95% confidence limits) mg/kg, s.c., for Lewis rats and 19.47 (14.25-30.51) mg/kg, s.c., for F344 rats, suggesting that F344 rats are much less sensitive to morphine than Lewis rats. This difference in sensitivity to morphine may be related to low preference for morphine and codeine, and the preference after the injection in F344 rats.

In conclusion, the Lewis strain seems to have greater preferences for morphine and codeine relative to the F344 strain, which makes Lewis strain a very interesting strain for future research in drug-seeking behavior. It is also suggested that genotype is an important determinant of preference for opioids.
in inbred strains of rats.

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References