EATING BEHAVIOR REVEALS RATS' PREFERENCE FOR MORPHINE

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Abstract—Using an automatic food intake measuring apparatus ("food intakometer"), we recorded the approach behavior to food, eating behavior and food intake of morphine dependent rats and examined the relationship among these factors and morphine dependence. Morphine dependent rats were produced by the drug-admixed food (DAF) method. In the choice trial of free feeding group, morphine dependent rats showed only the approach behavior both to drug-free diet and to morphine-admixed food, then ate the morphine-admixed food and approached the drug-free diet at the same period. Eating behavior in the case of morphine-admixed food was observed not only at night but also during the day time in the morphine dependent rats. In the choice trial of the limited feeding group, preference for morphine rapidly increased in every choice trial of each session and the preference rate became stable at about 60%. Eating patterns of these rats were similar to those in the free feeding group. When these rats were given morphine prior to the choice trial, eating behavior of those on the morphine-admixed food was inhibited dose-dependently, while eating behavior of these on the drug-free diet was enhanced dose-dependently. When these rats were allowed free access to drug-free diet for 1 hour previously to the choice trial, eating behavior of the rats on the morphine-admixed food in the choice trial was markedly enhanced. Thus, the rats clearly showed drug-seeking behavior and seemed to be able to distinguish between the need for morphine and satisfaction without it. Morphine dependent rats apparently can control their required maintenance dose.

Over the years a several techniques have been developed to examine drug-seeking behavior. A Y-maze with two goal boxes contrasting in physical as well as spatial characteristics was introduced by Beach (1), self-administration by oral route in rats (2–4), intraperitoneal route in rats (5), intragastric route both in rats (6) and monkeys (7), intravenous route both in rats (8) and monkeys (9) and intraventricular route in rats (10) are the most common means for inducing drug dependence and investigating drug-seeking behavior in laboratory animals. However, when drugs are insoluble in water, these self-administrations cannot be employed to examine drug-seeking behavior, except for oral self-administration, namely, the drug-admixed food (DAF) method. Using this method, preference for morphine, codeine, phenobarbital, diazepam and cocaine has been demonstrated in rats (4, 11–13).

Continuous recordings during the course of the experiment can be made of the behavior of drug self-administration. Hill and Stellar (14) and Kuribara et al. (15) developed an electric drinkometer for the purpose of continuously measuring water intake. Stolerman and Kumar (16) studied the preference for morphine by animals, using this electric drinko-
meter. More recently, Yanaura and Suzuki (17) have developed an automatic food intake measuring apparatus ("food intakometer") recording continuous variations in the approach behavior to food, eating behavior and food intake, and recorded these data during the morphine dependence-inducing period, during withdrawal of morphine, and on application of a narcotic antagonist to morphine dependent rats.

In the present series of experiments, behavior of rats on self-administration of drugs was analyzed. Accordingly, we examined preference for morphine from the eating pattern using "food intakometer" and discussed the relationship between the dependence on morphine and the eating pattern.

**MATERIALS AND METHODS**

Each group included 6 male Sprague-Dawley strain rats (Charles River Japan, Inc., Kanagawa), 5 weeks of age which were housed individually in a cage (21 x 25 x 15 cm) with two food cups equidistant from the water bottle. The place of the cup containing the drug was changed daily. Morphine hydrochloride and quinine sulfate were used in this experiment. Each drug was applied as an admixture with normal powder food (CA-1, Japan CLEA, Inc., Tokyo) at the concentration of 1 mg/g food. The light was turned on at 8:30 hours and off at 18:00 hours. During the experimental period, the animals were kept in a room air-conditioned to a temperature of 22 ± 1°C and a relative humidity of 55 ± 5%.

**Experiment 1.** (free feeding group)

All animals ingested morphine at the rate of 1 mg/g of food for 3 weeks. The pretreatment period was followed by 3 days withdrawal period during which only drug-free diet was provided. This withdrawal period was intended to motivate the rats to want the drug. The drug-admixed food was given again for 1 week. The eating pattern of these pretreated rats was recorded using an automatic food eating measuring apparatus ("food intakometer") (17) during the daily choice trial for 1 week (one cup containing drug-admixed food vs. one cup containing drug-free diet). The food intakometer can record continuous changes in approach to food (approach behavior), eating behavior and food intake. During these trials, the eating pattern and the preference rate for morphine were compared between the morphine and control groups. Preference rate was calculated as follows:

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\text{Preference rate} \left(\%\right) = \frac{\text{drug-admixed food intake (g)}}{\text{total food intake (g)}} \times 100
\]

In this experiment, rats had free access to food and water. Body weight and food intake were measured daily at 18:00. In the control experiments, the eating pattern of naive rats was similarly recorded using the food intakometer during the daily choice trial for 1 week.

**Experiment 2.** (limited feeding group)

Rats were allowed access to food only between 9:00–17:00, though they had free access to water. Body weight was also measured daily. Actual daily drug intake was calculated in mg/kg of body weight at 17:00/day.

1) **Eating pattern of morphine dependent rats during choice trials:** The experiment was
performed as follows: One experimental session consisted of the choice trial (the rats can choose the preferred of two kinds of food) and then two forced trials (in this case only one cup containing drug-admixed food was set). After 7 sessions, the choice trial was performed daily for 1 week. The eating pattern of these pretreated rats was recorded, using the food intakometer, during the daily choice trial for 1 week.

2) Changes in eating pattern during choice trials after morphine injection: The preference for morphine was stabilized at approx. 60% with all rats. Twenty-four rats were separated into four groups and were started on the choice trial 30 min after being given morphine (10, 30 and 50 mg/kg respectively) or saline s.c. In the choice trial, the eating pattern of these premedicated rats was recorded using the food intakometer and preference rate for morphine, total food intake, total water intake and body weight were measured and compared among the four groups.

3) Time course of feeding and previous access to drug-free diet: Six rats whose preference rate for morphine was stabilized at approx. 60% in the daily choice trial were used. The rats were allowed access to only the drug-free diet for 1 hour previous to the choice trial and then performed the choice trial for 7 hours. The eating pattern of these rats was recorded using the food intakometer. Preference rate for morphine in the choice trial, total food intake, total water intake and body weight were measured and compared with morphine dependent rats.

RESULTS

Experiment 1. (free feeding group)

Figure 1 shows the continuous records of the approach behavior, eating behavior and food intake of morphine dependent rats during the choice trials in the free feeding group. During the choice trial, morphine dependent rats first showed the approach behavior both to the drug-free diet and morphine-admixed food and then the eating behavior seen in the case of drug-free diet. Subsequently, the rats exhibited such an eating pattern that they ate the morphine-admixed food (1 mg/g food) very frequently. Even when the rats preferred the morphine-admixed food, they also approached the drug-free diet.

The time course of food intake by the morphine dependent rats (n=6) and that by the naive rats (n=6) on the morphine-admixed food and the drug-free diet are shown in Figs. 2 and 3. The average food intake of the morphine-admixed food and the drug-free diet at night, by the morphine dependent rats, was 9.7 g and 5.7 g per animal, which were 78.5% and 76.2% of the each daily food intake of 12.4±0.9 g and 7.5±1.5 g per animal. On the other hand, the average food intake of the rats eating the morphine-admixed food and the drug-free diet at night, by the naive rats, was 0.5 g and 12.3 g per animal, which were 64.0% and 64.4% of the each daily food intake of 0.8±0.4 g and 19.1±2.8 g per animal. The naive rats ate the drug-free diet for many hours, compared with the morphine dependent rats. The preference rate for the morphine-admixed food in the morphine dependent rats was 62.1±4.7%, this being significantly higher (P<0.001), than in the case of the naive rats, 3.8±1.8%. The morphine intake by the morphine dependent rats was 46.7±2.8 mg/kg/day
Fig. 1. Typical eating patterns of morphine dependent rat (top) and naive rat (bottom) during choice trial. The ordinate denotes the cumulative decreased amount of food, i.e., cumulative food intake (g); and the thick bar of the abscissa, the dark period, and the thin bar, the light period. The movement of the pen in increasing direction on the chart indicates the shutting off of the photocell set at the eating hole, i.e., it denotes the eating behavior in cases where food intake increases, and the approach behavior in cases where food intake does not vary. NF: drug-free diet. MAF: morphine-admixed food (1 mg/g food).

Fig. 2. Time course of food intake during choice trial in morphine dependent rats. The ordinate denotes the food intake (g); and the abscissa, time. The thick bar of the abscissa represents the dark period. Each plot is the mean of 6 animals. - - - : drug-free diet. - - - : morphine-admixed food (1 mg/g food).
per animal and that by the naive rats was 2.5 ± 1.2 mg/kg/day per animal.

**Experiment 2.** (limited feeding group)

1) Eating pattern of morphine dependent rats during choice trials: Fig. 4 shows the continuous records of the approach behavior, eating behavior and food intake of morphine dependent rats during the choice trial, in the limited feeding group. Morphine dependent rats rarely approached both the drug-free diet and the morphine-admixed food at the beginning of the choice trial and then abruptly showed a preference for the morphine-admixed food, whereas the rats ingesting the quinine-admixed food approached the drug-free diet and the quinine-admixed food at the beginning of the choice trial and then ate both drug-admixed food and drug-free diet for approx. 30 min. Subsequently, the rats mainly ate the drug-free diet. The time course of food intake by the morphine dependent rats and by the quinine treated rats is shown in Fig. 5. The morphine dependent rats showed the mean food intake of the morphine-admixed food of 4.9 ± 0.7 g for 1 hour after start of the choice trial, the mean food intake of the drug-free diet of 2.4 ± 0.5 g for 1 hour and then both intake of both foods decreased gradually. Total food intake of the morphine-admixed food and the drug-free diet was 12.9 ± 1.1 g, 5.0 ± 0.8 g, respectively. While the quinine treated rats showed the mean food intake of the quinine-admixed food of 1.0 ± 0.4 g for 1 hour after start of the choice trial, the mean food intake of the drug-free diet was 4.0 ± 1.0 g for the same time. However, the food intake of the quinine-admixed food was 0 g from 2–8 hours after the start of the choice trial. The food intake of the drug-free diet decreased gradually. Total food intake of the quinine-admixed food and the drug-free diet was 1.7 ± 0.6 g, 15.0 ± 1.1 g, respectively.
2) **Effect of morphine injection on eating pattern in morphine dependent rats:** In rats in which the preference rate for morphine had stabilized at approx. 60%, morphine or saline
was given s.c. 30 min before the choice trial and the approach behavior, eating behavior, food intake and preference rate during the choice trial were continuously recorded by the use of the food intakometer. In the choice trial, the preference rate for morphine was negatively related to the dose. However, body weight and total food intake changed little in each group.

Fig. 6. Effect of injection of morphine 30 min before the daily choice trials on eating pattern in morphine dependent rats during the choice trials. The ordinate denotes the food intake (g); and the abscissa, time. A: saline treated rat. B: morphine (10 mg/kg, s.c.) treated rat. C: morphine (30 mg/kg, s.c.) treated rat. D: morphine (50 mg/kg, s.c.) treated rat. NF: drug-free diet. MAF: morphine-admixed food.
The rats showed neither the approach behavior nor the eating behavior for several hours after the start of the choice trial. This duration was related to the dose. Thereafter, such behavior markedly increased. Such behavior in rats ingesting the morphine-admixed food was depressed dose-dependently, while on the contrary, the behavior of rats eating the drug-free diet was enhanced dose-dependently (Fig. 6.).

3) Time course of feeding and previous access to drug-free diet: In rats in which the preference rate for morphine had stabilized at approx. 60% and who had free access to drug-free diet for 1 hour before the choice trial, the mean food intake of drug-free diet was $3.7 \pm 0.3$ g. During the choice trial, the rats almost always chose the morphine-admixed food, and the rats showed the mean food intake of morphine-admixed food of $12.8 \pm 0.7$ g, the mean food intake of drug-free diet of $1.7 \pm 0.6$ g and the mean morphine intake of $47.8 \pm 2.5$ mg/kg/day. Preference rate for morphine was $88.2 \pm 3.7\%$ and this rate was significantly different from that of morphine dependent rats without the treatment ($P<0.01$). During the choice trial, the rats usually preferred the morphine-admixed food (Figs. 7, 8).

DISCUSSION

For the purpose of analyzing the eating pattern of animals by the drug-admixed food (DAF) method (18–21), an automatic food intake measuring apparatus (food intakometer) was developed (17). By this food intakometer, it was possible to substantiate the validity of the DAF method and, at the same time, to examine the time course of drug self-admin-
istration. Normal rats lose body weight during the daytime and gain it during the night period. The pattern appears to be the result of high motor activity and high food intake which prevail in this species during the night period. However, the eating time of morphine dependent rats was prolonged in comparison with that of naive rats, namely, the rats ate morphine-admixed food not only during the night period but also during the day, in the choice trial. This finding appears to result from the fact that when morphine is withdrawn from morphine dependent rats, weight loss begins to occur from about 8 hours after the withdrawal onward (17); if the morphine dependent rats, like naive rats, scarcely eat the food in the daytime, withdrawal signs will begin to evolve. Changes in the eating pattern may be the result of escape from withdrawal signs.

Stolerman and Kumar (16) have used drinkometers to monitor the consumption of morphine solution and water in the choice trial by rats. However, the drinkometer cannot be used to record the activity of approach to the bottles and continuous change of drinking pattern in rats. The food intakometer allows for continuous recording of food intake and the recording of eating and approach behavior. Consequently, we observed drug-seeking behavior in rats, namely, morphine dependent rats immediately approached both the morphine-admixed food and the drug-free diet after the beginning of the choice trial and then preferred the morphine-admixed food. These results support our previous report (4) that the rats searched for the one food cup containing morphine-admixed food from five food cups then ate the contents, thus indicating a clear drug-seeking behavior. Stolerman and Kumar (16) reported that the rats seemed to be able to discriminate between the needs for morphine and for water because they adjusted their behavior according to the conditions.

**Fig. 8.** Time course of food intake in morphine dependent rats in cases where hunger had been partially satisfied, during choice trial, in the limited feeding group. The ordinate denotes the food intake (g); and the abscissa, time. Morphine dependent rats were allowed access to only drug-free diet for 1 hour and then the choice trial was performed for 7 hours. Each plot represents the mean of 6 animals. ---: drug-free diet. --○--: morphine-admixed food (1 mg/g food).
of deprivation. The present experiments showed that when the rats were partially allowed to satisfy their hunger, the ingestion of the drug-free diet was reduced, while ingestion of morphine-admixed food was enhanced during subsequent choice trials (Figs. 7, 8) and morphine injected prior to choice trials can selectively depress the intake of the morphine-admixed food (Fig. 6). These results coincide with the reports of Stolerman and Kumar (16, 22). We found that morphine dependent rats showed the phenomenon of a circadian change in food intake of drug-free diet during the choice trial (Fig. 2). These results also suggest that the rats were able to discriminate between the needs for morphine and for food and that they can control their own required maintenance dose in cases of morphine dependence.

In the present study, when the rats were given morphine s.c. prior to choice trial, the rats did not show the eating behavior and the approach behavior in the early period of the choice trial. The abolishment of such behavior was dose-dependent (Fig. 6). Thereafter, the rats abruptly showed eating behavior. Kumar et al. (23) reported that large doses of morphine can facilitate eating and drinking in tolerant rats. Effect of morphine injection on eating, drinking and spontaneous motor activity in morphine dependent rats produced by systemic injection (23–25) might differ from the effect in morphine dependent rats produced by a variety of techniques, including intravenous self-administration (8), the implantation of morphine pellets and reservoir (26–28), continuous parenteral infusion (29) and oral self-administration (2–4, 30, 31). These differences may be attributed to the different methods, route, dose and frequency of administration and passive and active administration etc.

When observing the drug-seeking behavior in rats using a food intakometer, we found that the animals seemed to be able to discriminate between the needs for morphine and for drug-free diet. Apparently morphine dependent rats can control their own required maintenance dose.

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