Induction of Physical Dependence on Codeine in the Rat by Drug-Admixed Food Ingestion

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Abstract—The developmental process of physical dependence on codeine has been explored in rats treated with codeine-admixed food (0.5 mg/g food) during 1 to 7 days. In rats treated with codeine for more than 2 days, body weight loss was markedly observed after the abrupt codeine withdrawal. The intensity and time course of body weight loss increased according to the duration of codeine treatment. After the codeine withdrawal, behavioral signs such as diarrhea, ptosis and vocalization were observed. In the naloxone-precipitated withdrawal test, rats treated with codeine for 1 day manifested a loss of body weight after naloxone challenge, and the intensity of body weight loss increased according to the duration of codeine treatment. After naloxone injection, the codeine-treated rats showed abnormal behaviors such as diarrhea, ptosis, teeth chattering, salivation, body shakes, vocalization, nose bleed, irritability, lacrimation and writhing. The total score, evaluated by the ranking system for precipitated withdrawal behaviors, was correlated with the duration of codeine treatment. These results suggest that naloxone-precipitated withdrawal signs are powerful in comparison with that after codeine withdrawal, and the weight loss is a common index for quantitative assessment of physical dependence on narcotics in the natural and naloxone-precipitated withdrawal tests. It is concluded that the drug-admixed food ingestion method has the advantage of rapidly inducing a high degree of physical dependence on codeine.

Physical dependence on narcotics can quickly be induced in rodents by a variety of techniques, including implantation of a morphine pellet (1, 2), infusion of narcotics (3, 4), treatment with a slow release emulsion of morphine (5), implantation of a reservoir of morphine solution (6) and treatment with drug-admixed food (7, 8). Although a considerable amount of research has been reported on the induction of physical dependence on morphine in rodents, there has never been any report of the developmental process of physical dependence on codeine.

The drug-admixed food ingestion method is an easy way to produce physical dependence on narcotics (7, 8), barbiturates (7) and benzodiazepines (9). In a previous report, we have already reported the developmental process of physical dependence on and tolerance to morphine in rats treated with morphine-admixed food (8).

The purpose of the present study was to assess the method of codeine-admixed food ingestion as a means to reliably and rapidly produce a high degree of physical dependence on codeine in rats. Furthermore, the rate and degree of physical dependence development during the treatment of codeine-admixed food have been quantitatively examined.

Materials and Methods

Animals and drugs: Male Sprague-Dawley rats weighing between 140 g to 160 g at the beginning of the experiment were housed individually at a temperature of 22±1°C under a light-dark cycle of 12 hr (lights on at 8:30 A.M. and off at 8:30 P.M.). They were given food and tap water ad libitum. Codeine
phosphate was purchased from Sankyo Co.,
Tokyo; naloxone hydrochloride from Endo
Laboratories, New York. Codeine was
admixed with rat food (CA-1 powder, Clea
Japan Inc., Tokyo) at a drug/food ratio of
0.5 mg/g. In the naloxone-precipitated with-
drawal test, naloxone was dissolved in
physiological saline.

Natural withdrawal: Rats were divided into
8 groups (n=4–5) and were treated with
codeine-admixed food (0.5 mg/g food),
without a control group. Non-drug added
food was then given for 24 hr to observe
withdrawal signs after the treatment with
codeine-admixed food, and withdrawal signs
were observed at intervals of 2 hr. During
the treatment, body weight, food intake and
drug intake were measured daily at 5:00 P.M.
Figure 1A gives a summary of the treatment
and the withdrawal schedule in various
series.

Precipitated withdrawal with naloxone:
Rats were divided into 8 groups (n=4–5) and
were treated with codeine-admixed food
(0.5 mg/g food), without a control group.
The withdrawal signs were precipitated by
injecting naloxone (3 mg/kg, s.c.). The rats
were given naloxone at 5:00 P.M., and their
behaviors were observed for 60 min. Body
weight was measured at 15, 30, 60, 90, 120,
150 and 180 min after naloxone injection.
During the treatment, body weight, food
intake and drug intake were measured daily
at 5:00 P.M. Figure 1B gives a summary of
the treatment and the naloxone-precipitated
withdrawal schedule in the various series.

![Fig. 1. Experimental protocol used for the study of physical dependence on codeine. (A) withdrawal of codeine, (B) naloxone-precipitated withdrawal. Open and black columns correspond to normal food and codeine-admixed food, respectively. The hatched column indicates the measurement of body weight and the observation of behavior at intervals of 2 hr. The number of rats used in each group was 4–5. •: naloxone injection (3 mg/kg, s.c.)](image)

![Fig. 2. Time course of the changes in body weight (%) in the control group (---), during chronic codeine treatment (-----), and after codeine withdrawal (--•--). The withdrawal was done at 5:00 P.M. Each plot represents the mean of 4–5 animals. The duration of treatment (days) with codeine-admixed food is given in parentheses.](image)
Results

The relationship between the duration of codeine treatment and the intensity of the withdrawal signs was examined. During the treatment with codeine-admixed food, the animals did not show any signs of intoxication such as loss of body weight and anorexia. Codeine daily intake ranged from 19.7 to 38.1 mg/kg during the treatment. As shown in Fig. 2, the body weight of non-treated rats and codeine-treated rats increased during the night period and decreased during the daytime: namely, chronic codeine treatment did not alter the circadian rhythm of body weight. During the withdrawal of codeine in rats treated with codeine-admixed food for 1 or 2 days, the animals did not show a marked loss of body weight. However, rats treated with codeine-admixed food for 3 to

Table 1. Behavioral changes during codeine withdrawal in rats treated with codeine-admixed food

| Withdrawal signs   | 1   | 2   | 3   | 4   | 5   | 6   | 7 (days) |
|--------------------|-----|-----|-----|-----|-----|-----|----------|
| Diarrhea           | 1/5 | 1/5 | 5/5 | 5/5 | 4/4 | 5/5 | 5/5      |
| Ptosis             | 2/5 | 3/5 | 5/5 | 5/5 | 4/4 | 5/5 | 5/5      |
| Vocalization       | 0/5 | 0/5 | 0/5 | 0/5 | 1/4 | 2/5 | 2/5      |
| Irritability       | 0/5 | 0/5 | 0/5 | 0/5 | 1/4 | 0/5 | 0/5      |

Each value represents positive animals/used animals.
7 days manifested a marked loss of body weight after the withdrawal of codeine, and the body weight loss reached a maximum value at 20 to 24 hr after the withdrawal (Fig. 2). The magnitude of body weight loss 24 hr after the withdrawal was correlated with the duration of codeine treatment (Fig. 3). The spectrum of withdrawal signs are summarized in Table 1.

Naloxone induced withdrawal signs in rats treated with codeine-admixed food. The withdrawal signs included body weight loss, diarrhea, ptosis, teeth chattering, body shakes, salivation, vocalization, nose bleed, irritability, marked scratching, lacrimation and writhing. Body weight loss was observed in all groups treated with codeine-admixed food (Fig. 4), and the magnitude of body weight loss 3 hr after naloxone injection was correlated with the duration of codeine treatment (Fig. 5). Figure 6 shows relationship between the incidence of the naloxone induced withdrawal signs and the duration of codeine treatment.

**Discussion**

There exists a considerable number of reports on the induction of physical dependence on codeine (3, 10, 11), but there has never been any report of the developmental process of physical dependence on codeine. Using the drug-admixed food ingestion method, we examined fully the development of physical dependence on codeine in rats.

The results of this study show that a high degree of physical dependence on codeine which is usually regarded as one of opiates with little dependence-producing liability (3, 12), can be rapidly produced in rats by the drug-admixed food ingestion method. The degree of physical dependence was studied with respect to dose, frequency and period of drug administration, and frequency was found to be the most important factor (13). In this regard, the drug-admixed food ingestion method satisfies these factors, namely, the animals are continuously exposed to the drug. In this study, when the dose and frequency of codeine administration were maintained under the same conditions, the duration of codeine treatment became the most important factor in determining the degree of physical dependence. As shown in Figs. 3 and 5, when loss of body weight was used to quantitate the intensity of withdrawal signs, the degree of physical dependence on codeine was intensified according to the duration of codeine treatment. However, the intensity of withdrawal signs, body weight loss and behavioral signs induced by naloxone was powerful in comparison with that after codeine withdrawal. This result is in agreement with that of morphine as reported by Blasig et al. (14) and Suzuki et al. (8). In fact, naloxone-precipitated and natural withdrawal signs were clearly observed in rats treated with codeine-admixed food for 1 day and 3 days, respectively.

Some criteria used for quantifying the
intensity of the withdrawal signs were loss of body weight (7, 15–17) and behavioral signs (14, 18–20). In this study, body weight loss measured after naloxone injection and codeine withdrawal was correlated with the duration of codeine treatment, respectively. Consequently, the weight loss was found to be an objective and sensitive index for quantitative assessment of physical dependence on narcotics. However, the linear correlation between the weight loss and the duration of codeine treatment suggests that loss of body weight in rats treated with codeine for 5 to 7 days is scarcely intensified with the duration of codeine treatment to the extent that it is in rats treated with codeine for 1 to 5 days. The time of onset, peak intensity and duration of withdrawal signs vary with the degree of physical dependence on the drug (21). Consequently, the time of onset and duration of body weight loss need to be considered.

In the naloxone-precipitated withdrawal test, various withdrawal signs such as diarrhea, ptosis, body shakes, vocalization, teeth chattering, nose bleed, salivation, irritability, marked scratching, lacrimation and writhing were observed. As shown in Fig. 6, the appearance rate of each behavioral sign was related to the duration of codeine treatment. Suzuki et al. (8) reported a ranking system for precipitated withdrawal behavior which was devised to quantify the intensity of physical dependence on morphine by the drug-admixed food ingestion method. Using this scoring system, the relationship between the total score and the duration of codeine treatment was examined. As shown in Fig. 7, the total score was correlated with the duration of codeine treatment. The correlation is similar to the results of naloxone-precipitated withdrawal signs in rats treated with morphine-admixed food (8). On the other hand, the appearance rate of behavioral signs after codeine withdrawal was markedly lower than the naloxone-precipitated behavioral signs (Table 1 and Fig. 6). Furthermore, the continuous observation of the behavioral signs after drug withdrawal is very difficult. These results suggest that weight loss is a common index for quantitative assessment of physical dependence on narcotics in the natural and the naloxone-precipitated withdrawal tests.

It is concluded that the drug-admixed food ingestion method has the advantages of easily and rapidly inducing a high degree of physical dependence on codeine without causing toxicity to animals and in eliminating the need for excessive handling of animals.

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Fig. 7. Correlation between total score of withdrawal signs and the duration of codeine treatment. Withdrawal signs were scored according to Suzuki et al. (8).
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