STUDIES OF PHYSICAL DEPENDENCE ON CINEPAZIDE IN RATS

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Abstract......Physical dependence liability to cinepazide was studied, and the following were found:
1. Rats were applied cinepazide for 96 days by the cinepazide-admixed food method (DAF method) on gradually increasing dosage schedules from low cinepazide doses of 1/4 and 1/2 mg/g food (the average intake being 40 mg/kg/day) to 6 and 8 mg/g food (the average intake being 277 mg/kg/day) which caused toxic signs to evolve, e.g., suppressed righting reflex, urinary incontinence, dacryohemorrhrea, hypothermia, and weight loss. No abstinence signs evolved on withdrawal at any dosage level. 2. The challenge with levallorphan (2 mg/kg, s.c.) at each dosage level during the application of cinepazide on the gradually increasing dosage schedules precipitated no abstinence signs. 3. Cross-application at 3- to 6-hour intervals of 0 (the vehicle only), 10, 30, 100 and 300 mg/kg (p.o.) of cinepazide (doses prolonging the duration of hexobarbital-induced sleep) to animals with the manifestation of moderate to severe barbital abstinence signs, unlike the similar application of diazepam (10, 30 and 100 mg/kg, p.o.) as the positive control, failed to suppress but rather tended to aggravate the abstinence signs. 4. Cross-application at 3- and 6-hour intervals of 0, 10, 30, 100 and 300 mg/kg (p.o.) of cinepazide and similar s.c. application of 30 mg/kg of pethidine resulted in a significant suppression of weight loss due to withdrawal of morphine in the groups of animals treated with 100 and 300 mg/kg of cinepazide. Naloxone challenge at the stage when weight loss was suppressed significantly (p < 0.05) in the animals on the cross-application of pethidine precipitated the abstinence signs but no significant weight loss in the groups on the cross-application of cinepazide. Thus, the suppressive action of cinepazide on the morphine abstinence signs proved to be derived from its non-specific action on drug dependence, and failed to maintain morphine dependence. In conclusion, cinepazide cannot be considered either to have physical dependence liability or maintain barbital- or
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morphine-dependence.

Key words: Cinepazide, nonspecific action for dependence.

INTRODUCTION

In recent years, the safety of drugs is one of the worldwide controversial subjects. Under such a current situation, it is mandatory as a rule in Japan to test the “drugs acting on the central nervous system” for drug dependence liability. Since the research has not yet reached the level where this test should be made only of the drugs which have proved to produce some specified actions, there are an increasing number of new drugs requiring this test.

For this reason, it is desirable to establish the method for drug dependence liability test which, using small animals, is easy to accomplish and permits an accurate estimation. The authors have already reported the experimental systems using rats which permit studies not only of physical dependence on morphine-type drugs but also of the dependence on sedative-hypnotic agents (Yanaura, et al., 1975; Tagashira, et al., 1978, 1979 a-c). Following a previous paper (Yanaura, et al., 1978), the authors studied the utility of the experimental systems by the use of a drug which had proved to have a relatively weak action on the central nervous system in a study of its general pharmacology.

Cinepazide, a drug developed at Lab. Delalande, Courbevoie, France, is used chiefly in the treatment of cerebrovascular accidents and circulatory failure in the peripheral arteries of the four limbs (Marmo, et al., 1973; Bremer, 1974). This drug has been shown to have a central depressant action, e.g., depression of spontaneous motor activity, lying on the side, ptosis, potentiation of hexobarbital-induced sleep, lowering of body temperature and appearance of slow waves on the EEG (Kasai, 1979; Arauchi, 1979); hence, it appears to be a drug that falls in the “category of drugs which require animal experiments and clinical observations of drug dependence liability.” Therefore, the authors made a series of physical dependence liability tests of this drug with reference to the authors' background data on the drugs reported previously (Yanaura, et al., 1974; Yanaura, et al., 1975; Yanaura, et al., 1978). Because it was not clear whether this drug was a morphine-type drug or would suppress the barbiturate abstinence signs, cross-physical dependence liability was studied, using barbital- and morphine-dependent animals; the results were evaluated in 2 categories: suppression of abstinence and maintenance of drug dependence. In this study, a recently controversial subject, a new method for cross-physical dependence liability test, was discussed in relation to whether the suppressive action of the test drug on the morphine abstinence signs had been derived from the maintenance of morphine-dependence liability.

MATERIALS AND METHODS

Cinepazide maleate, chemically called 1-[(1-pyrrolidiny1-carbonyl)-methyl]-4-(3, 4, 5-trimethoxycinnamo1)-piperazine maleate, is a compound with such a chemical structure
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as is shown in Fig. 1. In all the experiments, 5-week-old male Sprague-Dawley strain rats (purchased from Tokyo Laboratory Animals Co., Tokyo) were used as experimental animals, and raised in a air-conditioned stable (temperature range: 22±2°; relative humidity range: 50–60%) throughout the experimental periods.

CH₂O
CH₂O
CH₂O
N
N
N
HCCOOH

CINEPAZIDE

Fig. 1. Chemical structure of cinepazide maleate Molecular formula: C₃₆H₅₈N₃O₉
Molecular weight: 533.6

1) General condition of naive rats on single-dose and repeated application of cinepazide: Cinepazide was dissolved in distilled water; 10, 30, 100 and 300 mg/kg of the drug were applied orally to the rats divided into groups each of 5 animals 11 times at 3-hour intervals (with only the 300 mg/kg dose applied at 6-hour intervals); and the animals were examined for general behaviors, changes in body weight and food intake.

2) Blood levels of cinepazide administered by the cinepazide-admixed food method, a preliminary test was made to check whether the drug would be absorbed when applied by the drug-admixed food method and how the blood levels of the drug would differ from those when applied orally through a stomach tube.

Assay of cinepazide: To 0.1-1 ml of plasma, 1 ml of M/15 phosphate buffer (pH 7.3) was added, and the mixture was extracted with 10 ml of chloroform containing 1 μg/ml of α-naphthol as the internal standard. Eight milliliters of the chloroform layer was measured into a 10-ml glass-stoppered test tube, and evaporated to dryness in the stream of dry nitrogen. The residue was dissolved in 200 μl of a mixture of 0.04 N hydrochloric acid and methanol (50/50, v/v), and 10 ml of the solution was used for high speed liquid chromatography (the Hitachi model 635 liquid chromatograph). A stainless steel column, 150 mm in length and 2.1 mm in caliber, was packed with Lichrosorb RP-18 (5 microns in diameter; E. Merck AG. West germany) with the aid of an equilibrated density slurry. A mixture of 0.1% aqueous solution of ammonium carbonate and methanol (50/50, v/v) was used for elution. All the procedures were accomplished at room temperature, with the flow rate of the eluting solution set at 0.3 ml/min. The effluent was observed continuously at a wavelength of 305 nm with the AFS having a full scale range of 0.02–0.16. The concentration of cinepazide in a plasma sample of an unknown cinepazide concentration was calculated by comparing its peak ratio with those of the standards likewise treated.

About 1 g of food was weighed, and dissolved in sufficient water while stirring to make 1 liter. To 20 ml of this solution, 10 ml of M/15 phosphate buffer (pH 7.3) was added, and the mixture was extracted with 10 ml of CHCl₃. The top layer was discarded, and the absorbance of the CHCl₃ layer at a UV wavelength of 305 nm was determined. Concomit-
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Antily, 20 ml each of 1 and 5 μg/ml solutions of cenepezide as standard solutions was likewise treated, and its absorbance was determined.

Fig. 2. Dosage schedules (doses, days of drug application and withdrawal period) of direct physical dependence liability test of cinepazide in rats (N = 6). W1: Withdrawal for one day. W3: Withdrawal for 3 days. Lev.: Levallorphan challenge (2 mg/kg, s.c.).

3) Direct physical dependence liability test of cinepazide: Cinepazide-admixed foods containing 0.5-8 mg of the drug per g were prepared by the conventional method, and as shown in Fig. 2, the animals were allowed free access to 2 foods of different concentrations at any stage for application of the drug on the gradedly increasing dosage schedules. The animals were allowed to eat the drug-admixed foods and drink water ad libitum for 24 hours a day. Four groups on different gradedly increasing dosage schedules were provided, i.e., a group on low doses for an extended period (group II), and groups on medium doses for longer periods (groups IV and V), to end with toxic doses, respectively. Cinepazide was withdrawn for 24-48 hours (by replacement with a drug-free food) at each dosage level during the drug application period, to observe the general behaviors of the animals, weigh them, and measure their food and water intakes. Also, because this drug is rapidly metabolized in vivo (Cameron, et al., 1976), the animals were weighed at 3- to 4-hour intervals for 24 hours after its withdrawal at each dosage level during the drug application on the gradedly increasing dosage schedules to see whether there occurred any abnormality in their circadian rhythm. Naive rats (group I) were used as the naive control group, and another group of rats from which the drug had not been withdrawn was used.
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as the dependent control group. Morphine was applied as the positive control drug by means of morphine-admixed foods containing 0.5 and 1 mg per g for 43 days; it was withdrawn for 48 hours at 7-day intervals during the application period, to check the animals for changes in general behavior, body weight, food intake and water intake.

4) Precipitation of abstinence signs with levallorphan: As shown in Fig. 2, challenge with 2 mg/kg of levallorphan (s. c.) was made at adequate stages during the application of cinepazide on the gradedly increasing dosage schedules. The naïve rats and morphine–dependent rats as controls were challenged with the same dose of levallorphan. The levallorphan challenge was, in all cases, made to the animals still on the drug-admixed foods, i.e., without withdrawal of the drug. The animals were checked for changes in general behaviors and body weight for 5 hours, starting immediately after each challenge.

5) Cross-physical dependence liability in barbital withdrawn rats: Severely barbital–dependent rats presenting typical clonic–tonic convulsion on withdrawal of barbital, which had been prepared by the method reported by Tagashira et al. (1978, 1979a), were used. Cross-application of cinepazide was begun at 17 hours of withdrawal (10:00 a.m.) when moderate barbital abstinence signs were manifested. Diazepam was used as the positive control drug. The oral cross-application of 10, 30 and 100 mg/kg of cinepazide was made at 3-hour intervals, and that of 300 mg/kg, at 3- to 6-hour intervals, depending on the evolution of toxic signs. The final cross-application of the drug was made at 48 hours of withdrawal when the abstinence signs were mostly maximal. The oral cross-application of 10, 30 and 100 mg/kg of diazepam (as a suspension in 0.5% carboxymethylcellulose) was made at 3-hour intervals as in the application of cinepazide. After the cross-application of the drug 10 times, the vehicle was applied to all the groups at the stage equivalent to the 11th application, and with this application as the initiation of complete withdrawal, the recrudescence of abstinence signs was observed.

6) Cross-physical dependence liability in morphine withdrawn rats: Rats which had been made sufficiently morphine dependent by feeding on foods containing 0.5 and 1 mg of morphine per g for not less than 21 days and which had presented diarrhea, hyperirritability and weight loss on withdrawal (by replacing with a normal food) for 2 days at 7-day intervals during the application period were used. The cross-application was initiated at 17-18 hours of morphine withdrawal when the abstinence signs were manifested in all animals, and the final application was made at 47 hours of the withdrawal (the application was made 11 times in total). The oral application of 0 (the vehicle only), 10, 30, 100 and 300 mg/kg cinepazide was made all at 3-hour intervals (Experiment I). However, the application of 300 mg/kg at 3-hour intervals resulted in the death of 4/6 rats due to overdosage; hence, 0, 10, 30 and 100 mg/kg of the drug were applied at 3-hour intervals, and 300 mg/kg at 6-hour intervals (Experiment II). Cross-application of 10, 20, 40 and 80 mg/kg of pethidine (s. c.) as the positive control drug was also made. In experiment II, both groups of rats cross-applied with 100 and 300 mg/kg of cinepazide and 30 mg/kg of pethidine as experiment II, were challenged with 2 mg/kg of naloxone (s. c., 16:00) to
observe whether the abstinence signs evolved and also whether the morphine dependence was maintained. Naive rats were used as controls, and likewise challenged with naloxone. The animals were examined for the signs and weighed at specified intervals for 6 hours, starting immediately after the naloxone challenge. In all of experiments I, II and III, 4-to 5-day complete withdrawal was provided after the cross-application, to observe the recrudescence of abstinence signs.

RESULTS

1) General behaviors of naive rats on single-dose and repeated application of cinepazide: Virtually no change was noted in general when 10, 30, 100 or 300 mg/kg of cinepazide were applied only once, respectively. From about the 5th application of either dose in the repeated application onwards, sedation and mild systemic muscle relaxation were observed in all groups, and salivation and hypothermia only in the high dose groups. No tolerance to these adverse effects of the drug was observed even the drug was applied 11 times in total at 3-hour intervals, and the animals exhibited neither weight loss nor decreased food intake, compared with the controls (Fig. 3).

![Time course changes with repeated administration of cinepazide in naive rats (N = 5)](image)

**Fig. 3.** Changes in body weight with frequent application to naive rats (N = 5). Doses, intervals and frequency of cinepazide application was the same dose schedule as substitution test (Fig. 7 and 8).
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2) Blood levels of cinepazide on its application by the DAF-method: The 3 different cinepazide-admixed foods, applied through a stomach tube, caused the signs of CNS suppression to evolve. Table 1 shows the blood levels of cinepazide on oral application of 300 mg/kg. The blood levels of this drug were more elevated, the higher was the drug concentration in the food: thus, it was demonstrated that the drug, even applied by the DAF method, was sufficiently transferred into blood. Also, the recovery rates of cinepazide from 3 samples taken randomly from each of 2 powder foods containing different concentrations of cinepazide were 100.1±5.5% and 98.0±0.93%, proving the homogeneous distribution of the drug in the foods (Table 1).

3) Direct dependence liability test of cinepazide: During the period of cinepazide application on the gradedly increasing dosage schedules shown in Fig. 2, 1-day withdrawal (W1) was provided at each dosage level; however, neither weight loss nor the abstinence signs occurred at any stage. At the stage of application of the final concentrations, 6 and 8 mg/g food, the animals began to exhibit marked sedation, systemic muscle relaxation, depression of spontaneous motor activity, ptosis, suppression or disappearance (in several animals) of righting reflex and reduced response to external stimuli, attended by the evolution of toxic signs, e.g., decreased food intake, loss in weight, urinary incontinence, dacryohemorrhage and hypothermia (Fig. 4). The evolution of these signs made further increase in drug concentration impossible. Fig. 5-A and 5-B show changes in body weight on withdrawal of cinepazide at the dosage levels of 4 and 6 mg/g food (group II) and 6 and 8 mg/g food (groups II and IV). At the dosage level of 4 and 6 mg/g food (the average intake being 227.9 mg/kg/day), the withdrawn animals showed virtually no change in body weight, compared with the naive controls or the dependent controls. At the dosage level of 6 and 8 mg/g food, the dependent controls showed a lower rate of nocturnal weight gain than the naive controls or the withdrawn group by replacing the drug-admixed foods with a normal food. Any of the groups, however, did not exhibit either a loss in body weight below the weight before the withdrawal or the abstinence signs within 24 hours of the withdrawal. The same groups neither presented any abnormality in circadian rhythm of body weight, compared with the naive controls. In the group on morphine application (On 0.5 and 1 mg/g foods; the average intake being 50-60 mg/kg/day), 8.0±1.4% weight losses occurred on natural withdrawal at 1 week of the application, and constant weight losses also occurred at 2, 3 and 4 weeks of the application. The rate of weight loss tended to increase, the longer became the morphine application period (Fig. 4). When the animals were put back to the same dosage level as before the withdrawal, they restored body weight completely to the level before the withdrawal in 24-48 hours, attended by the disappearance of abstinence signs, e.g., diarrhea, hyperirritability, piloerection and vocalization on handling.
Table 1. Plasma levels of cinepazide applied as cinepazide-admixed foods, 1, 5 and 10 mg/g food to rats.

<table>
<thead>
<tr>
<th>Drug-admixed food (average drug intake, mg/kg/day)</th>
<th>Plasma levels of drug (µg/ml)</th>
<th>Mean±S. E. M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/g food</td>
<td>2.5</td>
<td>2.00±0.29</td>
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<tr>
<td></td>
<td>1.6</td>
<td></td>
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<td></td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>5 mg/g food*</td>
<td>21.5</td>
<td>17.95±1.44</td>
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<tr>
<td></td>
<td>19.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.7</td>
<td></td>
</tr>
<tr>
<td>5 mg/g food</td>
<td>13.1</td>
<td>15.90±0.84</td>
</tr>
<tr>
<td></td>
<td>18.1</td>
<td></td>
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<td></td>
<td>15.2</td>
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<td></td>
<td>16.2</td>
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<td></td>
<td>16.9</td>
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<tr>
<td>10 mg/g food</td>
<td>30.0</td>
<td>33.55±2.50</td>
</tr>
<tr>
<td></td>
<td>38.1</td>
<td></td>
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<tr>
<td></td>
<td>28.5</td>
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<td></td>
<td>37.6</td>
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<tr>
<td>300 mg/kg (p. o.)**</td>
<td>127.8</td>
<td>85.23±15.10</td>
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<tr>
<td></td>
<td>85.1</td>
<td></td>
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<td></td>
<td>68.3</td>
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<td></td>
<td>59.7</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug-admixed food</th>
<th>Recovery rate (%)</th>
<th>Mean±S. E. M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/g food</td>
<td>104.9</td>
<td>100.1±3.18</td>
</tr>
<tr>
<td></td>
<td>101.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>94.1</td>
<td></td>
</tr>
<tr>
<td>5 mg/g food</td>
<td>96.9</td>
<td>98.0±0.54</td>
</tr>
<tr>
<td></td>
<td>98.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>98.4</td>
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All rats had been fasted for 16 hr prior to drug application. The method for determinations of cinepazide concentrations in plasma is shown in the Materials and methods of the monograph. Blood samples were collected 8 hr (*4 hr) after application of cinepazide with a stomach tube, or as cinepazide-admixed foods. **This dose of cinepazide was applied with a stomach tube, and the plasma was collected 1 hr after drug application. Samples of cinepazide-admixed foods were collected at random from three different food containers.
4) Precipitation of abstinence signs with levallorphan: As shown in Fig. 2, challenge with a narcotic antagonist was made at each of the low, medium and high dosage levels of cinepazide; however, neither the abstinence signs were precipitated nor did weight losses occur at any dosage level (Fig. 6). Levallorphan challenge at 2, 3 and 5 weeks of morphine application always resulted in a significant weight losses ($p < 0.01$) and the evolvement of the typical abstinence signs; however, the antagonism between morphine and levallorphan, unlike the maximum rate of weight loss on natural withdrawal, tended to be low, the longer was the morphine application period (A, B and C in Fig. 6).
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Time course changes in body weight by withdrawal of cinepazide in rats (N=6)

Fig. 5-A and -B. Time course changes in body weight, food and water intakes at 3-hr intervals during the first 24 hr after withdrawal of cinepazide. Control cinepazide-dependent rats were kept on the cinepazide-admixed food. Fig. A and B show the withdrawal stages of 4 and 6 mg/g food and 6 and 8 mg/g food, respectively, according to the gradedly increasing dosage schedules shown in Fig. 2.

5) Cross-physical dependence liability in barbital withdrawn rats: At 17 hours of barbital withdrawal, moderate to severe abstinence signs appeared, i.e., hyperirritability, tremor, ear twitching, ataxia and muscle fasciculation in all animals, and clonic-tonic convulsion or grand mal type convulsion and hyperkinesia in several of them. At this stage, the cross-application of cinepazide was initiated, but in any dosed group, the major abstinence signs were hardly suppressed. Two of the animals (2/6) dosed with 30 mg/kg died of convulsion after the 3rd application. The application was continued until 44 hours of barbital withdrawal, but the abstinence signs were not practically improved.

On the other hand, the initial application of 10, 30 or 100 mg/kg of diazepam as the positive control drug caused the dose-dependent suppression of the abstinence signs, and the dose of 100 mg/kg almost completely suppressed the abstinence signs. Some of the animals on 10 mg/kg exhibited convulsion at 3 hours, i.e., the time for the next application, but the application of another dose suppressed the convulsion in 15-20 minutes. The abstinence signs, especially, anorexia, were markedly improved, and as the decreased food
Fig. 6. Time course changes in body weight (calculated as percentage of the pre-precipitation level) during the first 5 hr after injection of levallorphan (2 mg/kg, s.c.) to rats at three different stages of morphine dependence and those at six different stages of cinepazide dependence (N = 6). The morphine-dependent rats were acquired by feeding the animals on morphine-admixed foods (0.5 and 1 mg/g food) for five weeks. Lavallorphan challenge was made at 2 (A), 3 (B) and 5 (C) weeks of the morphine application, and also at several stages (A–E) of the cinepazide application on the dose schedules shown in Fig. 2.

intake was restored to normal, the weight loss was suppressed or the animals tended to gain weight (Fig. 7). Complete withdrawal followed the cross-application of cinepazide 10 times (at 47 hours of withdrawal). The control animals on natural withdrawal tended to recover from the abstinence signs already at 17 hours of the complete withdrawal, while in the group on 100 mg/kg of cinepazide, moderate abstinence signs, e.g., hyperirritabilitly, vocalization or resistance on touch persisted, and a few animals of the group on 300 mg/kg continued to exhibit convulsion and pass soft feces (or watery feces), attended by an emaciating tendency. The aggravation of these abstinence signs continued until 41 hours of the complete withdrawal, and then recovered. In the groups on 10, 30 and 100 mg/kg of diazepam, on the other hand, the abstinence signs recurred in a dose-dependent fashion or in a manner related to its suppressive action on the abstinence signs from 17–24 hours of the complete withdrawal onwards. Especially, the abstinence signs that recrudesced in the
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Fig. 7. Effects of cross-application of cinepazide and diazepam on the body weight of barbital-withdrawn rats. At the time marked 0 (17:00), the barbital-admixed foods were replaced with a drug-free normal food. The withdrawal was continued after substitution test of both drugs (complete withdrawal).

group on 100 mg/kg in which the cross-application of cinepazide completely suppressed the signs were similar in kind and severity to those appearing at 17 hours of barbital withdrawal, i.e., the animals exhibited rapid losses in body weight, with the losses reaching a maximum at 72 hours of the complete withdrawal as observed in the control group on natural withdrawal at the stage of substitution. The pattern of changes in body weight also showed cinepazide to be entirely free of cross-dependence on barbital.

6) Cross-physical dependence liability in morphine withdrawn rats: The application of 10 mg/kg of cinepazide rather aggravated the diarrhea and weight loss during morphine withdrawal as a whole. The application of 30 mg/kg slightly suppressed the diarrhea, hyperirritability and weight loss, compared with the control group on the withdrawal; however, this suppression proved of no significance. The application of 100 or 300 mg/kg kept the rats sedated, hardly suppressed the diarrhea or soft feces, and suppressed the abstinence signs, e.g., hyperirritability, aggressiveness, vocalization on handling, from the early stage of application or the 4th-5th application onwards. The weight loss continued to be suppressed from the 4th application onwards in the group on 100 mg/kg, and from the 6th application in the group on 300 mg/kg (Fig. 8). There was hardly a difference in
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Fig. 8. Effects of cross-application of cinepazide on the body weight of morphine-withdrawn rats. At the time marked 0 (17:00), the morphine-admixed foods were replaced with a drug-free normal food. The withdrawal was continued after substitution test of the drug (complete withdrawal).

The signs until 12 hours after the final cross-application of cinepazide between the group on 10 or 30 mg/kg of the drug and the control group on withdrawal, but in the groups on high doses, i.e., 100 and 300 mg/kg, more animals tended to pass soft feces or diarrheal ones (watery feces) than those in the control group. This tendency was more prominent in the group on 100 mg/kg than in the group on 300 mg/kg. During the 24 hours following the final cross-application of cinepazide, the control group and the groups on 10 and 30 mg/kg gained body weight in a similar manner to each other, while the groups on 100 and 300 mg/kg lost body weight rapidly. Slightly more animals tended to pass soft feces and diarrheal feces in these groups.

The cross-application of 10–40 mg/kg of pethidine suppressed the morphine abstinence signs and weight loss dose-dependently until the 5th application (Fig. 9). At the 3rd-4th application of 80 mg/kg of this drug, 2/6 animals so treated died from overdosage. The pattern of weight loss suppression showed a reduction in the effect of the drug (tolerance) from the 5th application onwards, with similar weight losses to those in the control group on natural withdrawal; hence, 1.5 times each dose was applied at the final application.
Fig. 9. Effects of cross-application of pethidine on the body weight of morphine-withdrawn rats. Substitution of pethidine was started at 18 hr of morphine withdrawal. The last doses of the cross-application were increased to 1.5 times the preceding doses.

The doses of 15 and 30 mg/kg caused a transiently increased suppression, but the other doses, i.e., 60 and 120 mg/kg, failed to potentiate the suppression; and the doses of not less than 60 mg/kg, including the aforementioned dose of 80 mg/kg, proved to be overdosages. Rapid weight losses occurred in the animals at 3-15 hours of withdrawal following the cross-application, and the losses continued until 40 hours of the withdrawal in the group on 80 mg/kg; thus, the rate of weight loss proved to be dose-related. It was at 20 hours of complete withdrawal in the group on 20 mg/kg, at 24 hours in the group on 40 mg/kg and at 40 hours in the group on 80 mg/kg that a maximum weight loss occurred, attended by the longer durations of the abstinence signs that recrudesced dose-dependently.

Then, challenge with 2 mg/kg of naloxone was made after the repeated cross-application of 100 and 300 mg/kg of cinzapazide and also of a similar s.c. application of 30 mg/kg of pethidine, both of which, as described above, suppressed the weight loss during morphine withdrawal: it precipitated a significant weight loss (p < 0.05) in the pethidine-treated group but not in the cinzapazide-dosed groups, compared with the naive controls and the group on natural withdrawal (Fig. 10).
**DISCUSSION**

The routes of application, doses, application intervals and application periods are the major problems inherent in the direct dependence liability test of drugs. In case of a drug which, like cinepazide used as the test drug in this study, gives rise to obvious CNS-suppressing signs, e.g., suppressed spontaneous motor activity, animals' lying on the abdomen, ptosis and systemic muscle relaxation, it is of importance to apply the drug for a long time on application schedules which allow mild to moderate signs of CNS suppression to persist. This is described by the authors in previous papers (Tagashira, et al., 1978, 1979 a) in relation to the method for the physical dependence liability test of sedative-hypnotics. As described in paper on the cross-physical dependence on pentazocine (Tagashira, et al., 1978), there are some drugs whose action mechanisms in low and high doses are reversed, i.e., acting agonistically in a low dose but antagonistically in a high dose. For this reason, it is important to perform even the direct dependence liability test...
not only on one application schedule but on schedules including low, medium and high doses over different periods. In this study, the application of cinepazide was initiated with the low dosage levels of 1/4 and 1/2 mg/g food which hardly give rise to any change in the signs (the average intake being about 40 mg/kg/day, equivalent to about 4 times its clinical dose), which was gradedly increased over a period of 96 days to the highest dose by which not only the signs of CNS suppression but also general toxic signs evolved. Therefore, the cinepazide application schedules employed in this study may be considered to have been good enough for the dependence liability test of cinepazide.

Because cinepazide is rapidly metabolized in vivo (Cameron, et al., 1976), the present authors, with reference to their experience in the dependence liability tests of pethidine (Yanaura, et al., 1975) and ifenprodil (Yanaura, et al., 1978), considered changes in body weight withdrawal and the stages when the abstinence signs seemed to evolve, by means of determining the disappearance rate of cinepazide from serum sequentially at 0, 12, 24, 36 and 48 hours. As a result, it was observed that cinepazide, even when applied repeatedly in high doses, scarcely remained in blood from 12 hours of withdrawal onwards as when applied only once. It may be concluded therefore that no abstinence signs evolved even at the stage when cinepazide disappears entirely from the body.

From various signs of CNS suppression that evolved at the dosage levels of 6 and 8 mg/g cinepazide-admixed foods, this drug was suspected of an action to suppress barbital abstinence signs. Chlorpromazine and phenytoin as already reported by the authors (Tagashira, et al., 1977) are prone to be judged as having an apparent action to suppress the abstinence signs, respectively, from changes in part of the signs. However, when the signs not only during its cross-application period but also during complete withdrawal after its final cross-application were evaluated as a whole, the drug was demonstrated as entirely incapable of maintaining barbiturate dependence but as rather aggravating the barbiturate abstinence signs. This drug, contrary to expectation, proved to rather have a possibility to suppress the morphine abstinence signs. Moreover, in all the 3 cross-dependence tests (experiments I, II and III), 100 and 300 mg/kg of this drug suppressed the body weight loss at morphine withdrawal. Furthermore, as it was not certain whether the high incidence of diarrhea during the complete withdrawal was due to the recrudescence of the abstinence signs or to the aggravation of the signs resulting from the cross-application of cinepazide, a new experimental system was tried in this study. There are recent reports (Karkalas and Lal, 1973; Gold, et al., 1978; Fielding, et al., 1978; Baldino, et al., 1979), purporting that drugs with no dependence liability suppressed the morphine or heroin abstinence signs, and also were effective against “drug craving.” Although the methods described in these reports differed from the authors' cross-dependence liability test used in this study, it is very likely that drugs with such characteristics will be found in the newly developed drugs in the future. For this reason, challenge with naloxone (as a new experimental system) was made in place of the final cross-application at 47 hours of morphine withdrawal; the stage when morphine had almost completely disappeared from the body (Mulé and Woods,
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1962), to study whether the suppression of withdrawal signs results from a specific action on dependence of a drug like pethidine or an apparent action of a drug with non-specific dependence liability.

REFERENCES


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