CROSS-PHYSICAL DEPENDENCE LIABILITY OF PSYCHOTROPIC DRUGS IN RATS DEPENDENT ON BARBITURATES

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Abstract—Rats were rendered physically dependent on phenobarbital by phenobarbital-admixed foods. Barbital, phenobarbital, ethanol, diazepam, nitrazepam, meprobamate, methaqualone, chlorpromazine, diphenylhydantoin, mephenesin, reserpine and clonidine were cross-administered to evaluate the mode of suppression of withdrawal signs and cross-physical dependence liability. The drugs were administered several times during the period from 17-18 hr (when withdrawal signs began to appear) to about 48 hr after the withdrawal of phenobarbital. From the mode of suppression and severity of relapsed withdrawal signs, these drugs were classified into the following 3 types: Type I: drugs suppressing withdrawal signs of phenobarbital (WSP) almost completely and followed by the relapse of severe WSP. Type II-a: drugs suppressing WSP partially and followed by the relapse of moderate WSP. Type II-b: drugs suppressing WSP partially and followed by the relapse of only mild or practically no signs. Type III (III-a and -b): drugs practically failing to suppress or rather aggravate WSP. Consequently, we found that it was possible to evaluate precisely the cross-physical dependence on sedative-hypnotics by means of investigation for the method of suppression of WSP and the relapse of such signs upon their withdrawal.

In the previous papers (1, 2), we reported a correlation between physical dependence liability to phenobarbital (PhB) or barbital in rats fed on a food containing such a drug (barbiturate-dependence model) and its concentration in the brain and blood capable of maintaining the dependence. In the same papers, we described that systemic muscle rigidity, fascicular twitching, hyperirritability, ataxia, hyperkinesia, clonic-tonic convulsion and even grand mal-type convulsion appeared during the period from 17 or 18 hr to 48 hr after the withdrawal. When the drug had disappeared almost completely in the blood, some rats died immediately after the spontaneous convulsion (1). These kinds of withdrawal signs, time course of their appearance and their duration were similar to those not only in man (3), but also similar to those in large animals such as dogs (4) and monkeys (5). We considered that the rat was useful as a model for physical dependence on sedative-hypnotics.

There are reports of cross-physical dependence liability to sedative-hypnotics in dogs (6), monkeys (7), rats (8) and mice (9-11). However, the methods for the evaluation of such physical dependence liability in small animals and their usefulness have not yet been demonstrated sufficiently (12, 13). In their studies, Goldstein (10) and Belknap et al. (11) investigated the effects of cross-administered drugs on convulsive seizure using the alcohol- or PhB-dependence model in mice. Our aforementioned model is characterized not only by less variations in...
withdrawal signs, but also by the appearance of convulsions in rats. In general, the drugs cross-administered showed different manner of actions on the withdrawal signs. In mice, however, the duration of withdrawal signs is short, and it becomes not possible to administer the test drugs sufficiently enough to discriminate the apparent inhibition of withdrawal signs from cross-physical dependence liability (14). Clinically, there are many patients dependent on alcohol or on sedative-hypnotics treated with substitution drugs for a week or so. The WHO Technical Reports (12, 13) recommend the daily cross-administration of test drug for 6 days as the authorized method for the evaluation of cross-physical dependence liability to sedative-hypnotics.

To evaluate cross-physical dependence of a few kinds of centrally acting drugs, in this study, drugs were examined both for inhibition of PhB withdrawal signs and for maintenance of physical dependence on it. The results were compared with the findings in other species of animals. Furthermore, changes in the body weight of rats during and after the cross-administration period were examined for usefulness as an index for the evaluation of cross-physical dependence liability to sedative-hypnotics.

Materials and Methods

Five- to six-week old Sprague-Dawley (S.D.) rats were rendered physically dependent on phenobarbital (PhB) by PhB-admixed food of gradedly increasing dosage schedule (1). PhB-admixed food was gradedly increased from the initial level of 0.5 and 1 mg/g food to the final level of 4 mg/g food over 39 days. At 5:00 P.M. on the final dosing day, the PhB-admixed food was replaced with a drug-free normal food (natural withdrawal). At 17-18 hr of withdrawal, the rats had already begun to exhibit moderate to severe withdrawal signs (1) such as clonic convulsion, weight loss, etc. At 24 to 48 hr of withdrawal, they exhibited severe withdrawal signs: clonic-tonic convulsion and grand mal-type convulsion.

The rats were cross-administered with the test drugs several times as a rule from 10:00 A.M. the following morning (17 to 18 hr of PhB withdrawal) to 48 hr of PhB withdrawal when almost all natural withdrawal signs had already diminished. Intervals of cross-administration with the test drugs were determined according to speeds of metabolism, the state of inhibition of withdrawal signs, the duration of the inhibition, and also by observing the general behaviors of the rats. Such doses of the test drugs as proven in a preliminary study to be capable of maintaining moderate CNS depression but not to be overdoses in terms of general behaviors were chosen for the cross-administration. After the final cross-administration, the rats continued to be withdrawn from the test drugs and were examined for the relapse of PhB withdrawal signs or the maintenance of PhB dependence.

The rats were weighed, and their food consumptions were measured at each cross-administration and also after the end of the cross-administration at appropriate intervals.

1. Dose-related inhibition of withdrawal signs using changes in body weight as an index: The rats maintained PhB-dependent with 4 mg PhB/g food (maintenance dose: average 200 mg/kg/day) were cross-administered orally with 5, 10, 20 or 40 mg/kg of PhB, 6 times at 6-hr intervals from 18 hr of withdrawal onward. PhB was suspended in carboxymethyl cellulose-Na (CMC) in concentrations at which the respective doses were adjusted to a volume of 0.5 ml/100 g of body weight.

2. Cross-physical dependence on test drugs: The rats were likewise cross-administered with the following test drugs: barbital (4 and 6 mg/g food immediately after PhB
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withdrawal or 40 to 80 mg/kg, p.o., at 4–6 hr intervals), pentobarbital (50 to 100 mg/kg, p.o., at 4–6 hr intervals), ethanol (1.75 to 3.5 g/kg, p.o., at 3–6 hr intervals), diazepam (20 to 40 mg/kg, p.o., at 4–6 hr intervals), nitrazepam (2 mg/g food immediately after PhB withdrawal or 10, 30 and 100 mg/kg, p.o., at 6 hr intervals), meprobamate (300–450 mg/kg, p.o., at 6 hr intervals), methaqualone (10, 30 and 100 mg/kg, p.o., at 6 hr intervals), chlorpromazine (30 and 60 mg/kg, p.o. and i.p., at 4–8 hr intervals), diphenylhydantoin (80 to 160 mg/kg, p.o., at 6 hr intervals), mephenesin (200 to 300 mg/kg, p.o., at 6 hr intervals), reserpine (0.3 and 1 mg/kg, s.c., once 24 hr before or immediately after PhB withdrawal) and clonidine (0.03, 0.1, 0.3 and 1 mg/kg, i.p., at 8–12 hr intervals).

The withdrawal signs were examined for severity according to our classification of barbiturates withdrawal signs (1). Because changes in body weight with the passage of time well represent the severity of various withdrawal signs objectively and allow the quantitative evaluation of the severity, the animals were weighed frequently in this study, together with the observation for changes in general behaviors.

Results

1. Dose-related inhibition of withdrawal signs of phenobarbital using changes in body weight as an index

The first cross-administration with 5, 10, 20 or 40 mg/kg of PhB already caused a dose-related inhibition of withdrawal signs. At the 2nd to 3rd administration with 5 or 10 mg/kg, severe withdrawal signs of PhB (WSP) such as muscle rigidity and twitching and clonic-tonic convulsion were inhibited. However, moderate signs such as tremor, hyperirritability and ataxia tended to persist. The cross-administration with 20 mg/kg or more once or twice already inhibited WSP as a whole. The cessation of the cross-adminis-

Fig. 1. Dose-related suppression of body weight loss by removal of phenobarbital-admixed food with injection of phenobarbital. Phenobarbital was administered 6 times from 17 hr to 48 hr after removal of phenobarbital-admixed food. Subsequent relapse of body weight loss and withdrawal signs were observed following cessation of phenobarbital injection. Arrows denote administration of phenobarbital. Changes in body weight (%) are in reference to the prewithdrawal level of body weight as 0%.

Body wt. response following graded reduction in maintenance dose (N=6)

Phenobarbital

40 mg/kg (□)

20 mg/kg (○)

10 mg/kg (△)

5 mg/kg (◆)

0 mg/kg (■)

Cross-administration

Withdrawal

WO W17 W48 hr 3 4 5 6 7 8 (days)

Body weight change (%)

-32 -24 -16 -8 -0 2

0
Fig. 2. Dose-response relationship between graded reduction of maintenance dose of phenobarbital dependence and suppressing action on the withdrawal signs, and relapse of withdrawal signs following cessation of the drug injection. Body weight was measured 24 hr (closed circles) and 48 hr (open circles) after removal of phenobarbital-admixed food (left panel) and 24, 48 and 72 hr after cessation of the drug injection (right panel). Phenobarbital (40 mg/kg) and the vehicle control refer to 100% and 0% of suppression of withdrawal signs and relapse of withdrawal signs, respectively.

In other words, there was a mostly positive correlation between the intensity of withdrawal signs suppression during the cross-administration and the relapse of the signs after the cross-administration in terms of changes in body weight as a whole (Figs. 1 and 2).

2. Inhibition of withdrawal signs of phenobarbital and maintenance of physical dependence on phenobarbital by cross-administration with the test drugs

Barbital: The cross-administration with barbital inhibited almost all mild to severe WSP, with even anorexia, the mildest of all signs, recovering to almost the state before the withdrawal. The WSP relapsing after cessation of the cross-administration were just similar in time course and severity to those from the control at 17 hr of PhB withdrawal. In the control group, 1 out of 6 rats died of convulsions at 17 hr of PhB withdrawal, and another died of weakness 6 days later. In the group cross-administered with barbital, 1 out of 6 rats died 7 days after the cessation of cross-administration (Fig. 3).

The treatment of barbital-admixed foods from immediately after PhB withdrawal likewise inhibited the WSP completely and was followed by the typical pattern of relapse of signs after its withdrawal (Fig. 3).

Pentobarbital: This test drug, unlike barbital, failed to inhibit the severe withdrawal signs unless a dose near to the sleep-inducing dose to rats was administered. One hr after its cross-administration when the rats were still awake, tremor and systemic muscle twitching were seen in all rats, and clonic-tonic convulsion was seen in a few of them. The cross-administration with pentobarbital at these doses resulted in the im-
mediate inhibition of these signs. However, due to its weak inhibiting action on anorexia, the food consumption remained low, resulting in less recovery of body weight loss. The cessation of cross-administration was therefore followed by mild WSP, with only clonic convulsion appearing sporadically. Moderate withdrawal signs relapsed on the whole.

Ethanol: Ethanol, like pentobarbital, did no inhibit WSP completely unless a marked behavioral depression was maintained. During the cross-administration with this drug, almost all the signs were inhibited with the rats in a coma-like state. However, in the mildly depressed stage exhibiting ptosis, many of them bit the penis which was erect. They showed a tendency to weakness due to bleeding from the penis and anorexia. Most of the rats died within a week following the cessation of the cross-administration due mainly to weakness (Fig. 4).

In general, ethanol caused only partial inhibition of WSP, and a high dosage was necessary to inhibit severe grades of WSP, including convulsions.

Diazepam: The cross-administration of 20 to 40 mg/kg of diazepam inhibited severe grades of WSP such as muscle twitch, convulsions, hyperirritability, etc. and sedated the rats dose-relatedly. However, this drug failed to inhibit the signs as completely as did barbital and nitrazepam, with some of the signs such as tremor, ataxia and anorexia persisting during the cross-administration. Especially, diazepam proved to be less inhibitory to anorexia, with the food consumption of the cross-dosed group increasing only slightly over that of the withdrawal control group, and weight loss was more intense than with barbital and nitrazepam.

Relapse of convulsions was seen in a few rats and was only sporadic. Diazepam proved less contributory to the maintenance of PhB dependence than barbital and nitrazepam.

Nitrazepam: Almost all mild to severe WSP was inhibited with cross-administration with nitrazepam. Rats ingested preferably nitrazepam-admixed food immediately after
PhB withdrawal. Cross-administration, moreover, of various doses of nitrazepam inhibited WSP dose-relatedly. Potency of the inhibiting action of nitrazepam was approximately 1/2 that of barbital (40 mg/kg barbital approximately equal to 100 mg/kg nitrazepam).

Withdrawal signs after cessation of cross-administration with nitrazepam was relapsed also dose-relatedly and was almost the same as those of the withdrawal control group observed from 0 hr to 48 hr after PhB withdrawal (Fig. 5).

Meprobamate: Neither did meprobamate inhibit severe WSP unless a high dose was used to cause severe CNS depression such as loss of righting reflex. However, because it failed to recover and maintain normal food
consumption and water intake, the rats tended to show weakness in general. Meprobamate proved to exert only partial suppression of WSP.

Methaqualone: The cross-administration of 10 to 100 mg/kg of methaqualone suppressed WSP dose-relatedly. The inhibition of the signs with 100 mg/kg of methaqualone. The mode of inhibition was also similar to that with nitrazepam which suppressed almost all the signs with such doses as to cause mild to moderate sedation. The relapse of the withdrawal signs came to its peak 48 hr after the withdrawal of the cross-administration. The relapse of signs of 100 mg/kg of methaqualone. The suppression of the withdrawal signs and the severity of relapse were intimately correlated (results not shown).

Other drugs: The cross-administration with chlorpromazine, diphenylhydantoin or mephenesin, for its sedative and systemic muscle relaxant actions, obviously suppressed the severe signs — especially, tonic convulsion. However, a high dose which seemed to be an overdosage in terms of general behaviors (e.g., hypothermia, dysuria, lying on the abdomen, etc.) was required to suppress the signs continuously. The cross-administration of a low dose was associated with frequent sudden occurrence of clonic convulsion, grand mal-type convulsion or hyperkinesia. Vocalization on touching, ataxia, piloerection, aggressiveness and tremor persisted even in the state of severe CNS depression. Anorexia was scarcely inhibited with any of these drugs. WSP were aggravated after the cessation of the cross-administration on the whole, with the rats tending to be more weakened due to prolonged duration of the signs (Figs. 6 and 7).

Reserpine obviously intensified WSP as a whole — especially withdrawal convulsions remained likely to appear.

Clonidine at the doses causing severe CNS depression inhibited WSP such as hyperirritability, hyperreflexia, tremor, etc. However, this drug in no way inhibited anorexia. The cessation of this cross-administration was followed by aggravation of the withdrawal signs on the whole, leading to retarded recovery.
Fig. 7. Effects of cross-administration of diphenylhydantoin on body weight loss and food consumption (closed column: vehicle control, open column: diphenylhydantoin group) after phenobarbital withdrawal. S, I and M in lowest part of the figure denote Severe, Intermediate and Mild grades of phenobarbital withdrawal signs (Ref. 1), respectively. Short and long arrows during cross-administration denote injection of 80 mg/kg and 160 mg/kg of the drug, respectively.

Discussion

The cross-physical dependence test has been widely used to evaluate the physical dependence liability of sedative-hypnotics because it can be done quickly and economically (6-11). However, it is sometime difficult to determine which type the test drugs belong to, the morphine or sedative-hypnotics type. For this reason, both types of the cross-physical dependence test were required (15, 16). In the previous papers (15-17), we fully described the aspects of the physical dependence test of the morphine type drugs by the drug-admixed food (DAF) method (15-17). Here we have reported the method for the cross-physical dependence test on rats made phenobarbital-dependent by the DAF method and the method for the evaluation of the cross-dependence liability.

Observation of relapse of the withdrawal signs following cessation of the cross-administration is a useful indicator to confirm the maintenance liability of barbiturate dependence with a test drug. In general, relapse of withdrawal signs after cross-administration peaks at 48-72 hr after withdrawal of the test drugs (15, 16). From the time course changes in withdrawal signs of long lasting barbiturates (e.g., PhB and barbital), most typical withdrawal signs, i.e., spontaneous clonic-tonic convulsions, were elicited and lasted from 24 to 48 hr after withdrawal from the drugs. After 48-72 hr, withdrawn rats showed gradual recovery from withdrawal convulsions (1, 2).

However, in the rats made very severely dependent on PhB in the present study, withdrawal convulsions were indeed elicited from 24 to 48 hr after withdrawal of PhB; but in many cases, some of the rats in the withdrawal-control group died immediately after convulsion (1), and other rats further showed long-lasting losses in body weight due to weakness that eventually lead to death (Figs. 1, 4, 5 and 7). Therefore, long-lasting loss in body weight observed later than 48-72 hr after the cessation of cross-administration is not a specific effect of the test drugs on PhB withdrawal signs. On the
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other hand, barbital-dependent rats (1, 15, 16, 18-20) in the same period of drug administration rarely died even after several time of convulsion, and loss in body weight recovered to pre-withdrawal levels on 7-8 days after the withdrawal of barbital. From these viewpoints, it is rational and accurate for evaluation of maintenance of barbiturate dependence to observe the relapse of the withdrawal signs within 48-72 hr after the cessation of cross-administration with the test drugs.

The 13 drugs used both in this study and in the previous reports (15-20) could be classified into the following 3 types by both the mode of inhibition of WSP and the intensity of the relapsed signs within 48-72 hr after the cessation of the cross-administration with those test drugs (Fig. 8):

Type I: Drugs which inhibit WSP almost completely followed by the relapse of severe withdrawal signs by cessation of cross-administration of the test drugs.

Type II-a: Drugs which inhibit WSP partially and is followed by the relapse of moderate withdrawal signs by the cessation of cross-administration of the test drugs.

Type II-b: Drugs which inhibit WSP partially and is followed by the relapse of only mild or no marked signs, i.e., apparent inhibition of the withdrawal signs due to nonspecific action on dependence. In our experience, ifenprodil and isoxsuprine (15), therapeutic agents for cerebrovascular failure, and PhB-antipsychotics combinations (18) inhibited some kinds of withdrawal signs in some animals without maintaining barbiturate dependence.

Type III-a: Withdrawal control, i.e., elicitation of spontaneous convulsions, but no death during withdrawal. Withdrawal signs recovering 7-8 days after withdrawal with use of small doses of the test drugs (Fig. 8, III) that have no cross-physical dependence with barbiturates. These drugs practically do not inhibit WSP, and they do not recover food and water intake. No relapse of withdrawal signs can be followed, and almost all rats recover from withdrawal signs 7-8 days after withdrawal from the test drugs.

Type III-b: Withdrawal-control rats made very severely dependent on barbiturates that

Fig. 8. Classification of several centrally acting drug from the point of view of both suppression of phenobarbital withdrawal signs and relapse of withdrawal signs within 2 (or 3) days following cessation of cross-administration of the test drugs. 1) Withdrawal from barbiturates. 2) Withdrawal from the test drugs after cross-administration. 3) Differences between III-a and III-b are explained in the discussion of the main text.
elicit severe and durable clonic-tonic convulsion. Some of the withdrawn rats die immediately after convulsions or long lasting weakness. In the animals cross-administered at high doses (e.g., severe CNS depression) of these drugs (Fig. 8, III), weakness, the excretion of water-like feces, aggravation of general behavioral signs and longlasting loss in body weight can be observed. No relapse of withdrawal signs can be followed. These animals sometimes need more time for recovery from the withdrawal signs compared with that needed for control withdrawn animals (15, 16, 18).

Abnormal metabolism of the brain monoamines is known to play an important role in the appearance of barbiturate withdrawal convulsion (19-21). Norton (8) reported that reserpine aggravated barbital withdrawal convulsions (audiogenic seizure). More recently, we have reported that p-chlorophenylalanine intensified the withdrawal convulsions and that the decrease in the brain catecholamines induced by α-methyl-p-tyrosine or disulfiram resulted in the inhibition of the convulsions (22). Goldstein (10), on the other hand, studied the effect of monoamine-related compounds on alcohol withdrawal convulsions in mice. In a previous study (20), we found β-adrenoceptor blockers, propranolol and pindolol, inhibit withdrawal convulsions of barbital suggesting that withdrawal convulsions might be derived from imbalance between the activities of noradrenergic and serotonergic neurons. However, mechanisms of various withdrawal signs other than convulsions has to be elucidated, and the role of monoamines in brain must await further studies.

From the viewpoint of drug dependence, there is a principle that withdrawal signs cannot be inhibited with any drug other than the drug depended upon. We believe it is most adequate to evaluate the effect of a test drug on withdrawal signs of barbiturate-type drugs in terms of both the inhibition of withdrawal signs and maintenance of dependence on the barbiturate.

In previous papers (17, 23), we described the results of the cross-physical dependence test using rats made more mildly dependent on phenobarbital than those used in this study. These rats exhibited only a few signs of mild hyperirritability. In mildly dependent animals, it is more difficult to discriminate the drugs apparently inhibiting withdrawal signs such as chlorpromazine, diphenylhydantoin and clonidine from the drugs which have practically cross-physical dependence to barbiturates. For the effective evaluation of cross-physical dependence liability to any test drug, it is important to investigate what kind of effect the drug exerts on the withdrawal signs close to those encountered clinically as in this study.

The results of this study are similar to the findings in dogs (6), monkeys (7) and humans (3, 24). The phenothiazine derivatives are often used clinically after alcohol withdrawal. It is said that the gradual switchover of an antianxiety drug (e.g., diazepam) to neuroleptics is rather aimed at preventing the acquisition of dependence on the antianxiety drug rather than for the efficacy of neuroleptics on withdrawal syndromes (25). Also from the results of this study, chlorpromazine indeed inhibited part of the withdrawal signs, but the cessation of its cross-administration was associated with the retarded recovery of the signs and also with a tendency for the aggravation of signs due to weakness on the whole. Diphenylhydantoin, when administered during PhB withdrawal, was found to have a similar action, which was well consistent with the clinical findings (3).

It was further found that changes in the body weight of rats during and after cross-administration of a test drug was useful as an index to evaluate the cross-physical dependence liability.
References


12) WHO Tech. Res. Ser. No. 287 (1964)


