EFFECTS OF NIFEDIPINE ON PHYSICAL DEPENDENCE ON BARBITAL OR DIAZEPAM IN RATS

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ABSTRACT — The effects of nifedipine on the development of physical dependence on barbitol and diazepam in rats were examined using the drug-admixed food method. Rats were chronically treated with either barbitol- or barbitol in combination with nifedipine-admixed food for 28 days, and with either diazepam- or diazepam in combination with nifedipine-admixed food for 26 days, on schedules of gradually increasing doses of barbitol or diazepam. Withdrawal was conducted by substituting normal food for drug-admixed food on the last day of the treatment. Co-administration of nifedipine with barbitol potentiated body weight loss and withdrawal scores after the termination of barbitol treatment. However, the withdrawal signs after the termination of diazepam treatment were not affected by co-administration of nifedipine with diazepam. These results suggest that nifedipine potentiates the development of physical dependence on barbitol but not diazepam. It is known that co-administration of dihydropyridine derivative nitrendipine suppresses the development of physical dependence on ethanol. Basing on the differences in sensitivity of central depressants, barbiturates, benzodiazepines and ethanol, to three types of voltage-dependent Ca^{2+} channels, such as L-, N- and T-types studied so far, the development of physical dependence on central depressants may be modified differently by L-type Ca^{2+} channel blockers, corresponding to respective depressants.

KEY WORDS : Diazepam, Barbitol, Physical dependence, Nifedipine, Drug interaction.

INTRODUCTION

Barbiturates, benzodiazepines and ethanol act on respective binding sites on GABA_A benzodiazepine receptor/ Cl^- channel complex and increase Cl^- influx into brain synaptosomes ; as a result, these drugs cause the central depressing effects (Morrow *et al.*, 1988 ; Rogawski and Porter, 1990). It is well known that these central

depressants suppress the depolarization-induced Ca^{2+} influx into brain synaptosomes (Blaustein and Ector, 1975 ; Leslie *et al.*, 1983 ; Taft and DeLorenzo, 1984), and that this suppression of Ca^{2+} influx correlates the magnitude of the central depressing effects of these drugs (Leslie *et al.*, 1986). Moreover, it has been reported that Ca^{2+} channel blockers potentiate several behavioral effects of these central depressants, e.g. hypnosis (Dolin and Little, 1986 ; Dolin *et al.*, 1991), ataxia (Dolin *et al.*, 1991 ; Isaacson *et al.*, 1985), anticonvulsion (Czuczwar *et al.*, 1990a, 1990b),

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and hypothermia (Draski *et al.*, 1985 ; Isaacson *et al.*, 1985). Since specific binding sites of Ca^{2+} channel blockers have been found in the brain (Gould *et al.*, 1985) and these blockers possess the central depressing effects (DeSarro *et al.*, 1988 ; Johnston *et al.*, 1986 ; Pucilowski, 1992), the potentiation by Ca^{2+} channel blockers has been thought to be due to the further reduction of the Ca^{2+} influx.

Chronic treatment with barbiturates, benzodiazepines and ethanol develops tolerance and physical dependence (Suzuki *et al.*, 1992a, 1992b). The development of tolerance to these central depressants is accepted to be due to down-regulation of the binding sites of these central depressants on GABA_A benzodiazepine receptor/ Cl^- channel complex (Miller *et al.*, 1988a, 1988b) and then to subsensitivity to the increase in Cl^- influx induced by these central depressants (Allan and Harris, 1987 ; Allan *et al.*, 1992a, 1992b). Moreover, it is known that the subsensitivity to the decrease in Ca^{2+} influx induced by these central depressants (Elrod and Leslie, 1980 ; Harris and Hood, 1980 ; Leslie *et al.*, 1980) and up-regulation of voltage-dependent Ca^{2+} channels take place when tolerance to these central depressants appears (Dolin and Little, 1989). Dolin and Little (1989) reported that dihydropyridine derivative nitrendipine, an L-type Ca^{2+} channel sensitive blocker, suppresses the development of tolerance to ethanol, owing to the prevention of up-regulation of L-type Ca^{2+} channel induced by chronic treatment with ethanol, and suggested that not only the functional changes in Cl^- channels but also voltage-dependent Ca^{2+} channels may be involved in the development of tolerance to ethanol.

On the other hand, the mechanisms of development of physical dependence on and appearance of withdrawal signs of these central depressants have not been clear. It has been recently reported that several Ca^{2+} channel blockers suppress the appearance of withdrawal signs of barbiturates (flunarizine, Suzuki *et al.*, 1993), benzodiazepines (cinnarizine, Chugh *et al.*, 1992 ; nitrendipine, Dolin *et al.*, 1990) and ethanol (nitrendipine, Little *et al.*, 1986 ; nimodipine, Littleton *et al.*, 1990). Moreover, Whittington *et al.* (1991) reported that dihydropyridine derivatives suppress the development of physical de-

pendence on ethanol, owing to the prevention of up-regulation of L-type Ca^{2+} channel induced by chronic treatment with ethanol. The up-regulation of voltage-dependent Ca^{2+} channel and resultant hyper-excitation may be involved in the development of physical dependence on and appearance of withdrawal signs of these central depressants. We previously reported that flunarizine, a T-type Ca^{2+} channel sensitive blocker, suppresses the development of physical dependence on barbitol (Suzuki *et al.*, 1993) and diazepam (Mizoguchi *et al.*, 1993), and suggested that T-type Ca^{2+} channel is involved in the development of physical dependence on barbiturates and benzodiazepines. However, the involvement of L-type Ca^{2+} channel in the development of physical dependence on barbiturates and benzodiazepines has not yet been clear. In the present study, the effects of dihydropyridine derivative nifedipine, an L-type Ca^{2+} channel blocker, on the development of physical dependence on barbitol and diazepam in rats were examined using the drug-admixed food method.

MATERIALS AND METHODS

Animals : Male Sprague-Dawley rats (Tokyo Animal Laboratories Inc., Tokyo, Japan) and Fischer 344 rats (Charles River Japan Inc., Atsugi, Japan) that weighed 180–210 g and 90–110 g at the beginning of the experiment, respectively, were used. Animals were housed in individual cages under a 12 hr light-dark cycle (light on 8 : 30 a.m. to 8 : 30 p.m.) with free access to food and tap water. The room temperature and the relative humidity were maintained at $22 \pm 1^\circ\text{C}$ and $55 \pm 5\%$, respectively.

Drug treatment : To prepare the drug-admixed food, barbitol (Wako Pure Chemical Ind., Tokyo, Japan), diazepam (Profarma Co., Milan, Italy) and nifedipine (Sigma Chemical Co., St. Louis, USA) were mixed with a normal powdered food (CA-1, Japan Clea, Tokyo, Japan) in a mortar. Each Sprague-Dawley rat was fed either barbitol- or barbitol in combination with nifedipine-admixed food for 28 days. On the other hand, each Fischer 344 rat was also fed either diazepam- or diazepam in combination with nifedipine-admixed food for 26 days. Animals could drink tap water *ad libitum*. The

concentration of barbital or diazepam in the food was gradually increased during the treatment, according to the methods of Mizoguchi *et al.* (1994) and Suzuki *et al.* (1993) with minor modifications (Table 1). The concentration of nifedipine in the food was fixed at 1.5 mg/g of food. Body weight and food consumption were measured every day at 16:00. Daily barbital or diazepam intake was calculated according to the method of Suzuki *et al.* (1992a, 1992b).

Motor incoordination: Motor incoordination during the treatment with barbital or diazepam was measured for 3 min using rotarod performance apparatus (9 cm in diameter, 7.5 rpm; Natsume Seisakusho Co., Tokyo, Japan). Each rat was trained to run on a rotarod until it could remain there for 3 min without falling. The rotarod performance test was carried out at an interval of 1–3 days. Motor incoordination was calculated as follows: motor incoordination (%) = $[180 \text{ (sec)} - \text{performance time (sec)}] \times 100/180 \text{ sec}$.

Withdrawal: Withdrawal was achieved by substituting normal food for drug-admixed food at 18:00 on the last day of the treatment. Body weight was measured and withdrawal signs were

observed after the termination of drug treatment. Changes in body weight after the withdrawal were calculated as percent changes from the body weight at the beginning of withdrawal. To quantify the intensity of physical dependence on barbital or diazepam, a rating score for withdrawal signs according to the methods of Mizoguchi *et al.* (1994) and Suzuki *et al.* (1993) with minor modifications was used (Table 2). Withdrawal scores were the sum of the rating scores of individual animals.

Statistical Analysis: Analyses of drug intake and motor incoordination during the chronic treatment and of the time course changes in body weight loss and withdrawal scores were performed by two factor (groups $\times$ times) repeated measures analysis of variance (ANOVA). All other analyses were carried out using Student's *t*-test.

RESULTS

During the treatment, daily barbital or diaze-

Table 1. Progressively increasing dosage schedules for development of physical dependence on barbital and diazepam in rats.

Barbital concentration (mg/g of food)	Duration (days)
0.5 and 1.0	4
1.0 and 2.0	6
2.0 and 4.0	6
4.0 and 6.0	6
6.0	6
Diazepam concentration (mg/g of food)	Duration (days)
1.0 and 2.0	3
2.0 and 4.0	3
4.0 and 6.0	4
6.0 and 8.0	4
8.0 and 10.0	3
10.0	3
12.0	6

Table 2. Score chart for barbital and diazepam withdrawal signs.

Characteristic signs	Score
Weight loss	
5–10%	1
10–15%	2
15% <	3
Piloerection	2
Vocalization	2
Irritability	2
Aggression	2
Diarrhea	2
Teeth-chattering	2
Muscle rigidity	2
Straub tail	2
Ear-twitch	2
Lacrimation	3
Nose-bleed	3
Fascicular-twitch	3
Tremor	3
Convulsion	4
Death	4

pam intake gradually increased in a barbitol or diazepam concentration-dependent manner. The mean barbitol intake at the final barbitol concentration (6 mg/g of food) in barbitol- or barbitol in combination with nifedipine-treated group was 330.5 ± 5.3 or 349.5 ± 8.6 mg/kg/day, respectively. On the other hand, the mean diazepam intake at the final diazepam concentration (12 mg/g of food) in diazepam- or diazepam in combination with nifedipine-treated group was 760.3 ± 9.5 and 748.9 ± 10.8 mg/kg/day, respectively. There was no significant difference in daily barbitol or diazepam intake during the treatment between the nifedipine-free and nifedipine-combined groups.

Motor incoordination induced by barbitol or diazepam was also gradually increased in a concentration-dependent manner during the treatment. The mean motor incoordination in all groups during the final concentration of barbitol or diazepam exceeded 90%, and there were no differences in the intensity of motor incoordination between the nifedipine-free and nifedipine-combined groups ($F[1,240] = 0.66$, $P > 0.05$ and $F[1,240] = 0.42$, $P > 0.05$, respectively).

After the termination of barbitol or diazepam treatment, several withdrawal signs were observed. These signs included body weight loss, piloerection, vocalization, irritability, aggression, muscle rigidity, Straub's tail, ear-twitching, teeth-chattering, fascicular-twitch, lacrimation, nose-bleed, tremor, convulsions and death. Figures 1 and 2 show the time course of changes in withdrawal scores after the termination of barbitol and diazepam treatments, respectively. Co-administration of nifedipine significantly potentiated the withdrawal scores after the termination of barbitol treatment ($F[1,143] = 10.815$, $P < 0.01$; Fig. 1), but not after the termination of diazepam treatment ($F[1,135] = 0.19$, $P > 0.05$; Fig. 2). As shown in Figures 3 and 4, body weight decreased after the termination of barbitol or diazepam treatment. The maximum weight loss after the withdrawal from barbitol was $8.7 \pm 1.6\%$ at 66 hr after the withdrawal in the barbitol-treated group and $8.1 \pm 0.5\%$ at 54 hr after the withdrawal in the barbitol in combination with nifedipine-treated group (Fig. 3). On the other hand, the maximum weight loss after the withdrawal from diazepam was $12.4 \pm 1.0\%$ at 51 hr

Fig. 1. Time course of changes in withdrawal scores after withdrawal from treatment with barbitol (BAR) or barbitol in combination with nifedipine (NIF; 1.5 mg/g of food). Each point represents the mean with S.E.M. of 6–7 observations. Withdrawal scores were calculated by adding the rating scores for individual animals. Withdrawal scores were significantly potentiated by co-administration of nifedipine.

Fig. 2. Time course of changes in withdrawal scores after withdrawal from treatment with diazepam (DZP) or diazepam in combination with nifedipine (NIF; 1.5 mg/g of food). Each point represents the mean with S.E.M of 5-6 observations. Withdrawal scores were calculated by adding the rating scores for individual animals.

Fig. 3. Time course of changes in body weight loss (%) after withdrawal from treatment with barbitol (BAR) or barbitol in combination with nifedipine (NIF; 1.5 mg/g of food). Each point represents the mean with S.E.M. of 6-7 observations. Body weight loss after the withdrawal was calculated as percent change from the body weight at the beginning of withdrawal. Body weight loss was significantly potentiated by co-administration of nifedipine.

Fig. 4. Time course of changes in body weight loss (%) after withdrawal from treatment with diazepam (DZP) or diazepam in combination with nifedipine (NIF; 1.5 mg/g of food). Each point represents the mean with S.E.M. of 5–6 observations. Body weight loss after the withdrawal was calculated as percent change from the body weight at the beginning of withdrawal.

after the withdrawal in the diazepam-treated group and $12.3 \pm 1.1\%$ at 45 hr after the withdrawal in the diazepam in combination with nifedipine-treated group (Fig. 4). Weight loss after the withdrawal from barbital but not diazepam was significantly increased by co-administration of nifedipine ($F[1,143] = 12.636$, $P < 0.01$ and $F[1,135] = 0.37$, $P > 0.05$, respectively).

DISCUSSION

In the present study, the modification of the development of physical dependence on barbital or diazepam by nifedipine was investigated in rats using the drug-admixed food method. We previously reported the effects of Ca^{2+} channel blockers, flunarizine and diltiazem, on the development of physical dependence on barbital in Sprague-Dawley rats, using the drug-admixed food method (Suzuki *et al.*, 1993). However, it is known that there are strain differences in the sensitivity to central depressants, barbiturates, benzodiazepines and ethanol and in the potential of development of physical dependence on these central depressants (Suzuki *et al.*, 1987, 1992a,

1992b). Although the development of a severe physical dependence on benzodiazepines is very difficult in Sprague-Dawley rats, it is easy to develop the dependence in Fischer 344 rats (Suzuki *et al.*, 1980, 1992a). Therefore, Sprague-Dawley rats were used in the experiment of barbital physical dependence and Fischer 344 rats were used in the experiment of diazepam physical dependence. Chronic co-administration of nifedipine potentiated withdrawal scores and body weight loss after the withdrawal from barbital treatment, but not diazepam treatment. It is known that withdrawal scores are related to body weight loss. However, in the present study, there is a difference in peak time between withdrawal scores and body weight loss after the termination of barbital or barbital in combination with nifedipine treatment. Although this reason is not clear, it may be a reason that the severe withdrawal signs such as convulsion and tremor were preceded the severe body weight loss after the withdrawal. Moreover, Okamoto *et al.* (1981) demonstrated individual variations in the drug elimination half-lives related significantly to the severity of physical dependence produced by

barbiturates: the longer half-life, the more intense the production of physical dependence by barbiturates. However, it is known that barbital is not readily metabolized and is excreted in rats (Ebert *et al.*, 1964). Therefore, influence of nifedipine on metabolism of barbital may eliminate as mechanisms of the potentiation of development of physical dependence on barbital by nifedipine. These results suggest that co-administration of nifedipine potentiates the development of physical dependence on barbital, but not on diazepam. The reports of Dolin *et al.* (1990) and our previous report (Mizoguchi *et al.*, 1993) which were examined in mice using benzodiazepine partial inverse agonist FG 7142 suggested that nifedipine did not affect the development of physical dependence on flurazepam and diazepam. Therefore, taken together, it is concluded that L-type Ca²⁺ channel blocker does not affect the development of physical dependence on benzodiazepines.

Functional changes in the GABA_A benzodiazepine receptor/Cl⁻ channel were considered to be mainly involved in physical dependence on these central depressants. However, maximal down-regulation of the binding sites and subsensitivity of Cl⁻ channel appears at 0 hr after the withdrawal from these central depressants (when tolerance to these central depressants appears), and these functional changes in the binding sites and Cl⁻ channel already disappear when withdrawal convulsion occurs (Ito *et al.*, 1989; Miller *et al.*, 1988b; Tietz *et al.*, 1986). These functional changes in the binding sites and Cl⁻ channel may therefore be directly involved in tolerance to and indirectly involved in the development of physical dependence on these central depressants.

Recently, it has seemed that chronic treatment with central depressants caused up-regulation of voltage-dependent Ca²⁺ channels (Dolin and Little, 1989; Whittington *et al.*, 1991). When withdrawal signs of the central depressants (especially convulsions) appear, the increase of neurotransmitter release and overstimulation of several neurons are caused (Hitchcott *et al.*, 1990). Voltage-dependent Ca²⁺ channels in the brain seem to contribute the spontaneous depolarization wave and rebound excitations in the brain (Takahashi and Akaike, 1991) and to regulate the neurotransmitter release from nerve

terminals (Miller, 1987). Therefore, the up-regulation and supersensitivity of voltage-dependent Ca²⁺ channels on the nerve terminals and cell bodies may contribute to the appearance of withdrawal signs of the central depressants (Little *et al.*, 1986); as a result, Ca²⁺ channel blockers may suppress the appearance of withdrawal signs of the central depressants. On the other hand, Ca²⁺ channel blockers also suppress the development of physical dependence on the central depressants (Table 3). Whittington *et al.* (1991) reported that dihydropyridine derivatives suppressed the development of physical dependence on ethanol, owing to the prevention of up-regulation of L-type Ca²⁺ channel which was induced by chronic treatment with ethanol. Therefore, Ca²⁺ channel blockers may suppress the development of physical dependence on the central depressants by preventing the up-regulation of voltage-dependent Ca²⁺ channels.

In contrast with T-type calcium channel sensitive blockers, L-type Ca²⁺ channel sensitive blockers suppress the development of physical dependence on ethanol but not on barbiturates and benzodiazepines (Table 3). It is known that the types of voltage-dependent Ca²⁺ channels through which barbiturates, benzodiazepines and ethanol decrease Ca²⁺ influx are different among them. For example, benzodiazepines selectively suppress the Ca²⁺ influx through the T-type Ca²⁺ channel by mediating the central-type benzodiazepine receptor (Watabe *et al.*, 1993). On the other hand, barbiturates suppress Ca²⁺ influx through the N- and L-type Ca²⁺ channels, but not T-type Ca²⁺ channel (Gross and Macdonald, 1988). Chronic treatment with these central depressants may selectively induce the up-regulation of the voltage-dependent Ca²⁺ channel which acute treatment with these central depressants selectively suppresses. In fact, chronic treatment with ethanol induces the up-regulation of L-type Ca²⁺ channel (Whittington *et al.*, 1991), but chronic treatment with benzodiazepines did not (Dolin *et al.*, 1990).

In the present study, nifedipine potentiated the development of physical dependence on barbital. There is another report concerning potentiating effect of L-type Ca²⁺ channel sensitive blockers on the physical dependence on barbiturates. Yutzenka and Reynen (1991) reported

Table 3. Modification of the development of physical dependence on central depressants by two types of Ca^{2+} channel blockers.

	L-type Ca^{2+} channel sensitive blockers		T-type Ca^{2+} channel sensitive blockers
	Nitrendipine Nifedipine	Diltiazem	Cinnarizine Flunarizine
<i>Ethanol</i>	suppression (Whittington <i>et al.</i> , 1991)	—	—
<i>Barbiturates</i>			
Pentobarbital	—	potentiation (Yutrzenka and Reynen, 1991)	—
Barbital	potentiation (present results)	no effect (Suzuki <i>et al.</i> , 1993)	suppression (Suzuki <i>et al.</i> , 1993)
<i>Benzodiazepines</i>	no effect (present results)	no effect (Mizoguchi <i>et al.</i> , 1993)	suppression (Mizoguchi <i>et al.</i> , 1993)

that diltiazem potentiated the development of physical dependence on pentobarbital. In the case of benzodiazepines which selectively suppress Ca^{2+} influx through T-type Ca^{2+} channel (Watabe *et al.*, 1993), these potentiations do not appear. In contrast to benzodiazepines, barbiturates suppress Ca^{2+} influx through both N- and L-type Ca^{2+} channels (Gross and Macdonald, 1988). Moreover, barbiturates are divided into 2 groups by the characteristics of inhibition of Ca^{2+} influx through L- and N-type Ca^{2+} channels (Gundersen *et al.*, 1988). One group of barbiturates, pentobarbital, secobarbital and amobarbital, suppresses mainly Ca^{2+} influx through L-type Ca^{2+} channel but slightly that through N-type Ca^{2+} channel (Gundersen *et al.*, 1988). On the contrary, another group, barbital, phenobarbital and hexobarbital, suppresses mainly Ca^{2+} influx through N-type Ca^{2+} channel but slightly that through L-type Ca^{2+} channel (Gross and Macdonald, 1988). The potentiation of the development of physical dependence on barbiturates by L-type Ca^{2+} channel sensitive blockers may be related to the selectivity of barbiturates to L- and N-type Ca^{2+} channels and to the chronic interaction between L- and N-type Ca^{2+} channels. It is well known that there is an allosteric interaction between dihydropyridines and diltiazem or verapamil (Glossmann *et al.*, 1985). The dihydropy-

ridines and diltiazem or verapamil reduce the blocking effects of L-type Ca^{2+} channel each other. Moreover, the increase of intracellular Ca^{2+} concentration potentiates the binding and blocking effects of dihydropyridines to L-type Ca^{2+} channel, but suppresses that of diltiazem or verapamil. Chronic interaction between binding sites of dihydropyridines and diltiazem is not clear. However, the potentiating effects of diltiazem and nifedipine on the development of physical dependence on pentobarbital and barbital, respectively, may also involve the chronic allosteric interactions between the respective binding sites of Ca^{2+} channel blockers and the selectivity of central depressants to Ca^{2+} channels, because the effects of diltiazem on the development of physical dependence on pentobarbital (Yutrzenka and Reynen, 1991) and barbital (Suzuki *et al.*, 1993) are different.

The present study indicated that in contrast to ethanol physical dependence, L-type Ca^{2+} channel blocker nifedipine potentiates the development of physical dependence on barbital but not on diazepam. Unlike T-type Ca^{2+} channel blockers, the modification of the development of physical dependence on the central depressants by L-type Ca^{2+} channel blockers is derived from the differences in the selectivity of central depressants to three types of voltage-dependent

Ca²⁺ channels.

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