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Effects of Flunarizine and Diltiazem on Physical Dependence on Barbital in Rats

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SUZUKI, T., H. MIZOGUCHI, H. NOGUCHI, T. YOSHII AND M. MISAWA. *Effects of flunarizine and diltiazem on physical dependence on barbital in rats.* PHARMACOL BIOCHEM BEHAV **45**(3) 703–712, 1993.—The effects of flunarizine and diltiazem both on development of physical dependence on barbital and on barbital withdrawal signs in rats were examined using the drug-admixed food (DAF) method. Rats were chronically treated with barbital or barbital in combination with flunarizine (fixed at 1.5 mg/g of food) or diltiazem (fixed at 0.75 mg/g of food)-admixed food on the schedule of gradually increasing doses of barbital. Motor incoordination during the treatment was potentiated by coadministration of flunarizine, but not by coadministration of diltiazem. After the termination of drug treatment, the body weight loss and withdrawal scores were significantly suppressed in the group coadministered flunarizine, but not in that coadministered diltiazem. There were no significant differences in plasma barbital levels after the withdrawal between groups. In the substitution test, flunarizine (20 and 40 mg/kg, IP) significantly suppressed the body weight loss and withdrawal scores after the withdrawal, but diltiazem (20 mg/kg, IP) did not. These results indicated that flunarizine suppressed both the development of physical dependence on barbital and barbital withdrawal signs, mainly according to the suppression of convulsions, but not diltiazem, which is known to poorly penetrate into the brain. Therefore, the present findings suggest that central calcium channels may be involved in both the development of physical dependence on barbital and the appearance of barbital withdrawal signs.

Barbital Physical dependence Rat Flunarizine Diltiazem Calcium channel blockers

CALCIUM channel blockers are widely used as vasodilator and antiarrhythmic agents. Specific binding sites for these drugs have been found in the periphery and the brain (21), and recently effects of these drugs on the central nervous system (CNS) have been reported. Several studies suggested that calcium channel blockers have some CNS depressing effects such as anticonvulsant (8–12), ataxic (24) and anxiolytic effects (2).

On the other hand, acute treatments with barbiturates (4, 17,19), ethanol (18,20,22,26,35), and benzodiazepines (27,28, 37) decrease calcium influx or calcium uptake, suggesting that CNS depressing effects of these drugs may result from reduction of central calcium concentration (19,20,27,28). It is known that calcium channel blockers potentiate the anesthetic effects of ethanol, pentobarbital, and midazolam (13,15), the anticonvulsant effect of phenobarbital (8,9), the hypothermia induced by ethanol and diazepam (16,23), and the motor incoordination by ethanol, midazolam, and clonazepam (15,23). Thus, calcium channel blockers seem to potentiate the CNS depressing effects of CNS depressants that affect GABA·benzodiazepine receptor/chloride channel complex.

Chronic administration of barbiturates, ethanol, and benzodiazepines develops physical dependence and tolerance. Administration of calcium channel blockers, especially dihydropyridines, during chronic ethanol treatment prevents development of tolerance to ethanol (14,45). Furthermore, calcium channel blockers suppress ethanol withdrawal signs, when these drugs were administered systemically to ethanol-dependent rats at the termination of ethanol treatment (29, 30). However, effects of calcium channel blockers on physical dependence on barbiturates and benzodiazepines have been scarcely investigated.

In the present study, we examined the effects of two types of calcium channel blockers, diltiazem and flunarizine, on both the development of physical dependence on barbital and the barbital withdrawal signs. It is well known that flunarizine possesses a property of high penetration into the brain (34), but diltiazem possesses a property of poor penetration (32).

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Therefore, we discussed the role of central and peripheral calcium channels in physical dependence on barbital.

METHOD

Animals

Male Sprague-Dawley rats (Tokyo Animal Laboratories Inc., Tokyo, Japan), weighing 180–230 g at the beginning of the experiment, were used. Animals were housed in individual cages under a 12L : 12 D cycle with free access to food and water. The room temperature was maintained at 22 ± 1°C, and the relative humidity was maintained at 55 ± 5%. The rats were allowed to adapt to their environment for a period of 1 week.

Drug Treatment

For preparing the drug-admixed food, barbital (Wako Pure Chemical Ind., Tokyo, Japan), barbital and diltiazem hydrochloride (Sigma Chemical Co., St. Louis, MO), or barbital and flunarizine dihydrochloride (Sigma Chemical Co., St. Louis, MO) were mixed with a normal powdered food (CA-1, Japan Clea, Tokyo, Japan) in a mortar (36,47). Each rat was allowed to eat the barbital or barbital in combination with diltiazem or flunarizine-admixed food and to drink tap water ad lib. The concentration of barbital in the food was gradually increased during the treatment. When rats were treated with one dose of barbital, there was one food container in a cage. When rats were treated with two doses (e.g., 0.5 and 1.0 mg/g of food), there were two food containers with each dose in a cage. The concentrations of diltiazem and flunarizine in the food were fixed at 0.75 and 1.5 mg/g of food, respectively. Body weight and food consumption were measured every day at 16:00. Daily barbital intake was calculated as follows:

$$\text{barbital intake (mg/kg/day)} = \frac{\text{food intake (g/day)} \times \text{drug concentration (mg/g of food)}}{\text{body weight (kg)}}$$

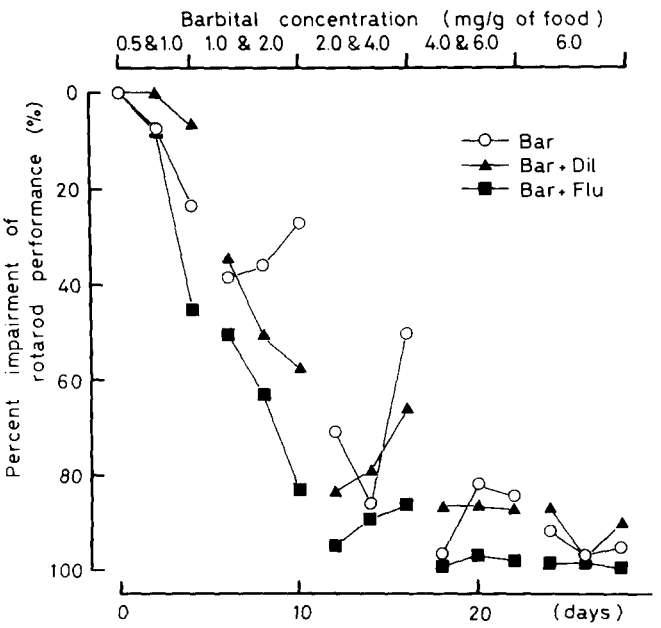

FIG. 1. Motor incoordination (percent) during barbital or barbital in combination with diltiazem or flunarizine-admixed food treatment on a schedule of gradually increasing barbital dosage for 28 days in rats. Each point represents the mean of eight observations. Bar: barbital treated group. Bar + Dil: barbital in combination with diltiazem (0.75 mg/g of food) treated group. Bar + Flu: barbital in combination with flunarizine (1.5 mg/g of food) treated group.

TABLE 1

SCORE CHART FOR BARBITAL 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
Dysuria	3
Hematuria	3
Fascicular-twitch	3
Jerk	3
Tremor	3
Convulsion	
Handling type	3
Spontaneous type	4
Death	4

Measurements of Motor Incoordination

Motor incoordination in barbital and barbital in combination with diltiazem or flunarizine-treated rats 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 every other day.

Withdrawal

Withdrawal was conducted by substituting normal food for barbital and barbital in combination with diltiazem or flunarizine-admixed food at 18:00 h 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, a rating score for withdrawal signs was used (Table 1): these grades of withdrawal signs were according to the method of Suzuki et al. (36) with minor modifications.

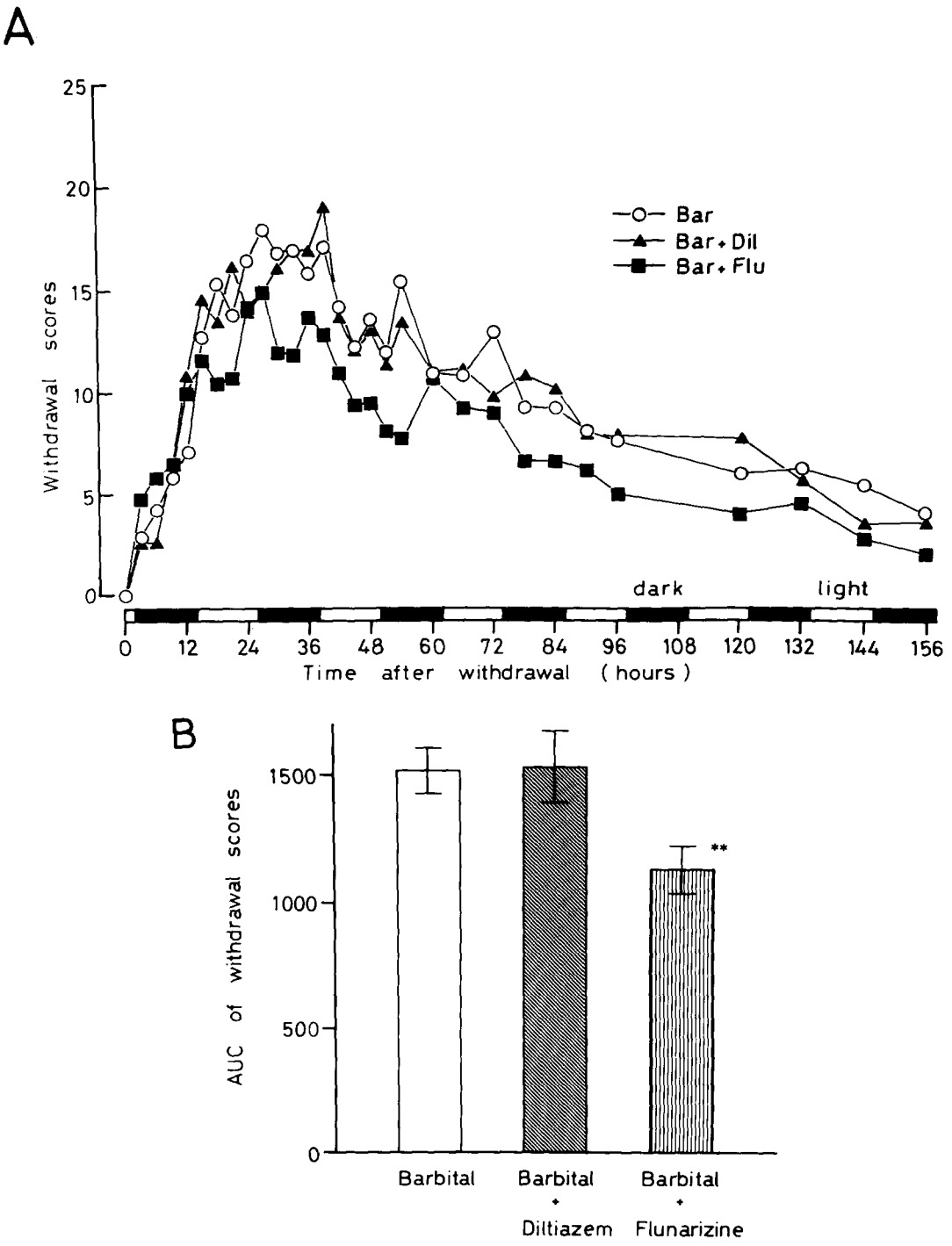

FIG. 2. Withdrawal scores after withdrawal from barbital or barbital in combination with diltiazem (0.75 mg/g of food) or flunarizine (1.5 mg/g of food) treatment. (A) Time course changes in withdrawal scores after the withdrawal. Each point represents the mean of eight observations. Bar: barbital treated group. Bar + Dil: barbital in combination with diltiazem treated group. Bar + Flu: barbital in combination with flunarizine treated group. (B) Area under the curve of withdrawal scores after the withdrawal. Each column represents the mean with SE of eight observations. $*p < 0.05$ vs. barbital treated group.

Substitution of Calcium Channel Blockers

After the withdrawal, barbital-treated rats were divided into nine groups. Three groups were injected with diltiazem (20 and 40 mg/kg, IP) or saline from 17 h to 57 h after the withdrawal at intervals of 4 h. Furthermore, other four groups were injected with flunarizine (20, 40, and 80 mg/kg, IP) or vehicle from 17 h to 53 h after the withdrawal at intervals of 6 h. Diltiazem was dissolved in saline, and flunarizine was dissolved in vehicle consisting of 9% Tween 80 (Kishida Chemical Co., Osaka, Japan) in saline.

Determination of Plasma Barbital Levels After the Withdrawal

Plasma barbital levels were measured four times at 8 h intervals after the withdrawal. Rats of barbital and barbital in combination with diltiazem or flunarizine-treated groups, were drawn blood samples of 400 μl from a tail caudal vein using hematocrit tubes (100 μl, Drummond Scientific Co., PA). Blood samples of 400 μl were centrifuged at 5000 rpm for 10 min and then plasma samples of 100 μl were separated. The plasma barbital levels were analyzed by high performance liquid chromatography according to the method of Kabra et al. (25).

Statistical Analysis

Analysis for the incidence of withdrawal signs was performed by the chi-square (2 × 2) test. Analysis for time course changes in motor incoordination, body weight loss, and withdrawal scores was performed by two factor (groups × times) repeated measures analysis of variance (ANOVA). All other analyses were carried out using the Student's *t*-test.

RESULTS

Effects of Calcium Channel Blockers on Motor Incoordination

There was practically no significant difference in daily barbital intake during the barbital treatment between barbital-treated group and barbital in combination with diltiazem or flunarizine-treated groups. The mean barbital intake at the final barbital concentration (6 mg/g of food) was 389.3 ± 6.9 mg/kg/day for barbital-treated group, 374.2 ± 13.6 mg/kg/day for barbital in combination with diltiazem-treated group, and 374.6 ± 18.7 mg/kg/day for barbital in combination with flunarizine-treated group.

During the treatment, motor incoordination gradually increased in a barbital concentration-dependent manner (Fig. 1). By coadministration of flunarizine, barbital-induced motor incoordination was significantly potentiated, $F(1, 240) = 19.634, p < 0.01$. However, barbital-induced motor incoordination was not affected by coadministration of diltiazem.

Effects of Coadministrations of Calcium Channel Blockers on Barbital Withdrawal Signs

After the termination of barbital treatment, several signs of barbital withdrawal were observed. These signs included piloerection, vocalization, irritability, aggression, muscle rigidity, Straub's tail, ear-twitching, diarrhea, teeth-chattering,

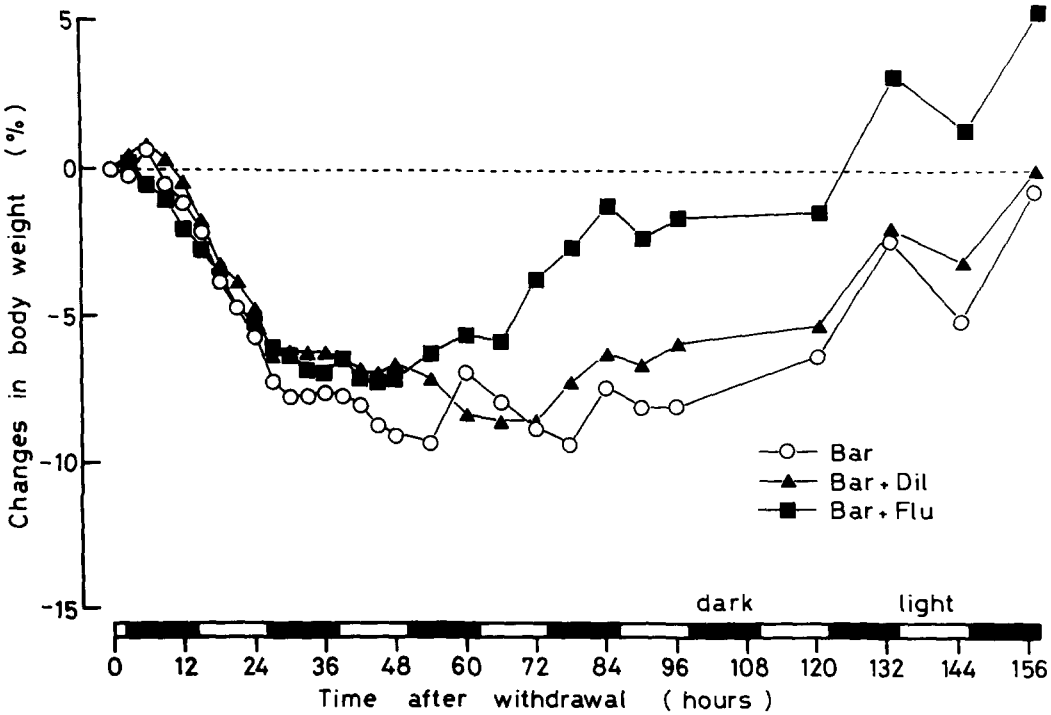

FIG. 3. Time course changes in body weight loss (percent) after withdrawal from barbital or barbital in combination with diltiazem or flunarizine treatment. Each point represents the mean of eight observations. Bar: barbital treated group. Bar + Dil: barbital in combination with diltiazem (0.75 mg/g of food) treated group. Bar + Flu: barbital in combination with flunarizine (1.5 mg/g of food) treated group.

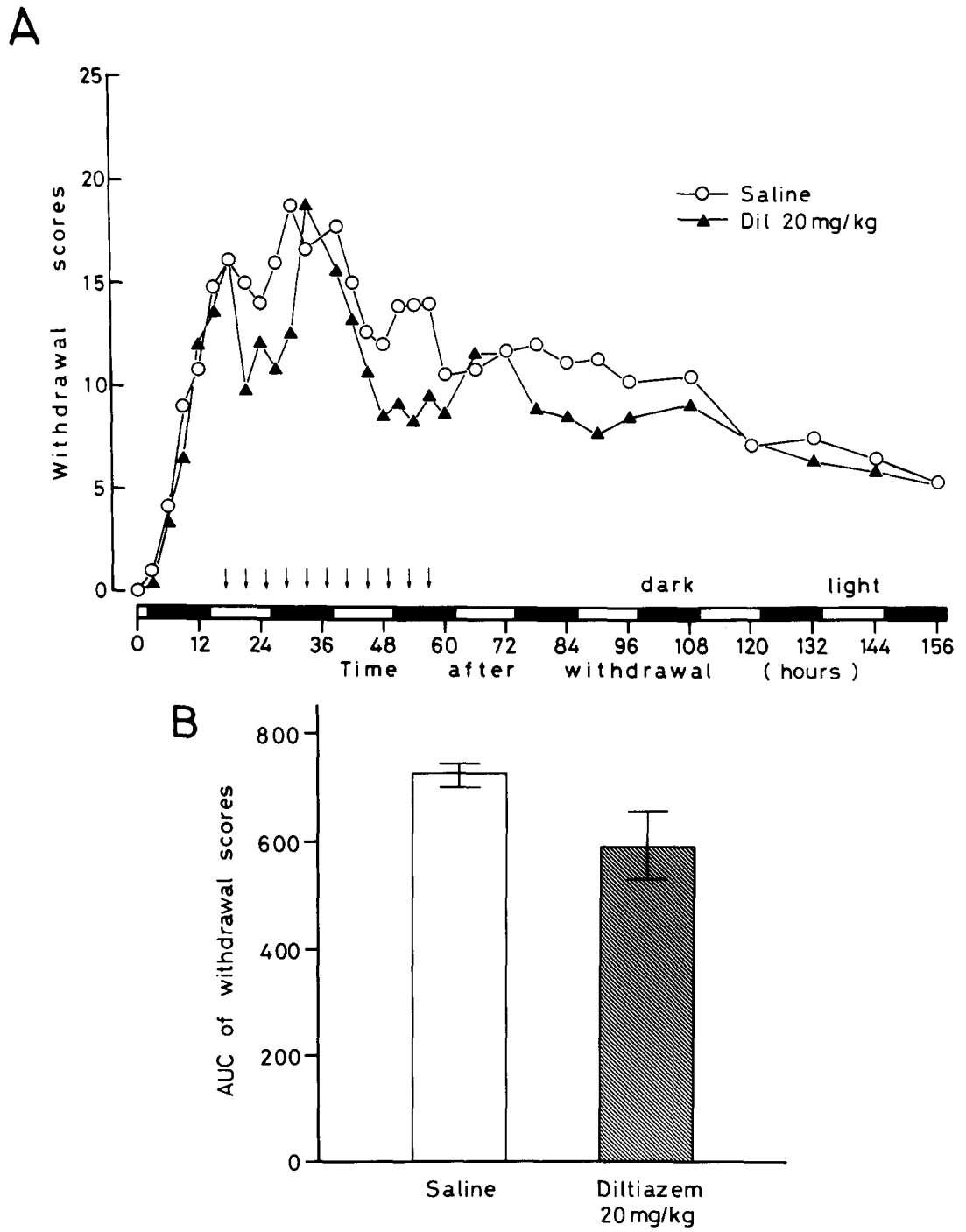

FIG. 4. Effect of substitution of diltiazem on withdrawal scores after withdrawal from barbital treatment. (A) Time course changes in withdrawal scores after the withdrawal. Each point represents the mean of six observations. Each arrow represents the injection of saline or diltiazem. Saline: saline substitution group. Dil 20 mg/kg: diltiazem 20 mg/kg substitution group. (B) Area under the curve of withdrawal scores after the withdrawal. Each column represents the mean with SE of six observations.

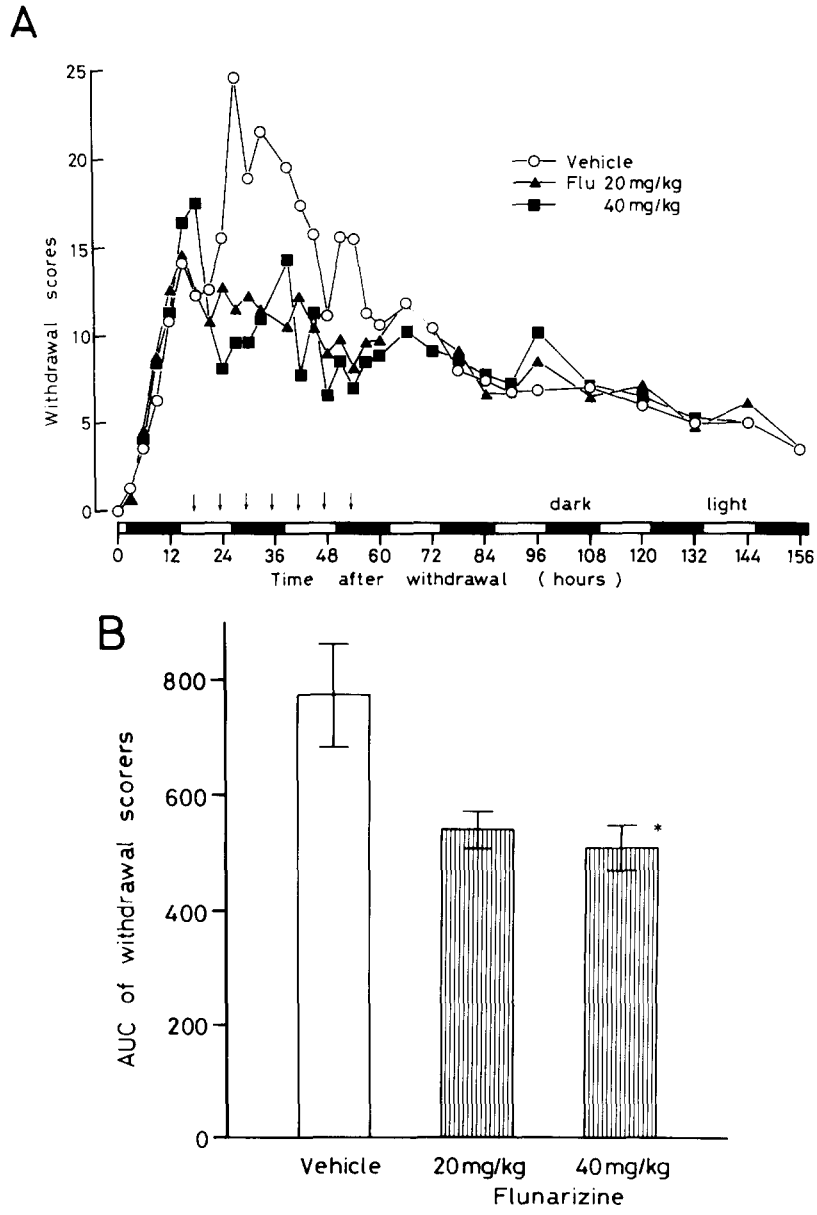

FIG. 5. Effect of substitution of flunarizine on withdrawal scores after withdrawal from barbital treatment. (A) Time course changes in withdrawal scores after the withdrawal. Each point represents the mean of six observations. Each arrow represents the injection of vehicle, or flunarizine 20 or 40 mg/kg (IP). Vehicle: vehicle substitution group. Flu 20 mg/kg: flunarizine 20 mg/kg substitution group. Flu 40 mg/kg: flunarizine 40 mg/kg substitution group. (B) Area under the curve of withdrawal scores after the withdrawal. Each column represents the mean with SE of six observations. $*p < 0.05$ vs. vehicle substitution group.

fascicular-twitch, lacrimation, nose-bleed, dysuria, hematuria, jerk, tremor, handling-elicited convulsions, and spontaneous convulsions.

Figure 2A shows the time course changes in withdrawal scores after the termination of barbital treatment. Withdrawal scores were significantly suppressed by coadministration of flunarizine, $F(1, 168) = 20.425$, $p < 0.01$. However, withdrawal scores were not affected by coadministration of diltiazem. Moreover, the areas under the curve (AUC: score × hour) of withdrawal scores were 1520 ± 87 for barbital-treated group, 1535 ± 140 for barbital in combination with diltiazem-treated group, and 1126 ± 94 for barbital in combination with flunarizine-treated group (Fig. 2B). The AUC of withdrawal scores was significantly lower in barbital in combination with flunarizine-treated group than that in barbital-treated group ($p < 0.01$).

As shown in Fig. 3, body weight of animals in all groups increased a little immediately after the barbital withdrawal,

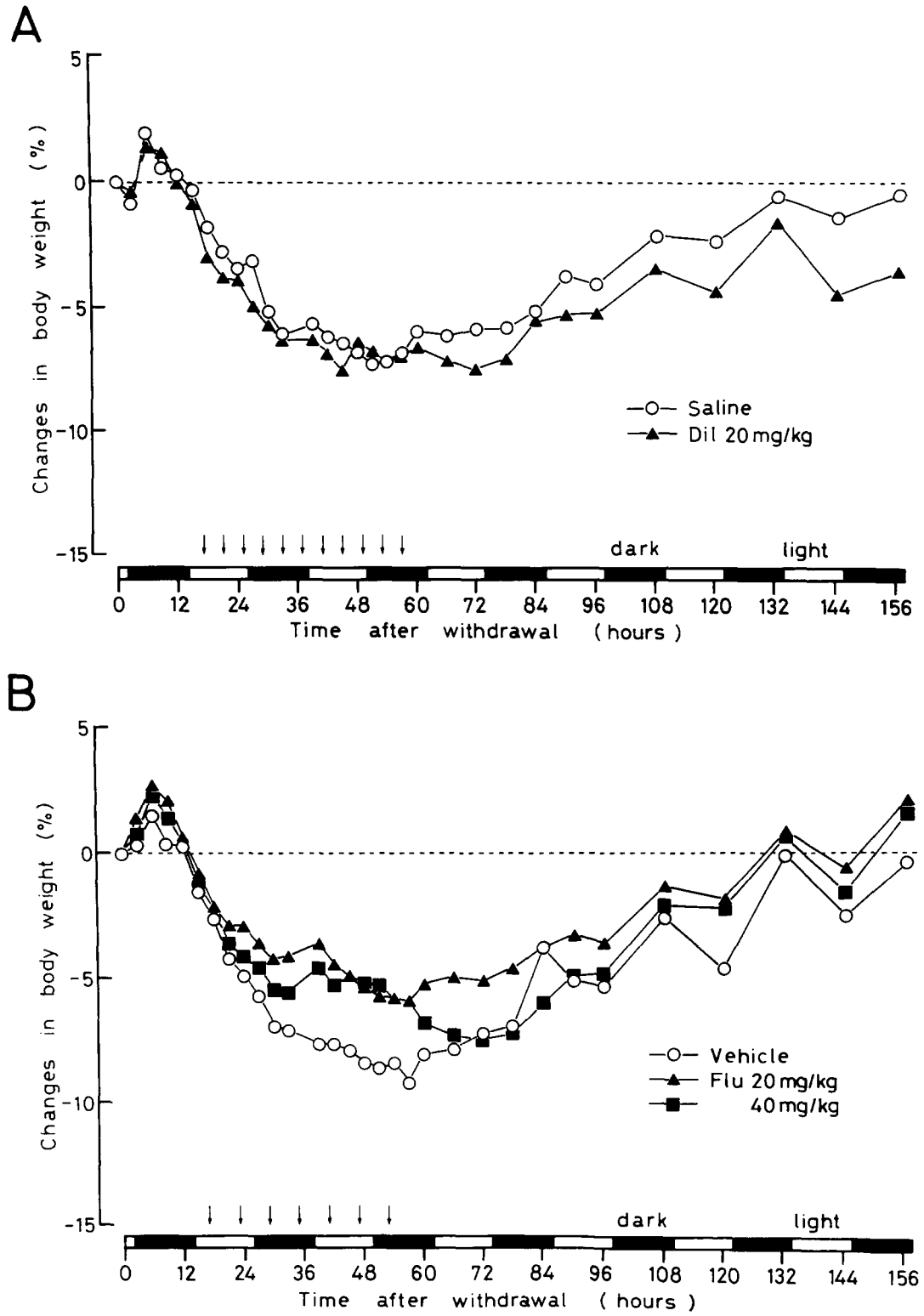

FIG. 6. Time course changes in body weight loss (percent) after withdrawal from barbital treatment. Each point represents the mean of six observations. (A) Substitution of saline or diltiazem. Each arrow represents the injection of saline or diltiazem 20 mg/kg (IP). Saline: saline substitution group. Dil 20 mg/kg: diltiazem 20 mg/kg substitution group. (B) Substitution of vehicle or flunarizine. Each arrow represents the substitution of vehicle, or flunarizine 20 or 40 mg/kg (IP). Vehicle: vehicle substitution group. Flu 20 mg/kg: flunarizine 20 mg/kg substitution group. Flu 40 mg/kg: flunarizine 40 mg/kg substitution group.

and then decreased abruptly. The maximum weight loss was 9.49 ± 1.20% at 51 h after the withdrawal in barbital-treated group, 8.55 ± 1.53% at 66 h after the withdrawal in barbital in combination with diltiazem-treated group and 7.23 ± 2.45% at 45 h after the withdrawal in barbital in combination with flunarizine-treated group. Weight loss after the withdrawal was significantly reduced by coadministration of flunarizine, $F(1, 168) = 20.074$, $p < 0.01$, but not by coadministration of diltiazem.

Effects of Substitutions of Calcium Channel Blockers on Barbital Withdrawal Signs

After the termination of barbital treatment, several signs of barbital withdrawal were observed. The incidences of withdrawal signs during the substitution (from 18 h to 60 h after withdrawal) were affected by substitution of diltiazem or flunarizine. Flunarizine 20 mg/kg significantly suppressed tremor (3/6, $p < 0.05$) and spontaneous convulsions (0/6, $p < 0.01$) as compared with vehicle treatment (6/6 and 5/6, respectively). Flunarizine 40 mg/kg significantly suppressed hematuria (0/6, $p < 0.05$) and handling-elicited convulsions (1/6, $p < 0.05$) as compared with vehicle treatment (4/6 and 5/6, respectively). On the other hand, diltiazem 20 mg/kg significantly suppressed handling-elicited convulsions (0/6, $p < 0.05$) as compared with saline treatment (4/6), but did not affect to other withdrawal signs.

Figure 4A and 5A show the time course changes in withdrawal scores after the termination of barbital treatment. Substitution of flunarizine or diltiazem significantly suppressed withdrawal scores during the substitution [flunarizine 20 mg/kg: $F(1, 150) = 34.239$, $p < 0.01$, flunarizine 40 mg/kg: $F(1, 150) = 39.673$, $p < 0.01$, diltiazem 20 mg/kg: $F(1, 150) = 19.502$, $p < 0.01$]. While diltiazem partially suppressed the withdrawal scores during the substitution (Fig. 4A), flunarizine constantly suppressed the withdrawal scores (Fig. 5A). Figures 4B and 5B show the AUC of withdrawal scores during the substitution. The AUCs of withdrawal scores during the substitution of flunarizine (20 mg/kg: 543 ± 32, $p < 0.05$, 40 mg/kg: 511 ± 39, $p < 0.05$) were significantly lower than that during the substitution of vehicle (775 ± 89) (Fig. 5B). However, there was no significant difference in the AUC of withdrawal scores during the substitution between saline group (722 ± 22) and diltiazem 20 mg/kg group (593 ± 63) (Fig. 4B).

Figure 6 shows the time course changes in body weight loss after the barbital withdrawal. During the substitutions, weight loss was significantly reduced by flunarizine [20 mg/kg: $F(1, 150) = 28.179$, $p < 0.01$, 40 mg/kg: $F(1, 150) = 20.168$, $p < 0.01$], but not by diltiazem.

More than half barbital-withdrawn rats died because of diltiazem 40 mg/kg or flunarizine 80 mg/kg substitution, and these rats showed intraperitoneal hemorrhage, but not in naive rats (data not shown). As a result, these dosage of diltiazem or flunarizine may be toxic dosage for barbital withdrawn rats.

Changes in Plasma Barbital Levels After the Withdrawal

Plasma barbital levels after the withdrawal are shown in Fig. 7. There were no significant differences in plasma barbital levels among groups.

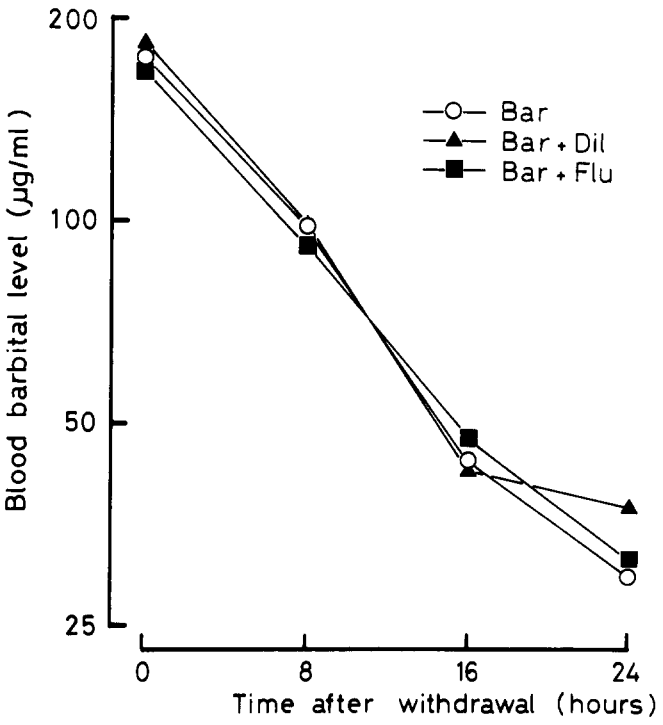

FIG. 7. Time course changes in blood barbital levels after withdrawal from barbital or barbital in combination with diltiazem or flunarizine treatment. Each point represents the mean of five to six observations. Bar: barbital treated group. Bar + Dil: barbital in combination with diltiazem (0.75 mg/g of food) treated group. Bar + Flu: barbital in combination with flunarizine (1.5 mg/g of food) treated group.

DISCUSSION

CNS depressants such as barbiturates, benzodiazepines, and ethanol are known to affect GABA·benzodiazepine receptor/chloride channel complex, and to develop tolerance and physical dependence. Recently, several reports have indicated that some calcium channel blockers prevented the development of tolerance to ethanol (14,45), and the ethanol withdrawal signs (29,30), and suggested that the functional changes in central calcium channel may be involved in the developments of tolerance to and the withdrawal signs of ethanol (14,18,20,22,26,29,30,45). In the present study, we examined the effects of calcium channel blockers on barbital physical dependence.

In general, it is known that the severity of physical dependence on sedative hypnotics such as barbiturates, relates to the magnitude of CNS depression during treatment (38,39). For example, Tagashira et al. (40) reported that when the magnitude of CNS depression with phenobarbital was enhanced due to its combination with dependence liability-free drugs (such as chlorpromazine); consequently, the combination potentiated the withdrawal signs of phenobarbital. On the contrary, in the present study, although coadministration of flunarizine, a calcium channel blocker, potentiated the barbital-induced motor incoordination, it suppressed the development of physical dependence on barbital. The potentiating effect of chlorpromazine on CNS depression and the development of phenobarbital physical dependence may result from the inhibition of monoamine reuptake by chlorpromazine (40). On the other hand, it has been suggested that the CNS depressing effects of ethanol, barbiturates, or benzodiazepines may result from reduction of central calcium concentration

(19,20,27,28), and that calcium channel blockers potentiate the CNS depressing effects of sedative hypnotics by further reducing the CNS calcium concentration (8,9,13,15,16,23). Although the mechanism of suppressing effect of flunarizine on the development of barbital physical dependence is not clear, it is known that tolerance to and physical dependence on sedative hypnotics may well relate to functional changes in central calcium channels (14,17,18,20,22,26,28-30,45). Therefore, the suppressing effect of flunarizine on the development of physical dependence on barbital may result from some functional changes in central calcium channel rather than from changes in central calcium concentration.

Boisse and Okamoto (5) reported that barbiturate withdrawal signs may be inversely related to residual blood barbiturate concentrations. There is a possibility that coadministration of flunarizine affects the disappearance rate of barbital. Therefore, we measured plasma barbital levels after the withdrawal. However, there were no differences in plasma barbital levels after the withdrawal between barbital-treated group and barbital in combination with flunarizine-treated group. These results suggest that the suppression of development of physical dependence on barbital by flunarizine is not ascribable to a pharmacokinetic interaction.

Chronic treatment with CNS depressants such as ethanol, barbiturates, and benzodiazepines, develops tolerance and physical dependence, and induces an upregulation of central calcium channel and/or an increase in central calcium influx (14,17,18,20,22,26,28-30,45). Furthermore, ethanol withdrawal signs may be caused by hypersensitivity of nerve terminals to calcium (29,30). From these findings, it is considered that functional changes in central calcium channel induced by chronic administration of CNS depressants may be involved in the development of tolerance to and physical dependence on CNS depressants. Chronic treatment with some calcium channel blockers, for example, nifedipine, verapamil, etc., induces a downregulation of central calcium channel (33). Dolin and Little (14) reported that nitrendipine prevents development of tolerance to ethanol ataxic action, as a result of prevention of upregulation of dihydropyridine receptors which is induced by chronic ethanol treatment. Therefore, the suppression of development of physical dependence on barbital by flunarizine may be due to a prevention of functional changes in central calcium channel.

Unlike flunarizine, diltiazem did not affect the barbital-induced motor incoordination and the development of physical dependence on barbital. Moreover, the suppression of barbital withdrawal signs by diltiazem was partial and weak. These results suggest that diltiazem may be unable to efficiently suppress the development of physical dependence on barbital and the barbital withdrawal signs. It is well known that diltiazem possesses a property of poor penetration into the brain (32) and that flunarizine possesses a property of high penetration into the brain (34). Then, central calcium channels but not peripheral calcium channels may be involved in the development of physical dependence on barbital and appearance of barbital withdrawal signs.

On the other hand, flunarizine potently suppressed the barbital withdrawal signs, especially convulsions. Flunarizine affects T-type calcium channel, rather than L-type calcium channel (1,31,41,43,44). Moreover, phenytoin, an antiepileptic drug and a selective T-type calcium channel blocker (42, 46), suppresses withdrawal convulsions induced by barbital (40). Therefore, the suppression of barbital withdrawal signs by flunarizine may result from blockade of T-type calcium channel.

Little et al. (29) and Littleton et al. (30) reported that flunarizine prevents ethanol withdrawal convulsions, and Chugh et al. (6) also reported that cinnarizine, a selective T-type calcium channel blocker, prevents diazepam withdrawal signs. Because withdrawal signs of ethanol and diazepam are caused by increases in evoked transmitter release owing to hypersensitivity of the nerve terminals to calcium, calcium channel blockers may prevent the hypersensitivity to calcium. As a result, calcium channel blockers may prevent these withdrawal signs. In the present study, flunarizine suppressed the barbital withdrawal signs. This suppression by flunarizine may be through the same mechanism as ethanol, and diazepam withdrawal signs are suppressed by T-type calcium channel blockers.

Flunarizine has an anticonvulsant property in laboratory animals and humans (3,10,11). Because phenytoin and ethosuximide are T-type calcium channel blockers (7,42,46), antiepileptic effects of these drugs may result from T-type calcium channel blockade. In the present study, flunarizine suppressed the development of physical dependence on barbital. From the above findings, we, therefore, considered that the adequate coadministration of T-type calcium channel blockers and barbiturates has an important mean for producing a potent antiepileptic action with decreasing physical dependence potential of barbiturates.

In conclusion, we found that flunarizine suppresses both the development of physical dependence on barbital and the appearance of barbital withdrawal signs, mainly according to the suppression of convulsions, but diltiazem does not. The differences between flunarizine and diltiazem may be due to the difference in the ability to penetration into the brain, and central T-type calcium channel may be involved in physical dependence on barbital.

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