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European Journal of Pharmacology 316 (1996) 7–14

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Diltiazem inhibits naloxone-precipitated and spontaneous morphine withdrawal in rats

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Received 20 May 1996; revised 7 August 1996; accepted 13 August 1996

Abstract

The effects of diltiazem, a Ca^{2+} channel blocker, on naloxone-precipitated and spontaneous morphine withdrawal were studied in male Sprague-Dawley rats. In naloxone-precipitated withdrawal, body weight loss and plasma corticosterone elevation were dose dependently inhibited by diltiazem injected 4 or 2 and 4 h before naloxone, respectively. Three administrations of diltiazem (17, 11 and 5 h before naloxone) did not reduce the above withdrawal signs. Diarrhea was dose dependently inhibited by all schedule of diltiazem treatments. In spontaneous withdrawal, body weight loss and plasma corticosterone elevation were dose dependently inhibited by two (6 and 12 h) or three (6, 12 and 18 h after the last morphine) treatments with diltiazem at 6-h intervals after the last morphine, but not by a single diltiazem injected 18 h after the last morphine.

Keywords: Diltiazem; Naloxone-precipitated withdrawal; Morphine withdrawal, spontaneous; Corticosterone, plasma; Body weight loss; Diarrhea; (Rat)

1. Introduction

Physical dependence is developed by sustained or repeated exposure to morphine and a withdrawal syndrome occurs in morphine dependent animals following the administration of a opiate antagonist, naloxone (naloxone-precipitated withdrawal) or the cessation of chronic morphine treatment (spontaneous withdrawal). This process complicates the therapeutic use of morphine. Biochemical studies have demonstrated that chronic administration of morphine increased brain synaptosomal Ca^{2+} levels (Yamamoto et al., 1978; Guerrero-Munoz et al., 1979) and this increased Ca^{2+} content rapidly returned to normal values after morphine withdrawal (Yamamoto et al., 1978). L-type Ca^{2+} channel blocker (dihydropyridine) binding sites exist in the brain (Triggle and Janis, 1987) and these binding sites were somewhat increased in the cerebral cortex of rats implanted with morphine pellets (Ramkumar and El-Fakahany, 1988), whereas they were markedly increased in morphine-abstinent rats (Antkiewicz-Michaluk et al., 1990). These findings suggest that L-type Ca^{2+} channels are functionally linked to the development of physical dependence on morphine or to the expression of morphine withdrawal.

It has been reported that L-type Ca^{2+} channel blockers, dihydropyridines (e.g., nimodipine, nifedipine, nicardipine) and phenylalkylamine (e.g., verapamil), protect against naloxone-precipitated morphine withdrawal in mice (Ramkumar and El-Fakahany, 1988; Barrios and Baeyens, 1991) and rats (Bongianni et al., 1986; Baeyens et al., 1987; Ramkumar and El-Fakahany, 1988; Antkiewicz-Michaluk et al., 1990; Colado et al., 1993). In addition, there have been a few reports on the effects of benzothiazepine (e.g., diltiazem), another L-type Ca^{2+} channel blocker, on naloxone-precipitated withdrawal in morphine dependent mice (Caro et al., 1988; Barrios and Baeyens, 1991) and rats (Colado et al., 1993). However, effects of L-type Ca^{2+} channel blockers on spontaneous withdrawal have not been reported as far as we know, although the suppression of spontaneous withdrawal signs is quite important for the therapy of opioid abuse or withdrawal from clinically prescribed opioid analgesics.

Body weight loss and diarrhea have been used as a reliable indicator of morphine withdrawal in rodents (Akera and Brody, 1968; Wei et al., 1973; Kishioka et al., 1994). On the other hand, there are several lines of evidence that plasma corticosterone elevation, which reflects the activity of hypothalamo-pituitary-adrenal axis, is also a sensitive and quantitative indicator of both naloxone-precipitated

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PII S0014-2999(96)00651-6

and spontaneous morphine withdrawal (Yamamoto et al., 1973; Eisenberg, 1983; Kishioka et al., 1994).

In this study, the effects of diltiazem on both naloxone-precipitated and spontaneous withdrawal were investigated in morphine dependent rats, and measurements of not only body weight loss and diarrhea but also plasma corticosterone elevation were taken as indicators of morphine withdrawal.

2. Materials and methods

2.1. Experimental animals

Male Sprague-Dawley rats (Nihon Clea, Osaka, Japan) weighing 200–250 g at the start of morphine treatment, were used in all experiments and were housed two per cage in an animal room with controlled temperature (22–24°C), humidity (60–70%) and light-dark cycle (on 07:00–19:00), prior to use for at least 2 weeks. They were given laboratory chow (MF; Oriental Yeast, Tokyo, Japan) and water ad libitum.

2.2. Experimental protocol

Morphine was administered in two ways; (1) in the naloxone precipitated withdrawal study, morphine (20 mg/kg, s.c.) was injected once daily (10:00) for 5 days (subacute morphine) and (2) in the spontaneous withdrawal study, morphine was injected twice daily (10:00 and 16:00) at increasing doses for 6 days, followed by 4 times daily administration (10:00, 16:00, 22:00 and 04:00) for 2 days (chronic morphine), i.e., 20 mg/kg × 2/day (day 1–2), 40 mg/kg × 2/day (day 3–4), 60 mg/kg × 2/day (day 5–6), 40 mg/kg × 4/day (day 7–8), and the last dose of morphine (40 mg/kg) was injected at 10:00 of day 9.

Morphine withdrawal was elicited by the injection of naloxone (naloxone-precipitated withdrawal) or by the cessation of chronic morphine administration (spontaneous withdrawal). The magnitude of withdrawal was evaluated by body weight loss, diarrhea and plasma corticosterone elevation.

In the naloxone-precipitated withdrawal study, naloxone (2 mg/kg, s.c.) was injected 23 h (i.e., 09:00) after the last morphine administration and withdrawal signs were measured at 1 h after naloxone. The diltiazem treatments (40 and 80 mg/kg, s.c.) were carried out; (1) 6, 12 and 18 h after the last morphine administration (i.e., 6-h intervals after the last morphine), (2) 19 h after the last morphine administration or (3) 21 h after the last morphine administration. The blood samples for the plasma corticosterone assay were collected between 10:00 and 11:00 by cardiopuncture under pentobarbital anesthesia (1–2 min after 600–900 mg/kg of pentobarbital, i.p.) in order to exclude the effect of a circadian rhythm on circulating plasma corticosterone. The collected blood samples were centrifuged at 2000 × g for 30 min at 4°C, and the plasma was separated and stored at −20°C until the time of assay. The plasma corticosterone level was estimated fluorometrically according to the method of Zenker and Bernstein (1958).

In the spontaneous withdrawal study, chronic morphine administration was discontinued, that is, the last morphine injection was carried out at 10:00 of day 9. The diltiazem treatments (10–40 mg/kg, s.c.) were carried out at 6, 12 and 18 h after the last morphine injection (i.e., 6-h intervals after last morphine). Body weight changes and plasma corticosterone concentrations were assessed 6, 12, 18 and 24 h (or only at 24 h) after the last morphine administration. In contrast to naloxone-precipitated withdrawal, diarrhea was not elicited in the spontaneous withdrawal. The procedure for the collection of blood samples and the estimation of plasma corticosterone levels was the same as mentioned above.

2.3. Drugs

The drugs used were morphine hydrochloride (Takeda, Osaka, Japan); naloxone hydrochloride, diltiazem hydrochloride (Sigma, St. Louis, MO, USA); sodium pentobarbital (Nembutal; Dinabot, IL, USA); heparin sodium (heparin sodium inj.; Fuso, Osaka, Japan); chloroform, sulfuric acid, sodium hydroxide (special grade, Katayama, Osaka, Japan); and ethanol (special grade, Wako, Osaka, Japan). Morphine, naloxone and diltiazem were dissolved in saline; the solutions were freshly prepared immediately before use. The injection volume was 0.2 ml/100 g body weight. Doses of drugs were given in terms of their salts.

2.4. Data analysis

Values are presented as the means ± S.E.M. In analyzing the data, Student's t-test was used to determine the differences between two means. With three or more means, a one-way analysis of variance (ANOVA) was used first and then the observed significant differences were confirmed with the Newman-Keuls test. The diltiazem effects on naloxone-induced diarrhea were analyzed by the χ^2-test. Statistical analyses were performed by the use of the computer programs described by Tallarida and Murray (1987) (Pharmacologic Calculation System, version 4.0).

3. Results

3.1. Naloxone-precipitated and spontaneous morphine withdrawal

After the subacute morphine administration, naloxone (2 mg/kg, s.c.) caused body weight loss and plasma corticosterone elevation, compared to saline control in

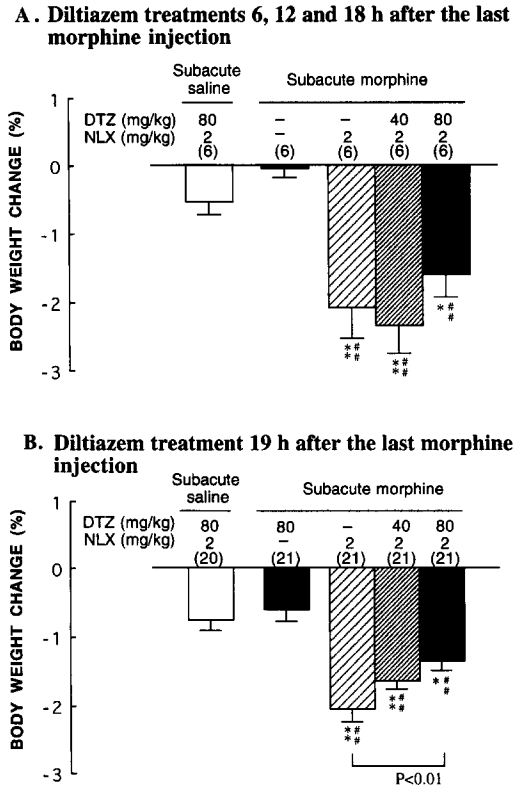


Fig. 1. Effects of diltiazem (DTZ) on naloxone-precipitated body weight loss in subacute morphine-exposed rats. DTZ was administered 6, 12 and 18 h after the last morphine injection (A) and 19 h after the last morphine injection (B). Naloxone (NLX; 2 mg/kg, s.c.) was injected 23 h after the last morphine injection and the percentage change of body weight during the first hour following NLX was evaluated. Numbers in parentheses indicates the number of rats. Each column represents the mean and vertical bars indicate the S.E.M. Differs from subacute saline control, ** $P < 0.01$, * $P < 0.05$ (Newman-Keuls test). Differs from subacute morphine control (without NLX), $^{\#\#}$ $P < 0.01$ (Newman-Keuls test).

morphine-exposed rats (Figs. 1–3). Diarrhea was also induced by naloxone but not by saline in these rats (Table 1). After the chronic morphine administrations, body weight loss and plasma corticosterone elevation were observed by the cessation of morphine administration, compared to chronic saline-exposed controls (Figs. 4–7), although diarrhea was not observed (data not shown). These results indicate that the naloxone-precipitated and the spontaneous withdrawal were elicited in subacute and chronic morphine treated rats, respectively.

3.2. Effects of diltiazem on naloxone-precipitated withdrawal

3.2.1. Body weight loss

In saline-exposed rats with diltiazem (80 mg/kg, s.c.) treatments (Fig. 1A,B), a small decrease in body weight was observed within 1 h of naloxone administration. Similar body weight changes were observed in drug-naive rats (Kishioka et al., 1994), indicating that neither naloxone nor diltiazem affected the body weight change in morphine-naive rats.

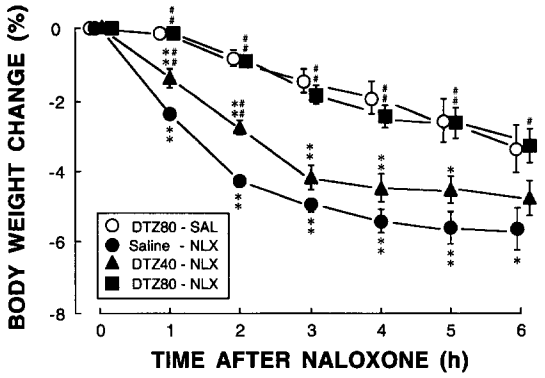


Fig. 2. Effects of diltiazem (DTZ) on naloxone-precipitated body weight loss in subacute morphine-exposed rats. All rats were exposed to subacute morphine. DTZ 40 mg/kg (DTZ40) or 80 mg/kg (DTZ80) was subcutaneously administered 21 h after the last morphine injection. Saline (SAL) or naloxone (NLX; 2 mg/kg, s.c.) were injected 23 h after the last morphine injection and the percent change of body weight was observed up to 6 h after NLX. Each point represents the mean and vertical bars indicate the S.E.M. of 7–8 rats. Differs from DTZ80-SAL (open circle), ** $P < 0.01$, * $P < 0.05$ (Newman-Keuls test). Differs from saline-NLX (closed circle), $^{\#\#}$ $P < 0.01$, $^{\#}$ $P < 0.05$ (Newman-Keuls test).

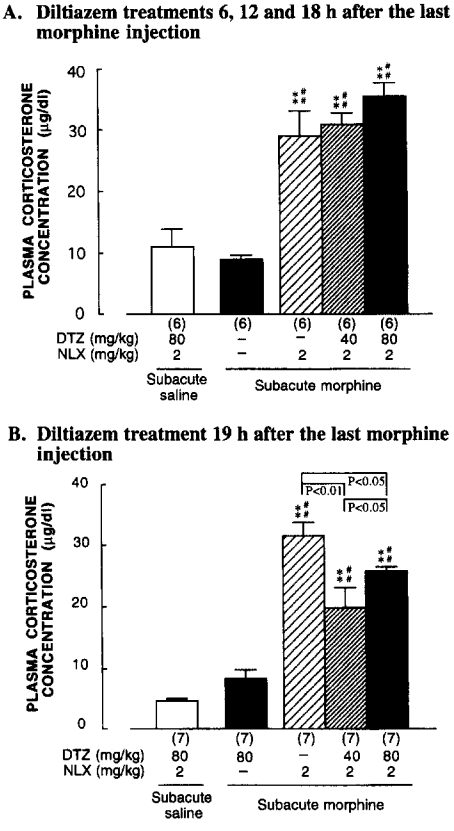


Fig. 3. Effects of diltiazem (DTZ) on naloxone-precipitated plasma corticosterone elevation in subacute morphine-exposed rats. DTZ was administered 6, 12 and 18 h after the last morphine injection (A) and 19 h after the last morphine injection (B). Naloxone (NLX; 2 mg/kg, s.c.) was injected 23 h after last morphine and the plasma corticosterone level was evaluated 1 h after NLX. Differs from subacute saline control, ** $P < 0.01$ (Newman-Keuls test). Differs from subacute morphine control (without NLX), ## $P < 0.01$ (Newman-Keuls test).

Table 1
Inhibitory effects of diltiazem (DTZ) on diarrhea induced by naloxone in morphine-dependent rats

Treatment [a]	Incidence of diarrhea [b]	
DTZ treatment at 6, 12 and 18 h after the last morphine injection		
Subacute saline + DTZ 80 mg/kg + NLX	0/6 (0%)	
Subacute morphine + saline + saline	0/6 (0%)	
Subacute morphine + saline + NLX	5/6 (83.3%)	
Subacute morphine + DTZ 40 mg/kg + NLX	4/6 (66.7%)	
Subacute morphine + DTZ 80 mg/kg + NLX	1/6 (16.7%)	
DTZ treatment at 19 h after last morphine injection		
Subacute saline + DTZ 80 mg/kg + NLX	0/20 (0%)	
Subacute morphine + DTZ 80 mg/kg + saline	0/21 (0%)	
Subacute morphine + saline + NLX	18/21 (85.7%)	}
Subacute morphine + DTZ 40 mg/kg + NLX	6/19 (31.6%)	} [c]
Subacute morphine + DTZ 80 mg/kg + NLX	4/21 (19.0%)	}
DTZ treatment at 21 h after the last morphine injection		
Subacute morphine + DTZ 80 mg/kg + saline	0/8 (0%)	
Subacute morphine + saline + NLX	8/8 (100%)	}
Subacute morphine + DTZ 40 mg/kg + NLX	3/7 (42.9%)	} [c]
Subacute morphine + DTZ 80 mg/kg + NLX	0/7 (0%)	}

[a] Morphine (20 mg/kg, s.c.) was injected once daily for 5 days to induce morphine dependence. Naloxone (NLX, 2 mg/kg, s.c.) was injected 23 h after the last morphine injection and diarrhea was observed during 1 h after NLX. [b] 'Diarrhea rats/total rats' and the incidence of diarrhea in parentheses. [c] $P < 0.01$ (the χ^2-test).

In morphine-exposed rats, the body weight change after saline was not affected by diltiazem (80 mg/kg, s.c.) treatments (Fig. 1B). Although the naloxone-precipitated body weight loss was not reduced by repeated treatments with diltiazem (three administrations of either 40 or 80 mg/kg, s.c., last dose of diltiazem was 5 h before naloxone) at 6-h intervals after the last morphine injection (Fig. 1A), a single diltiazem (80 mg/kg, s.c.) treatment 19 h after the last morphine (4 h before naloxone) significantly

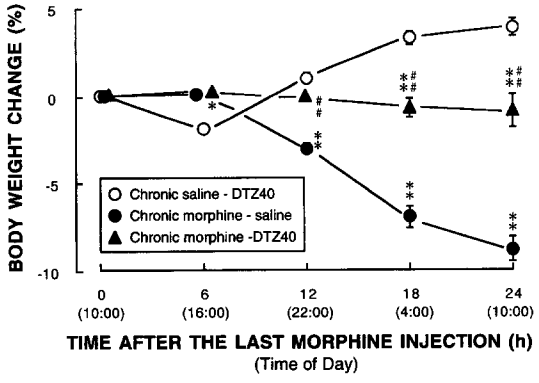
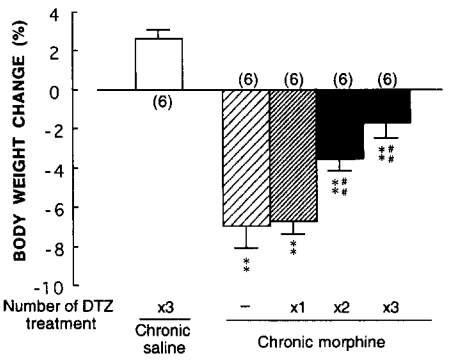


Fig. 4. Effects of diltiazem (DTZ) on the time course of body weight changes after the cessation of morphine treatment in chronic morphine-exposed rats. DTZ 40 mg/kg (DTZ40) was subcutaneously administered 6, 12 and 18 h after the last morphine injection. Each point represents the mean and vertical bars indicate the S.E.M. of 8 rats. Differs from chronic saline-DTZ40 (open circle), ** $P < 0.01$, * $P < 0.05$ (Newman-Keuls test). Differs from chronic morphine-saline (closed circle), $^{\#\#}$ $P < 0.01$ (Newman-Keuls test).

A. Diltiazem (40 mg/kg, s.c.) treatments with 1 - 3 times at 6 h intervals after the last morphine injection



B. Dose-dependency of diltiazem treatments at 6 h intervals after the last morphine injection

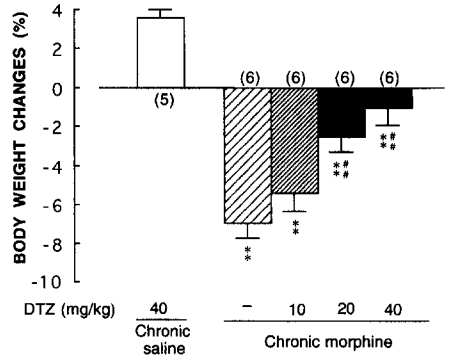


Fig. 5. Effects of diltiazem (DTZ) on body weight loss by spontaneous withdrawal in chronic morphine-exposed rats. DTZ (40 mg/kg, s.c.) was administered one (18 h after last morphine), 2 (12 and 18 h after last morphine) and 3 (6, 12 and 18 h after last morphine) times (A) and the various doses of DTZ (10, 20 or 40 mg/kg, s.c.) were administered 6, 12 and 18 h after the last morphine injection (B). The percentage change in body weight was evaluated 24 h after the last morphine injection. Differs from chronic saline + DTZ 40 mg/kg, ** $P < 0.01$ (Newman-Keuls test). Differs from chronic morphine + saline instead of DTZ, $^{\#\#}$ $P < 0.01$ (Newman-Keuls test).

inhibited the naloxone-induced body weight loss in a dose-dependent manner (Fig. 1B). These results suggested that inhibitory effects of diltiazem on naloxone-induced body weight loss were related to the interval between diltiazem and naloxone administration and the dose of diltiazem, and not to the frequency of diltiazem administration.

In order to clarify the inhibitory effects of diltiazem on the naloxone-precipitated body weight loss, diltiazem (40 or 80 mg/kg, s.c.) was administered 21 h after the last morphine injection (i.e., 2 h before naloxone) and the body weight change was successively observed for 6 h after naloxone (Fig. 2). Dose-dependent inhibitory effects of diltiazem on the naloxone-precipitated body weight loss were clearly detected in the time course of body weight loss after naloxone in morphine-exposed rats. In particular, the treatment with diltiazem 80 mg/kg completely blocked the body weight loss, and inhibitory effects of diltiazem

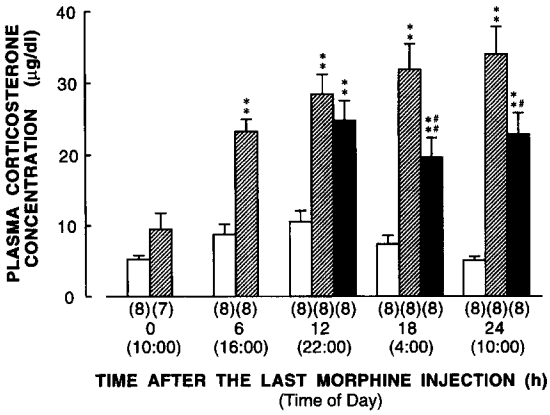


Fig. 6. Effects of diltiazem (DTZ) on the time course of plasma corticosterone elevation after the cessation of morphine treatment in chronic morphine-exposed rats. DTZ (40 mg/kg, s.c.) was treated at 6, 12 and 18 h after the last morphine injection. Open column; chronic saline with DTZ, hatched column; chronic morphine without DTZ, closed column; chronic morphine with DTZ treatment 6, 12 and 18 h after the last morphine injection. Differs from respective chronic saline + DTZ (open column), ** $P < 0.01$ (data at 6 h after the last morphine injection were analyzed by Student's t-test and others were analyzed by Newman-Keuls test). Differs from respective chronic morphine without DTZ (hatched column), $^{\#\#}$ $P < 0.01$, $^{\#}$ $P < 0.05$ (Newman-Keuls test).

were more potent than those of diltiazem injected 4 h before naloxone.

3.2.2. Plasma corticosterone elevation

In saline-exposed rats with diltiazem (80 mg/kg, s.c.) treatments (Fig. 3A,B), plasma corticosterone levels at 1 h after naloxone were similar to those observed in naive rats (Kishioka et al., 1994), indicating that neither naloxone nor diltiazem affected plasma corticosterone levels in morphine-naive rats.

In morphine-exposed rats, plasma corticosterone levels after saline were not affected by diltiazem (80 mg/kg, s.c.) treatments (Fig. 3B). The naloxone-precipitated plasma corticosterone elevation was not reduced by repeated treatments with diltiazem (three administration of either 40 or 80 mg/kg, s.c.) at 6-h intervals after the last morphine injection (Fig. 3A). However, a single treatment with diltiazem at 19 h after the last morphine injection significantly inhibited the naloxone-precipitated plasma corticosterone elevation, although the inhibitory effect of diltiazem 80 mg/kg was weaker than that of diltiazem 40 mg/kg (Fig. 3B).

The effects of diltiazem (40 and 80 mg/kg, s.c.) administered 21 h after the last morphine injection (2 h before naloxone) on naloxone-precipitated plasma corticosterone elevation could not be investigated, because at that time these doses of diltiazem alone increased plasma corticosterone levels in morphine-naive rats (data not shown).

3.2.3. Diarrhea

Diarrhea was elicited neither by naloxone in saline-exposed control rats treated with diltiazem (80 mg/kg, s.c.), nor by saline in morphine-exposed rats treated with saline or diltiazem (80 mg/kg, s.c.; Table 1).

Naloxone-induced diarrhea in morphine-exposed rats was reduced by diltiazem treatments at 6, 12 and 18 h after the last morphine dose, although the effect did not reach statistical significance. A significant and dose-dependent reduction of the incidence of the diarrhea was caused by diltiazem administered both at 19 h and at 21 h after the last morphine injection.

3.3. Effects of diltiazem on spontaneous withdrawal

3.3.1. Body weight loss

The effects of diltiazem treatments (40 mg/kg, s.c. at 6-h intervals after the last chronic administration) on the time-course of body weight changes in saline- or morphine-exposed rats are shown in Fig. 4. In saline-exposed control rats with diltiazem treatments, the body weight changes were similar to those observed in drug-naive rats,

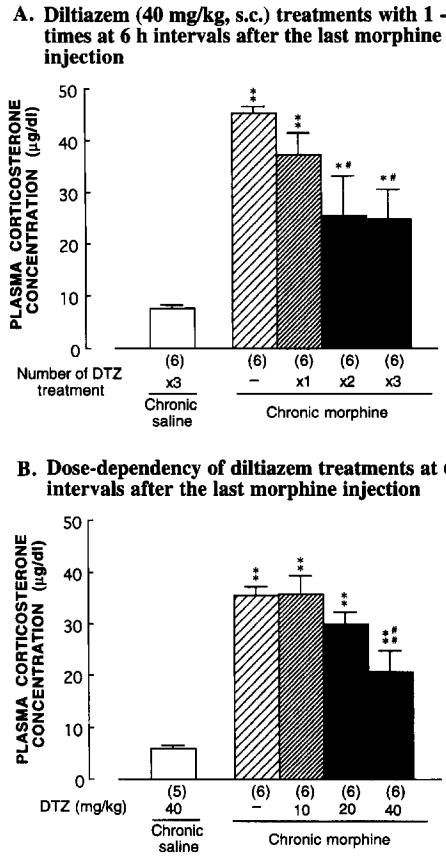


Fig. 7. Effects of diltiazem (DTZ) on plasma corticosterone elevation by spontaneous withdrawal in chronic morphine-exposed rats. DTZ (40 mg/kg, s.c.) was administered one (18 h after last morphine), 2 (12 and 18 h after last morphine) and 3 (6, 12 and 18 h after last morphine) times (A) and the various doses of DTZ (10, 20 or 40 mg/kg, s.c.) were administered 6, 12 and 18 h after the last morphine injection (B). The plasma corticosterone concentration was evaluated at 24 h after the last morphine injection. Differs from chronic saline + DTZ 40 mg/kg, ** $P < 0.01$, * $P < 0.05$ (Newman-Keuls test). Differs from chronic morphine + saline instead of DTZ, $^{\#\#}$ $P < 0.01$, $^{\#}$ $P < 0.05$ (Newman-Keuls test).

i.e., mild body weight loss during daytime followed by an increase during nighttime. After the cessation of chronic morphine, the body weight gradually decreased. The spontaneous withdrawal-induced body weight loss was significantly inhibited by diltiazem (40 mg/kg, s.c.) administered at 6-h intervals after the last morphine injection.

Then the effect of frequency of diltiazem administration (40 mg/kg, s.c.) on spontaneous withdrawal-induced body weight loss was investigated (Fig. 5A). The body weight loss induced by the spontaneous withdrawal was inhibited by two (12 and 18 h after last morphine; ×2) and three (6, 12 and 18 h after last morphine; ×3) treatments with diltiazem, but not by a single diltiazem treatment (18 h after last morphine; ×1), and the degree of the inhibitory effect of diltiazem was related to the frequency of the diltiazem treatments.

Dose dependence of diltiazem's effect on spontaneous withdrawal-induced body weight loss was examined (Fig. 5B). Diltiazem (10–40 mg/kg, s.c.) was administered 3 times at 6-h intervals after the last morphine injection. The spontaneous withdrawal-induced body weight loss was significantly inhibited by treatment with diltiazem 20 and 40 mg/kg in a dose-dependent manner.

3.3.2. Plasma corticosterone elevation

The effects of diltiazem treatments (40 mg/kg, s.c. at 6-h intervals after the last chronic administration) on time course of plasma corticosterone levels during the 24 h following chronic treatment with saline or morphine are shown in Fig. 6. In saline-exposed control rats treated with diltiazem 6, 12 and 18 h after the last chronic saline injection, a normal circadian variation in circulating plasma corticosterone was observed, i.e., low plasma corticosterone level during daytime and slightly higher level during nighttime. In morphine-exposed rats, plasma corticosterone gradually increased after the cessation of chronic morphine treatment. The spontaneous withdrawal-induced plasma corticosterone elevation at 18 and 24 h after the last morphine was significantly inhibited by diltiazem treatment.

In Fig. 7A, we demonstrated the relationship between the frequency of diltiazem (40 mg/kg, s.c.) treatments and the inhibitory effect of diltiazem on spontaneous withdrawal-induced plasma corticosterone elevation. The plasma corticosterone levels were measured 24 h after the last morphine injection. The plasma corticosterone level in saline-exposed control rats treated with diltiazem at 6, 12 and 18 h after the last chronic saline injection was similar to resting plasma corticosterone level in naive rats (Kishioka et al., 1994), indicating that these diltiazem treatments did not affect baseline plasma corticosterone level. The spontaneous withdrawal-induced plasma corticosterone elevation was reduced by two (12 and 18 h after last morphine; ×2) and three (6, 12 and 18 h after last morphine; ×3) diltiazem administrations, but not by a single administration of diltiazem (18 h after last morphine; ×1), and the extent of the inhibitory effect of diltiazem depended on the number of the diltiazem treatments.

The dose dependency of diltiazem's inhibitory effect on spontaneous withdrawal-induced plasma corticosterone elevation was examined (Fig. 7B). Diltiazem (10–40 mg/kg, s.c.) was administered 3 times at 6-h intervals after the last morphine injection. Spontaneous withdrawal-induced plasma corticosterone elevation was significantly reduced by the treatment with diltiazem 40 mg/kg and the inhibitory effect of diltiazem was dose-dependent.

4. Discussion

In the naloxone-precipitated withdrawal study, a single treatment with diltiazem 19 h after the last morphine injection (4 h before naloxone) significantly inhibited the withdrawal-induced body weight loss in a dose-dependent manner. Moreover, diltiazem treatment at 21 h after the last morphine (2 h before naloxone) markedly reduced the naloxone-precipitated body weight loss. Similarly, the withdrawal-induced plasma corticosterone elevation was also inhibited by the treatment with diltiazem 19 h after the last morphine injection, consistent with previous reports (Caro et al., 1988; Barrios and Baeyens, 1991; Colado et al., 1993). However, 3 times treatment with diltiazem, given at 6-h intervals after the last morphine injection (the last administration of diltiazem was 5 h before naloxone) did not inhibit the body weight loss and plasma corticosterone elevation. These results revealed that inhibitory effects of diltiazem on naloxone-precipitated withdrawal were related to the interval between diltiazem and naloxone administration rather than the number of pretreatments with diltiazem. In previous reports, L-type Ca^{2+} channel blockers were administered within 1 h before naloxone injection in order to alleviate the naloxone-precipitated withdrawal (Bongianni et al., 1986; Baeyens et al., 1987; Caro et al., 1988; Ramkumar and El-Fakahany, 1988; Antkiewicz-Michaluk et al., 1990; Barrios and Baeyens, 1991; Colado et al., 1993). These findings indicated that the diltiazem treatment in temporal vicinity to naloxone injection was necessary to achieve inhibition of the naloxone-precipitated withdrawal.

In the spontaneous withdrawal study, both body weight loss and plasma corticosterone elevation induced by the cessation of chronic morphine were dose dependently inhibited by the diltiazem (10, 20 and 40 mg/kg) treatments at 6, 12 and 18 h after the last morphine injection. Both body weight loss and plasma corticosterone elevation were significantly reduced by 40 mg/kg, but not by 10 mg/kg of diltiazem given at 6-h intervals after the last morphine injection. Meanwhile, body weight loss and plasma corticosterone elevation were inhibited by both two (6 and 12 h after the last morphine injection) and three (6, 12 and 18 h after the last morphine injection) treatments with 40

mg/kg of diltiazem, related to the frequency of diltiazem treatments but not inhibited by a single treatment with 40 mg/kg of diltiazem at 18 h after the last morphine injection. These results suggest that the exposure to diltiazem with higher plasma concentration during the initial stage of morphine withdrawal was an important factor for the inhibition of spontaneous withdrawal, consistent with the results of the naloxone-precipitated withdrawal experiment.

It has been suggested that the body weight loss induced by morphine withdrawal was likely due to diarrhea (Wei et al., 1973). However, in the present naloxone-precipitated withdrawal study, diltiazem treatments at 6-h intervals after the last morphine injection tended to inhibit the diarrhea but did not inhibit the body weight loss at all. Conversely, in the spontaneous withdrawal study, body weight loss was observed without diarrhea. These results suggest that the morphine withdrawal-induced body weight loss might not result solely from the occurrence of diarrhea. Although the mechanisms of withdrawal-induced body weight loss and diarrhea are not clear, Baeyens et al. (1987) demonstrated that naloxone-precipitated body weight loss was reduced by i.c.v. injection of the L-type Ca^{2+} channel blocker, verapamil, without inhibition of diarrhea in morphine-dependent rats, suggesting that the body weight loss was mediated by a central mechanism, whereas the diarrhea was mediated by the peripheral mechanism. High-affinity binding sites for L-type Ca^{2+} channel blockers (dihydropyridines) have been demonstrated in the brain (Triggle and Janis, 1987). Morphine analgesia was enhanced by diltiazem administered s.c. or i.c.v. in mice (Del Pozo et al., 1990). These facts indicated that the Ca^{2+} flux across L-type Ca^{2+} channels in central nervous system was functionally linked to activation of opioid receptors and that systemic administration of diltiazem might act on both peripheral organs and the central nervous system. Taken together, the present results suggest that the diltiazem s.c.-induced inhibition of morphine withdrawal signs was mediated through not only a peripheral mechanism (diarrhea) but also a central mechanism (body weight loss).

Naloxone-precipitated diarrhea (presumably a peripherally mediated effect) was inhibited by the treatments with diltiazem at 6, 12 and 18 h after the last morphine injection, although naloxone-precipitated body weight loss (presumably a centrally mediated effect) was not. These findings could be explained, at least in part, by the pharmacokinetic characteristics of diltiazem, since it has been shown that the diltiazem concentration in the brain was considerably lower than that in peripheral organs, following systemic administration (Naito et al., 1986).

Morphine withdrawal also led to a rapid increase in plasma corticosterone concentration (Yamamoto et al., 1973; Eisenberg, 1983; Kishioka et al., 1994) together with the increase of adrenocorticotropic hormone (ACTH) secretion (Donnerer and Lembeck, 1988), and these responses were the result of increased corticotropin-releasing factor (CRF) secretion in the hypothalamus (Harbuz et al., 1991). In morphine-exposed rats, plasma corticosterone elevation was also elicited by i.c.v. naloxone (Eisenberg, 1985). These results suggest that morphine withdrawal-induced plasma corticosterone elevation was mediated through a central mechanism. Although systemic injection of diltiazem might act on both peripheral organs and the central nervous system as mentioned above (in vitro study), L-type Ca^{2+} channel blocker (verapamil) inhibited neither the CRF-induced ACTH secretion from rat anterior pituitary cells (Giguere et al., 1982) nor the ACTH-induced corticosterone secretion from rat adrenocortical cells (Shima et al., 1979). These results suggest that the inhibitory effects of diltiazem on the increase in plasma corticosterone following morphine withdrawal were mediated through central mechanisms.

Clonidine, an antihypertensive drug, is also used for the treatment of opioid withdrawal (O'Brien, 1996) and has been reported to alleviate some of the naloxone-precipitated withdrawal signs, including diarrhea (Cervo et al., 1981), through a non-opioid mechanism (Katz, 1986). However, the elevation of plasma corticosterone precipitated by naloxone was not affected by clonidine in morphine-dependent rats (Eisenberg, 1983). On the other hand, diltiazem, in this experiment, inhibited both naloxone-induced diarrhea and plasma corticosterone increase. Thus, it appears that there may be differences between the inhibitory effects of diltiazem and clonidine on the morphine withdrawal signs.

In conclusion, systemic treatment with diltiazem inhibited not only some naloxone-precipitated but also spontaneous morphine withdrawal signs, i.e., body weight loss, diarrhea and plasma corticosterone elevation. The inhibitory effects of diltiazem on naloxone-precipitated withdrawal signs was related to the interval between diltiazem and naloxone administration and the dose of diltiazem. The inhibitory effects of diltiazem on spontaneous withdrawal signs were related to the frequency and the dose of diltiazem administered. The protective effects of diltiazem against both spontaneous and antagonist-precipitated morphine withdrawal suggests that L-type Ca^{2+} channel blockers may be useful therapeutic adjuncts in the management of opiate-dependent patients.

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